The female athlete triad profile of elite Kenyan runners and its future health implications

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Declaration

Prof. M.A. Monyeki (Promoter and co-author), professors J.H. De Ridder (Co-promoters and co-authors), A.L. Toriola, and M.K. Boit (Assistant-promoters and co-authors) hereby give permission to the candidate, Mrs Y Goodwin to include the articles as part of a doctoral thesis. The contribution of each co-author, both as promoter and candidate was kept within reasonable limits and included:

Mrs Y. Goodwin:	Developing the proposal, interpretation of the results, writing of the manuscript and the thesis;
Promoter/Supervisor:	Prof. dr M.A. Monyeki
Co-promoter:	Prof. dr Hans de Ridder;
Assistant Promoters:	Prof. dr Abel L Toriola (TUT) and Prof. dr Michael K. Boit (Kenyatta University)

This thesis, therefore, serves as fulfilment of the requirements for the PhD degree in Human Movement Science within Physical, Activity, Sport and Recreation (PhASRec) in the Faculty of Health Sciences at the North-West University, Potchefstroom Campus.

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ABSTRACT

The female athlete triad (FAT or the TRIAD) is a complex syndrome arising from associations among the trio of energy availability (EA), menstrual function (MF) and bone mineral density (BMD) along their respective continuums from health to disease state. It has been recognized that women whose energy intake (EI) does not meet the energy requirements for physiological functions subsequent to participation in exercise and physical activity could have low EA. In the TRIAD, low EA, an initiator in menstrual dysfunction (MD) and concomitant hypoestrogenism, indirectly results in low BMD. Therefore, the purpose of this study was to: (i) establish the status of EA, MF and BMD among elite Kenyan female athletes and non-athletes, (ii) explore associations between EA and MF in elite Kenyan female athletes and non-athletes, (iii) determine the relationships of EA and MF to BMD in elite Kenyan female athletes and non-athletes, and (iv) to determine the profile of the female athlete triad in elite Kenyan distance athletes and in non-athletes. Measurements of EA, MF and BMD were undertaken in 39 female participants (Middle distance athletes =12, Long distance athletes=13, Non-athletes=14). Energy intake minus exercise energy expenditure (EEE) and the remnant normalized to fat free mass (FFM) determined EA. Energy availability was determined through weight of all food and liquid consumed over three consecutive days. Exercise energy expenditure 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+. Fat free mass and BMD were assessed using dual energy x-ray absorptiometry (DXA). A nine-month daily temperature-menstrual diary was used to evaluate menstrual status. In addition, since psychological eating behaviour practice (EBP) contributes to low EA, the Eating Disorder Examination Questionnaire (EDE-Q) was used to determine presence of such practice among the participants and their relationship to EA. Overall, EA below 45 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$ was found in 61.53% of the participants (athletes=28.07±11.45$kcal \cdot kgFFM^{-1} \cdot d^{-1}$, non-athletes=56.97±21.38$kcal \cdot kgFMM^{-1} \cdot d^{-1}$). The ANOVA showed that there was a significant difference (p<0.001) in EA among the long and middle distance runners and non-athletes; and the Tukey's HSD revealed that the source of the difference were the non-athletes. Results of the EDE-Q showed almost negligible presence of psychopathological eating behaviour practice among the Kenyan participants. None of the TRIAD components showed significant relationship with EBP. Results of MF showed that whereas none of the athletes presented with amenorrhea, oligomenorrhea was present among 40% athletes and 14.3% non-athletes, and amenorrhea

among 14.3% non-athletes. However, there was no significant difference between athletes and non-athletes in MF. Low BMD was seen in 76% of the athletes and among 86% of the non-athletes. The analysis did not show significant difference in BMD *Z*-scores between athletes and non-athletes. The analysis did not show any significant association between EA and MF among the participants. The only significant relation of EA to any BMD dimension measured was between EA and total BMD in the long distance runners (r=0.560; p=.046). Significant relationship (*rho*=0.497; p=.001) was found between MF and BMD *Z*-scores among the athletes with middle distance highlighting the relationship further (*rho*=0.632; p=.027). Overall, the binary logistic regression revealed that MF did not predict BMD (OR=4.07, 95% CI, 0.8-20.7, p=.091). Overall, 10% of the participants (athletes=4, long distance athletes =3, middle distance athletes=1, non-athletes=0) showed simultaneous presence of all three components of the TRIAD. The independent sample t-test showed a significant difference (t=5.860; p=<.001) in the prevalence of the TRIAD between athletes and non-athletes.

Keywords: Energy availability, menstrual function, bone mineral density, exercise energy expenditure.

OPSOMMING

Die vroulike atleet triade (VAT of die TRIAD) is ’n komplekse sindroom wat ontstaan uit die assosiasies tussen die drietal van energie beskikbaarheid (EB), menstruele funksie (MF) been minerale digheid (BMD). In die triade, lei ‘n lae EB, ’n die inisieerder in menstruele disfunksie (MD), tesame met hipo-estrogenisme, indirek tot die ontstaan van lae BMD. Die doel van die studie was derhalwe om: (i) die status van EB, MF en BMD tussen elite Keniaanse vroulike atlete en nie-atlete vas te stel, (ii) die verwantskappe tussen EB en MF in elite Keniaanse vroulike atlete en nie-atlete te ondersoek, (iii) om die verwantskappe tussen EB en MB met BMD in elite Keniaanse vroulike atlete en nie-atlete te bepaal, en (iv) om die vroulike atleet triade-profiel tussen elite Keniaanse vroulike atlete en nie-atlete te bepaal. Metings tekende EB, MF en BMD is gedoen in 39 vroulike deelnemers (middel-afstand atlete =12, lang afstand-atlete = 12, nie-atlete = 14). Energie inname minus energie verbruik tydens oefening (EEE) en die oorblyfsel genormaliseer na vet vrye massa (VVM) bepaal EB. Beskikbaar energie is bepaal deur gewig van alle kos en vloeistof ingeneem oor drie aaneenlopende dae. Energie verbruik tydens oefening besteding is bepaal na isolasie en die aftrekking van energie verbrand in oefening of fisieke aktiwiteit bo leefstylvlak van die totale energie bestedings uitset soos gemeet deur Actigraph GT3X+. Vet vrye massa en BMD was bepaal deur gebruik te maak van duaal-energie x-straal-absorpsimetrie (DXA). ‘n Nege maandelange daaglikse temperatuur-menstruele logboek is gebruik om die menstruele status te evalueer. Aangesien byvoeging, sedert psigologiese eetgedrag (EG) tot lae EB, kan bydra is hou die energie teken in halwe gebruik om die voorkoms van sulke praktyke onder die deelnemers en hul verwantskappe tot EB vas te stel. In opsig is EB laer an 45 kkal/kgVVM^{-1}·d^{-1} gevind by 61.53% van die deelnemers (atlete = 28.07 ± 11.45 kkal/kgVVM^{-1}·d^{-1}, nie atlete=56.97 ± 21.38kkal/kgVVM^{-1}·d^{-1}). Die ANOVA het ’n betekenisvolle verskil (p<0.001) in EB onder die lang- en middel-afstand hardlopers en nie-atlete aangetoon, en die Tukey’s HSD het aangetoon dat die verskil met die nie-atlete was. Resultate van die EDE-Q wys ‘n bykans onbeduidende teenwoordigheid van psigopatologiese eetgedrag praktyk by die Keniaanse deelnemers. Geen van die TRIADE komponente het ’n betekenisvolle verwantskap getoon met EG getoon nie. Resultate van MF dat aangesien geen van die atlete amenorrhea, 40% van die atlete en 14.3% nie-atlete, en amenorrhea vertoon het met 14.3% van die nie-atlete me wat amenoriee vertoon het. Lae BMD is waargeneem in 76% van die atlete en

86% van die nie-atlete. Die enigste betekenisvolle verband van EB met enige BMD Dimensie gemeet was tussen EB en totale BMD in die lang-afstand atlete (*r*=0.560; *p*=.046). Betekenisvolle verhouding (*rho*=0.497; *p*=.001) is gevind tussen MF en BMD *Z*-tellings by die middel-afstandafstand atlete wat die verhouding verder beklemtoon(*rho*=0.632; *p*=.027). Oor die algemeen het die binêre logistiese-regressie aangetoon dat MF nie BMD (OR=4.07, 95% CI, 0.8-20.7, *p*=.091) voorspel het nie. Oor die algemeen 10% van die deelnemers (atlete = 4, lang-afstand atlete = 3, middel-afstand atlete = 1, nie-atlete = 0) gelyktydige aanwesigheid van al drie komponente van die TRAID aangetoon. Die onafhanklike t-toets toon ’n betekenisvolle verskil (*t*=5.860; *p*=<.001) in aanwesigheid van die triade tussen atlete en nie-atlete. As een van die eerste studies aangaande Keniaanse vroulike atlete, verskaf die leemtes wat in die studie voorkom matiging vir verder navorsing, wat direkte hormonale en sterodiale assessering insluit wanneer menstruale funksie bepaal word.

Sleutelwoorde: Energie-beskikbaarheid, menstruele-funksie, beenminerale digheid, oefening-energie verbruik.

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List of abbreviations and symbols

ACSM:	American College of Sports Medicine
ADA:	American Dietetic Association
AK:	Athletics Kenya
AMH:	Anti-mullerian hormone
AN:	Anorexia nervosa
ANOVA:	Analysis of variance
ASRM:	American Society for Reproductive Medicine
AA:	Athletic amenorrhea
ATP:	Adenosine triphosphate
BA:	Bone area
B.E.S.T.:	The Better Eating Safer Training Research Study
BUTE:	Bulimia Inventory Test Edinburgh
BMC:	Bone Mineral Content
BMD:	Bone Mineral Density
BMI:	Body Mass Index
BN:	Bulimia Nervosa
BSEA:	Border line sub-optimal energy availability
BSI:	Bone strength Index
CHO:	Carbohydrates
cm:	Centimetres
DC:	Dieticians of Canada
DE:	Disordered Eating
DHEAS:	Dehydroepiandrosterone sulphate

DXA: Dual-energy x-ray absorptiometry

E_2: Oestrogen/Estradiol

EA: Energy Availability

EAT-26: Eating Attitude Test-26

EB: Energy balance

EBP: Eating behavioural practice

ED: Eating Disorders

EDE-Q: Eating Disorder Examination Questionnaire:

EDI: Eating Disorder Inventory

EDNOS: Eating Disorder Not Otherwise Specified

EDTA: ethylenediaminetetraacetic acid

EEE: Exercise energy expenditure

EER: Estimated energy requirements

EI: Energy intake

ELISA: Enzyme-linked immunosorbent assay

FAT: Female Athlete Triad

$FFM.d^{-1}$: Free fat mass per day

FFM: Fat free mass

FHA: Functional Hypothalamic Amenorrhea

FSH: Follicle-stimulating Hormone

g/BW: grams per body weight

$g.kg^{-1}d^{-1}$: grammes/kilograms of body weight per day

GnRH: Gonadotropin-releasing Hormone

HC: Hormonal contraceptives

HEA: Healthy energy availability

Hgb: Haemoglobin

HPO:	Hypothalamic-pituitary-ovarian
HSD:	Honestly significant difference (Tukey's)
IGF-1/2:	Insulin growth factor-1/2
IGFBP-3:	Insulin-like growth factor binding protein-3
IOC:	International Olympic Committee
ISAK:	International Society for the Advancement of Kinathropometry
ISCD:	International Society of Clinical Densitometry
kg:	Kilogrammes
KU-ERC:	Kenyatta University Ethics Review Committee
LBM:	Lean body mass
LD:	Long Distance runners
LEA:	Low energy availability
LH:	Luteinizing Hormone
LPD:	Luteal Phase Defect/Deficiency
m^2:	Height squared
MD:	Middle Distance runners
MF:	Menstrual Function
ml:	millilitre
NA:	Non-Athletes/ Non-Athletic cohorts
NCST:	National Council for Science and Technology
OC:	Osteocalcin
PCOS:	Polycystic ovarian syndrome
PCr:	Phosphocreatine
PRO:	Protein
PTH:	Parathyroid hormone
QCT:	Quantitative computed tomography

RA:	Research assistant
REE:	Resting energy expenditure
rTfR:	Rat transferrin receptor
SCOFF:	Sick Control One-Stone Fat and Food Questionnaire
TEE:	Total energy expenditure
TFEQ:	Three Factor Eating Questionnaire
TRIAD:	The female athlete triad
TSH:	Thyroid-stimulating Hormone
UV:	Ultraviolet
uNTX:	Urinary N-telopeptide
WHO:	World Health Organization
±:	Plus or minus
α:	Alpha
<:	Less or below
>:	More or above
~:	Approximately

CHAPTER 1 PROBLEM STATEMENT, PURPOSE AND HYPOTHESES OF THE THESIS

1.0. INTRODUCTION

In a country where families survive on less than one US dollar per day (Kenya Economic Report, 2009:22), the financial rewards from participating in running competitions are tremendously motivating. Increasing number of girls and women in Kenya are taking up competitive running to elevate their families out of poverty for economic reasons (Onywera *et al.,* 2006:421). Despite phenomenal success achieved by Kenyan female athletes since their first international participation in 1965, the only study to have focused directly on the Kenyan female runners identified psychosocial factors as a possible cause for Kenyan female runners' disappearance into oblivion after just a couple of seasons (Mbaabu, 1997:8). Continuous rhythmicity of the menstrual cycle is unique to the female (Volk, 2010:1). Disruptions in these cycles could also affect the Kenyan female athlete's performance and health in the long term. As in all female athletes, these menstrual disruptions also need to be investigated (Manore *et al.,* 2007:s61-71).

1.1 PROBLEM STATEMENT

Suggestions of a possible association between physical activity and menstrual disorders date back to the 1970s. Bonen *et al.* (1979:15) highlighted reports and concerns about effects of intense exercise on normal menstrual cycle. A regular or normal menstrual cycle, referred to as eumenorrhoea lasts 28±7 days (Temme & Hoch, 2013:193). However, athletes can present menstrual disturbances such as primary amenorrhoea in which menarche or onset of first menses is delayed until about the age of 15 years (American Society for Reproductive Medicine [ASRM], 2008:S219); secondary amenorrhoea, when unless pregnant, there is absence of three or more consecutive menstrual cycles after menarche; oligomenorrhoea, in which the menstrual cycle is 35 days or longer (Torstveit & Sundgot-Borgen, 2005:142) and polymenorrhea, a shortened luteal phase with a complete cycle of less than three weeks (Sloane, 2002:92).

The menstrual cycle, beginning with the first day of bleeding and ending with the start of the next bleeding, is characterized by the follicular, ovulatory and luteal phases. The luteal phase is

characterized by increases in oestrogen/estradiol (E_2) and progesterone. Progesterone being thermogenic, causes an elevation in body temperature which lasts until start of the next cycle (Rosenblatt, 2007). Neuroendocrine dysfunction is the mechanism associated with exercise related ovarian dysfunction and results in functional hypothalamic amenorrhea (FHA) (Zanker, 2006:489). Athletes who start training, especially sports specific training, before menarche, tend to attain menarche later than the normal populations (Dusek, 2001:80, Baxter-Jones & Maffuli, 2002:14). Such delayed pubertal maturation has been associated with excessive exercise (Kasa-Vabu *et al.,* 2004:333). Hypothalamic dysfunction and disturbance of the gonadotropin-releasing hormone (GnRH) pulse generator initiate female athletes' reproductive abnormalities (Warren & Perlroth, 2001:3). Suppression of GnRh could result in infertility and irreversibly compromise bone density (Herrmann & Herrmann, 2004:1384; Birch, 2005:244).

Athletes with FHA tend to share some physical, physiologic, metabolic, and personality similarities with anorexic women - slender physiques and restricted eating practices (Zanker, 2006:489). Initial screening of menstrual dysfunction has been based on menstrual frequency and regularity using questionnaires that look for the number of appearances or non-appearances of monthly bleeding (Dusek, 2001:80) and menstrual patterns (Torstveit & Sundgot-Borgen, 2004:141). Recall of gynaecologic history has also been used to categorize menstrual status (Hagmar *et al.,* 2009:1242). Petrek *et al.* (2006:1046) used monthly bleeding calendars as surrogate for ovarian function. However, the serious health consequences of identified FHA ovarian dysfunction (Nattiv *et al.,* 2007:1870) requires more stringent laboratory investigations such as the analyses of the blood concentrations of thyroid-stimulating hormone (TSH), follicle stimulating hormone (FSH), prolactin, progesterone challenge test, estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEAS) to establish the exact nature of dysfunction (Lebrun, 2007:397). As cautioned by Loucks (2011), the necessity for repeated blood sampling every 10 to 20 minutes for 12 to 24 hours to assess functional status of luteinizing hormone (LH) could be expensive and not feasible.

By the early 1990s, the American College of Sports Medicine (ACSM) acknowledged that a significant number of female athletes were at risk of the female athlete triad or the TRIAD, the collective term ascribed to the inter-related syndrome comprising disordered eating, amenorrhea and osteoporosis (Abraham *et al.,* 2006:257; Manore *et al.,* 2007; Tietjen-Smith & Mercer, 2008:1). Osteoporosis is the bone condition typified by reduced bone mass and deteriorating

structural bone tissue that results in bone fragility with increased risk of fractures (Sundgot-Borgen & Torstveit, 2003:47; Health Encyclopedia, 2010). The problem of menstrual disturbances in general, and the female athlete triad in particular, was sufficiently widespread to warrant the focus of a consensus conference called by the ACSM's Task Force on Women's Issues (Otis *et al.*, 1997: i). Ten years later, Nattiv *et al.* (2007:1867) upgraded our understanding of the female athlete triad in the ACSM position stand and warned against the health risk of low energy availability with or without eating disorder, FHA and osteoporosis, individually or in combination. The broadened and updated definition of the triad from disordered eating, amenorrhea and osteoporosis to health spectrums of energy availability (EA), menstrual function (MF) and bone mineral density (BMD) has allowed the inclusion of more athletes with less severe conditions as having the components of the triad (Thein-Nessenbaum & Carr, 2011:108). Though not a component of the TRIAD, iron deficiency could be just as detrimental as FAT on the health and physical performance of female athletes. Fallon (2008:335) recommends special haemoglobin (Hgb) and ferritin examination in athletes entering elite sports. The problem is compounded when there is suboptimal dietary intake that combines with menstrual bleeding leading to low or negative iron balance and anaemia (McClung, *et al.,* 2009:124). The joint position statement from American Dietetic Association (ADA), Dieticians of Canada (DC) and ACSM advises that oxygen-carrying needs in distance runners could increase by 70% from normal levels for neural, muscular, behavioural and immune functions (ACSM *et al.,* 2009:716).

Despite the phenomenal success enjoyed by Kenyan female athletes, there is tremendous lack of investigation into issues that affect them or have impact on them. As revealed by literature, independently, collectively and inter-relatedly the three components of the Triad associated with nutritional, reproductive and skeletal status could have far reaching health and performance consequences for the female athlete. Competition longevity among the female runners has improved since Mbaabu's investigation in 1997. However, more needs to be done to improve and sustain performances of Kenyan female runners. In this wide information-gap concerning the female Kenyan runner, it became imperative to consider or exclude factors associated with the TRIAD that could affect health and performance by addressing the following questions: (a) What is the status of energy availability, menstrual function, and bone mineral density in elite Kenyan female athletes compared to non-athletes? (b) What is the association between energy availability and menstrual function in elite Kenyan athletes; and how does it compare to what is found in Kenyan non-athletes? (c) What is the relationship of energy availability and menstrual

function to bone mineral density in elite Kenyan runners, and how do they compare to what is found in Kenyan non-athletes? (d) What is the profile of the female athlete triad among elite Kenyan female athletes and non-athletes?

In view of the ever-increasing number of girls joining the running phenomenon in Kenya, responses to these questions would provide a scientifically sound basis for developing appropriate training programmes and diets for current and potential female athletes. The answers would provide Athletics Kenya, coaches, parents/guardians, Ministries of Primary and Secondary Education, and significant others involved in training girls and women, information about the TRIAD or its components amongst Kenyan athletes with a view to taking preventive, interventional or therapeutic measures. The answers would also provide students in the fields of physical education, recreation, exercise science, physiotherapy and nutrition with valuable knowledge about the female athlete triad and in turn, educate others about it. As the second exclusive study about the female athlete in Kenya, the results would generate extensive information for future research in the area.

1.2 OBJECTIVES

The objectives of this study were to:

2.1.1. Determine the status of energy availability, menstrual function, bone mineral density in elite Kenyan runners and non-athletes.

2.1.2. Determine the association between energy availability and menstrual functions among elite Kenyan athletes and non-athletes.

2.2.3. Investigate relationship of energy availability and menstrual function to bone mineral density in elite Kenyan runners and non-athletes.

2.2.4. Determine the profile of the female athlete triad and in the non-athletes.

1.3 HYPOTHESES

The study was based on the following hypotheses:

3.2.1. The status of energy availability, menstruation function, and bone mineral density would differ significantly between elite Kenyan female runners and non-athletes.

3.2.2. Significant association between energy availability and menstrual function would be found among elite Kenyan female athletes and non-athletes.

3.2.3. Significant relationship of energy availability and menstrual function to bone mineral density in elite Kenyan distance runners would be found.

3.2.4. Kenyan female athletes would show significantly higher profile of the female athlete triad than the non-athletes.

1.4. STRUCTURE OF THE THESIS

The thesis was submitted in the article format as approved by the senate of the North-West University, and was structured as follows:

Chapter 1: Introduction

Chapter 2: Literature review - Factors in The Female Athlete Triad.

Chapter 3: **Article 1:** Status of energy availability, menstrual function and bone mineral density in elite Kenyan female middle and long distance runners. This article was submitted to the *British Journal of Sports Medicine.*

Chapter 4: **Article 2**: The association between energy availability and menstrual function in elite Kenyan runners. This article was submitted for publication to the *African Journal for Physical, Health Education, Recreation and Dance.*

Chapter 5: **Article 3**: The relationship of energy availability and menstrual function to bone mineral density in elite Kenyan runners. This article was submitted for publication in the *BMC Public Health.*

Chapter 6: **Article 4**: Profile of the female athlete triad in elite Kenyan endurance athletes and in non-athletes was submitted to the *African Journal for Physical, Health Education, Recreation and Dance.*

Chapter 7: Summary, conclusions, limitations and recommendations**.**

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CHAPTER 2 FACTORS IN THE FEMALE ATHLETE TRIAD

2.0 INTRODUCTION

The 1972 passage of Title IX legislation in the United States of America (USA) was the impetus for tremendous surge in girls' and women's participation in sports and physical activity in North America (Beals & Meyer, 2007:69; Rumball & Lebrun, 2005:320). Participation in athletic activities has contributed greatly in health, cognitive, psychological and behavioural benefits gained by girls and women (Barrack & Van Loan, 2011:124). Regular participation in physical activity has been recognized as beneficial against early mortality and morbidity; and continual participation has been encouraged because of the substantial benefits that out-strip the manageable risks associated with physical activity (Tanji, 2000:175-176; American College of Sports Medicine, 2007:1867). The American College of Sports Medicine (ACSM) emphasizes regular programmed cardiorespiratory, resistance, flexibility and neuromuscular exercise training beyond daily activity as essential for enhancing physical fitness and health in most adults (ACSM, 2011:1334).

However, such encouragement and support for exercise is also accompanied by cautionary caveat from the International Olympic Committee (IOC) against two medical conditions that uniquely affect physically active women - the female athlete triad (FAT) or the Triad, and iron deficiency anaemia (IOC, 2009:546). Menstrual irregularity, being one of the most obvious and easiest signals for detecting presence of the female athlete triad, should raise warning signals (Raymond-Barker *et al.,* 2007:2). Reports that 90 percentage of women participating in the Tokyo Olympic Games in 1964 menstruated regularly; but, twelve years later, during the Montreal Olympic Games, 57% indicated irregular cycles, suggest that the condition may be escalating (Dusek, 2001:79).

Since protecting the health of the athlete is one of the highest priorities of the IOC, its consensus statement wonders whether, especially for the very elite athlete, the health benefits outweigh the long-term negative consequences of injury and disability (IOC, 2009:538). The IOC warns against low energy availability relative to the amount of energy expended during physical

activity, the consequential menstrual dysfunction and low bone density, which in extreme cases could result in osteoporosis (IOC 2009:546). This warning is particularly aimed at women and girls who participate in activities that favour slender or lean body types (Barrack & Van Loan, 2011:124), as they are the ones most likely to restrict energy availability (Manore *et al.*, 2007:S61). These concerns echoed warnings from the past regarding conditions such as infertility, stress fractures, eating disorders, and osteoporosis that could arise in the future from the presence of one or more preventable components of the Triad (Birch, 2005:244). Decreased endothelium-dependent vasodilatation, a precursor to cardiovascular disease, could be another serious potential health consequence of prolonged menstrual suppression in women presenting exercise-related amenorrhea (Hock *et al*., 2003:382).

2.1 HISTORICAL PERSPECTIVE OF THE FEMALE ATHLETE TRIAD

Understanding the female athlete triad, its prevention, treatment and management has evolved with ever-increasing research on the Triad including pathogenesis of its different interrelated dimensions (Nattiv, 2002:13). The ACSM initiated special Task Force on Women's Issues embarked on in-depth discussion of the Triad problem (Beals & Meyer, 2007:69). The three specific entities identified were disordered eating, as reflected in abnormal eating behaviour patterns (Sanborn *et al.,* 2000:200); amenorrhea or absence of three or more consecutive menses after menarche that were not due to pregnancy (Torstveit & Sundgot-Borgen, 2005a:142); and osteoporosis, manifest as increased bone fragility (Lerand & Williams, 2006:e12). Deliberations of the Task Force in 1992 led to official recognition of interrelatedness amongst the three distinct conditions of disordered eating, amenorrhea and osteoporosis, and terming of this three-condition syndrome as the 'female athlete triad' (Morgenthal, 2002:97). Taken separately, each entity has its own serious consequential morbidity and mortality (Rumball & Lebrun, 2004:153; Torstveit & Sundgot-Borgen, 2005b:184). It had been recognized that the etiological, pathogenic and resulting interrelatedness of the entities present serious negative prognosis for an athlete's health and performance (Torstveit & Sundgot-Borgen, 2005:1449).

The publication of its first Position Stand on the female athlete triad in 1997 by the ACSM, generated significant discussion and investigation into diagnosis, management and prevention of the Triad (Lebrun, 2007:397; Hock *et al.,* 2009:421). Much of the research focused on interrelatedness of individual components rather than on the Triad as a whole (Nichols *et al.,* 2006:137; Thein-Nissenbaum & Carr, 2011:109). Sceptical challenges that questioned the very

existence of the female athlete triad (DiPietro & Stachenfeld, 2006:490 - 493) were eloquently refuted (Loucks, 2007a:55-57). Misinterpretations, misrepresentations and misunderstanding about the Triad, its components and interrelatedness amongst them were clarified and corrected (De Souza *et al.,* 2007:58–59). Strong relationships found between disordered eating and menstrual irregularity were associated with low bone mineral density (BMD); and disordered eating without menstrual disturbances had also been associated with low BMD (Cobb *et al.,* 2003:711).

It was suggested that the large variations reported by researchers in prevalence of disordered eating, menstrual dysfunction and reduced bone density may have been due to differences in definitions, criteria, assessment methods and interpretation of the individual entities that made up the Triad (Nichols *et al.,* 2006:137-138). Khan *et al.* (2002:12) recommended the replacement of "osteoporosis" with what they considered the more appropriate, "osteopenia." Their analytical review on bone mineral density (BMD) had shown that compared to osteopenia, osteoporosis was relatively uncommon in the female athlete. Concerns had been voiced that the strict criteria set for each of the three Triad components excluded significantly large numbers of women and girls with less severe conditions but still at risk (Micklesfield *et al.,* 2007:679; Burrows *et al.,* 2007; Thein-Nissenbaum, 2011:108).

The better understanding of the Triad generated by prolific research, scientific evidence and discussion suggested need for review and an update of the ACSM Position Stand (Nattiv, 2002:13). It was admitted that perhaps each component in the Triad had been defined too narrowly (Loucks, 2005:S49). The revision of ACSM's Position Stand, began in 2003 by a team of experts in the Triad (Beals & Meyer, 2007:69), was published in 2007 (ACSM, 2007). The essence of interrelatedness among the three basic components of the Triad associated with dimensions of eating behaviour and food, menstrual status and skeletal health in the earlier Position Stand, has been reaffirmed in the new 2007 Position Stand (De Souza & Williams, 2010:1). However, cognizant of earlier concerns and based on stringent criteria for evaluating scientific evidence, each component was renamed and comprehensively redefined to shift focus from the extreme exclusionary clinical pathological end-point of clinical disorder to a more inclusive spectrum ranging from health to diseased state (Beals & Meyer, 2007:70; ACSM, 2007:1868). This revised definitive explanation of the female athlete triad as complex interrelationships along continuums from health to disease state amongst energy availability

(EA), menstrual function and bone health or BMD (Manore *et al.*, 2007:S61; Pantano, 2009:3) allows wider scope for inclusion of those with less severe conditions (Thein-Nissenbaum & Carr, 2011:108).

The term disordered eating has been replaced by energy availability, a concept that refers to a spectrum ranging from optimal healthy energy availability to low energy availability in the presence or absence of an eating disorder (ACSM, 2007:1868). The term amenorrhea, referring to the most severe menstrual dysfunction amongst physically active women (De Souza, 2003:1553), probably excluded many females at risk for not satisfying the strict amenorrhoeic criteria. The newer term, 'menstrual function', captures the spectrum of menstrual function ranging from eumenorrhea or optimal regular cycles to a wide spectrum of dysfunctions such as luteal suppression, anovulation, oligomenorrhoea, and primary and secondary amenorrhea (Gottschlich, 2012:2). Osteoporosis, the third component of the Triad in the earlier Position Stand has been associated with most severe deteriorating structural bone tissue resulting in bone fragility and increased risk of fracture (Health Encyclopedia, 2010). Hence, in the revised updated Position Stand, the term 'osteoporosis' has been replaced with the concept of bone mineral density making it all-inclusive of bone status ranging from optimal bone health to osteoporosis (ACSM, 2007:1868).

The Position Stand strongly recommends optimal energy availability, eumenorrhea and optimal bone health (ACSM, 2007:1868). Instead of the extreme conditions in each original Triad component, the revised stand emphasizes that athletes may present intermediate or sub-clinical aspects of each component; each component may develop at a different rate through its continuum from health to disease state; and though conditions could occur independent of each other, it is possible that an athlete experiencing deterioration in one component, may also have problems in the other components (De Souza & Williams, 2010:2).

2.2 THEORETICAL FRAMEWORK

2.2.1 The Female Athlete Triad (or the Triad)

Historically, recognition and confirmation of energy availability, menstrual function and bone mineral density along complex interrelated continuums from health to disease state as the three components in the female athlete triad in ACSM (2007) could be considered relatively recent. Researchers have reaffirmed interrelatedness amongst these components (American Academy of Pediatrics, 2000:610; Hobart & Smucker, 2000:3357; Cobb *et al.*, 2003:711; Nichols *et al.*, 2006:137; ACSM, 2007:1867; IOC, 2009:546). Theoretically, disturbances in each component have different potential causes. For example, energy deficit could result from intentional or non-intentional eating behavioural practices (Papanek, 2003:595). Anatomical anomalies in the ovaries, uterus or vagina, or disruptions in endocrine signals could all cause menstrual disorders (Pfeifer & Patrizio, 2002:3; Redman & Loucks, 2005:750). Any one factor or more from among genetics, nutrition, hormones, weight-bearing exercise, alcohol consumption, and cortisol levels could be implicated in deviations from optimal BMD (Papanek, 2003:600). However, as seen in the adapted *Figure 1* (Nattiv *et al.*, 2007:1868), there appears to be a sequential pattern in the cascade of events in the composite Triad (Beals & Meyer, 2007:71). Inadvertent, unintentional or psychopathological low energy availability seems to be the instigating factor impairing menstrual/reproductive and skeletal health (Nattiv *et al.*,2007:1867) that manifest as functional hypothalamic amenorrhea (FHA) (Zanker, 2006:489); and subsequent low bone density (Pfeifer & Patrizio, 2002:6).

2.2.2 Energy Availability

Energy availability in the Triad refers to the available energy from dietary energy intake (EI) after deducting energy expended in exercise or exercise energy expenditure (EEE) (Manore *et al.*, 2007:S61). This residual available energy, termed resting energy expenditure (De Souza *et al.*, 2007:971), is what supports all other bodily functions including reproductive and endocrine (Loucks, 2007b:1467; De Souza *et al.*, 2007:971; Pantano, 2009:3). Energy balance in healthy,

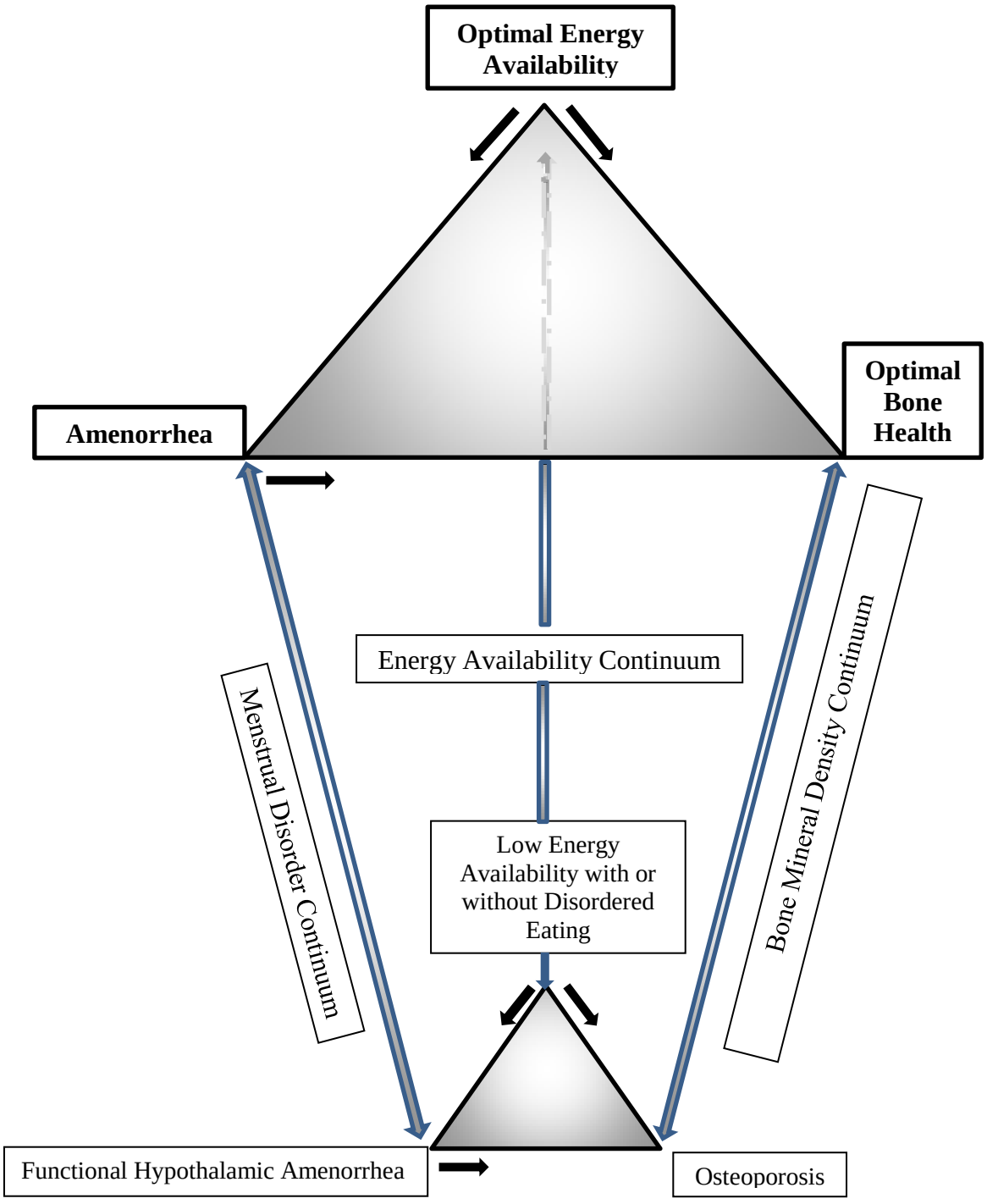

Figure 1: The female athlete TRIAD (Adapted from: Nattiv, A., Loucks, A. B., Manore, M.M., Sanborn, C. F., Sundgot-Borgen, J. & Warren, M. P. 2007. The female athlete triad. *Medicine and Science in Sports and Exercise*. 39:1867 – 1882).

young adult women occurs when energy availability is approximately 45kcal/kgFFM/d (free fat mass/day) and resting metabolic rate is approximately 30kcal/kgFFM/d. If energy availability falls below the resting metabolic threshold, reproductive function and subsequently bone formation is disrupted (Loucks & Nattiv, 2005:S49; Manore *et al.,* 2007:S61-71; West *et al.,* 2009:137).

Energy deficit in athletes occurs through deliberate or unintentional restriction in dietary energy intake relative to energy expenditure. Athletes experience energy deficiency by increasing exercise expenditure relative to dietary intake (ACSM, 2007:1869) or by adopting, counter-productive and ineffective disordered eating behaviours (Pantano, 2008:4). Some athletes experience inadvertent energy deficiency due to ignorance about adjusting diet and nutrition in relation to energy expenditure (Pantano, 2009:3).

Purposive energy restriction resulting in low energy availability and presenting as disordered eating (DE) (Torstveit *et al.,* 2008:108) has been identified as the first phase of energy deficiency in the female athlete triad (Teijen-Smith & Mercer, 2008:1). Disordered eating is viewed as the precursor to the more clinical eating disorder of bulimia nervosa (BN) that subsequently leads to the extreme disorder of anorexia nervosa (AN) (Symanski-Saunders, 2010:2; Nazem & Ackerman, 2012:305). Eating disorders, clinically conceptualized on a continuum, range from milder disordered eating followed by sub-clinical eating disorders of BN to the most serious AN. Eating Disorders Not Otherwise Specified (EDNOS) are deemed to be present in instances when disordered eating has not been present for sufficient duration or often enough, or when just a couple of criteria for BN or AN are detected (Torstveit *et al*., 2008:108). Eating disorders and ensuing menstrual disturbances have been associated with hypercortisolism, a factor known for its negative impact on skeletal health and osteoporosis (Naesse'n *et al.,* 2006:245). The energy continuum spectrum, which ranges from optimal to low availability, is not exclusive to an eating disorder; it can also occur in the absence of an eating disorder (ACSM, 2007:1872; Thein-Nissenbaum & Carr, 2011:109) or through ignorance (Pantano, 2009:3). Irrespective of the cause of energy deficit, powerful hormonal and metabolic effects are induced as a result of exercise provoked energy imbalance (Hagobian *el al.,* 2008:R233) that affect reproductive function and subsequently bone formation (West *et al.,* 2009:137).

An uninterrupted fuel supply of oxidizable substrates is required by every cell for optimal function (Wade & Jones, 2004:R1281). Energy, metabolized from food intake, is distributed according to priority among competing cellular functions. Cellular functions such as cell maintenance, circulation and neural activity, all considered essential to life, get priority over activities such as thermoregulation, locomotion and growth which are considered secondary to survival. Functions of reproduction and fat storage considered expendable, receive the lowest priority in energy distribution (Wade & Jones, 2004:R1278) (Figure 2).

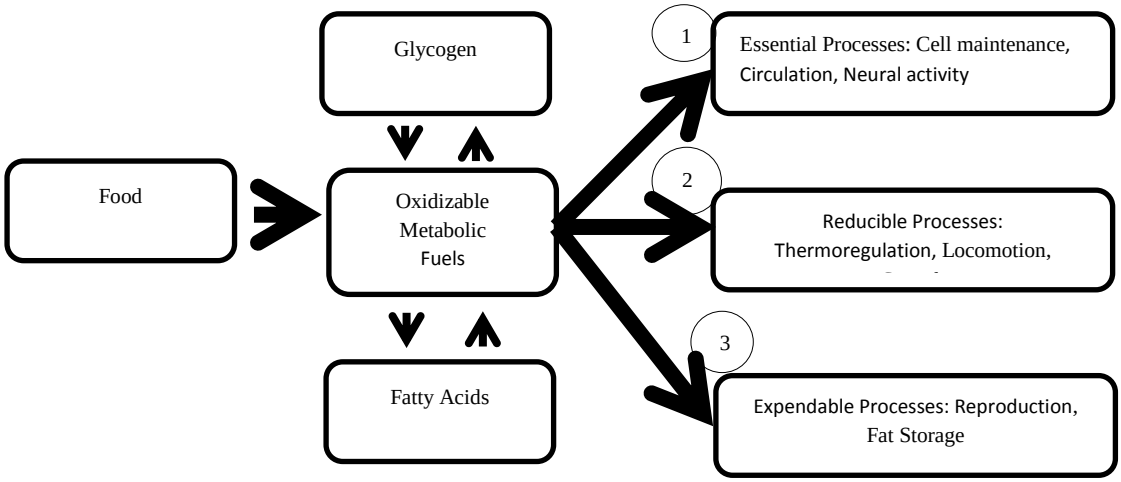

Figure 2: Energy Partitioning (Adopted from: Wade & Jones, 2004:R1278

Nutritionally, reproduction is dependent on metabolic fuels as opposed to an energy depot (Mircea *et al.,* 2007:888). As explained by Loucks and Thuma (2003:309), available evidence suggests that rather than depending on general energy availability, the brain depends on glucose availability, particularly from liver glycogen stores. This puts the brain in direct competition against skeletal muscle for available carbohydrate. The authors exemplified that working muscle could consume as much glucose in a 2 to 3 hour (h) marathon as would the brain in a week (Loucks & Thuma, 2003:309). Chronic energy deficit or metabolic challenge in the brain ultimately suppresses hypothalamic function and ovulation (Mircea *et al.,* 2007:891). In exercising or athletic women, such chronic short-fall in the resting energy expenditure (REE) necessitates energy conservation (De Souza *et al.,* 2007:971) and manifests as menstrual disorders (Loucks, 2003:144; Schneider, 2004:308). Severity of energy-associated menstrual dysfunction across the continuum reflects corresponding proportional magnitude of deficiencies in energy availability and adjustments in metabolic hormones. This association between energy availability status and reproductive function suggests that, regardless of menstrual dysfunction

severity across the continuum in exercising women, a dose-response relationship exists between categories of menstrual dysfunction and energy availability (De Souza *et al*., 2007:974).

2.2.3 Menstrual Function

Sloane (2002:65) indicated that the dominant role played by the hypothalamus in regulating reproduction was not recognized until the mid-1950s. Normal pulsatile secretion of GnRH from the hypothalamus and subsequent pulsatile release of luteinizing hormone (LH) in the pituitary are critical to optimal reproductive function (Loucks & Thuma, 2003:297). In the event of energy deficit, especially during exercise, there is great risk of disruption in the key reproductive cellular function of LH pulsatility (Loucks, 2003:147). Functional hypothalamic amenorrhea (FHA) has been identified as the distinct characteristic resulting from suppression of the hypothalamic-pituitary-ovarian (HPO) axis because of disruption in pulsatile secretion of gonadotropin releasing hormone (GnRH) (Gordon, 2010:3650). Chronic energy short-fall suppresses release of GnRH from the hypothalamus and subsequent pulsatile release of LH and FSH, effectively diminishing ovarian stimulation in amenorrhea (Warren & Goodman, 2003:874).

Though each menstrual cycle does not conform to identical duration in all women, a 28-day cycle with ovulation occurring on the 14th day has become the general practice in describing a cycle (Ganong, 2001:419). The regular uterine menstrual cyclic phenomenon in preparation for optimal pregnancy outcome (Brosens *et al.,* 2009:615e1) that begins with first day of bleeding in each cycle, is organized into three phases - follicular, ovulatory and luteal (Ganong, 2001: 419; Sloane, 2002:82). While the ovarian cycle prepares the ovum for fertilization, the uterine/endometrial cycle prepares an enabling environment for nourishing and maintaining pregnancy in the uterus (Sloane, 2002:82). As seen in Figure 3, simultaneous close coordination among rhythmically fluctuating hormones from the hypothalamus, anterior pituitary and ovaries, together with structural changes in the uterus and the ovaries govern female fertility (Goodenough & McGuire, 2012:340).

Secretion of FSH during the follicular phase promotes development of follicles in the ovary, which in turn, secretes oestrogen (E_2) and some progesterone, both of which enhance endometrial preparation (Goodenough & McGuire, 2012:343). By the stage when a dominant oestrogen–producing follicle has been selected for possible fertilization, the rising E_2 level exerts its inhibitory negative feedback on FSH secretion, thus, suppressing further follicular development (Mader, 2004:301). However, midway through the cycle, continuing rise in E_2 which reaches critical blood level approximating 200picograms/ml that is sustained for up to 50 hours, causes a surge in LH. This surge is followed by ovulation that denotes release of secondary oocyte about 34 to 36 hours later (Sloane, 2002:84). As the released secondary oocyte or egg moves along the oviduct, luteinization transforms the remaining follicle cells into a corpus luteum which continues releasing E_2 along with progesterone (Goodenough & McGuire, 2012:343). Progesterone's thermogenic qualities keep the body temperature elevated until start of menses and can be useful in estimating occurrence of ovulation (Rosenblatt, 2007:5). In the absence of fertilization, about four days before the start of the next menses in a 28-day cycle, the corpus luteum begins to degenerate. The accompanying reduction in E_2 and progesterone denies hormonal sustenance to the endometrium forcing its breakdown and eventual sloughing as menstruation/menses (Ganong, 2001:421). Lowered E_2 and progesterone during menses sensitize the anterior pituitary gland to resume secretion of FSH and subsequent follicle preparation of the next cycle. So, while the endometrium in an unsuccessful cycle is being discarded, preparation of the follicle and the endometrium for the next possible fertilization has already begun (Goodenough & McGuire, 2010:342).

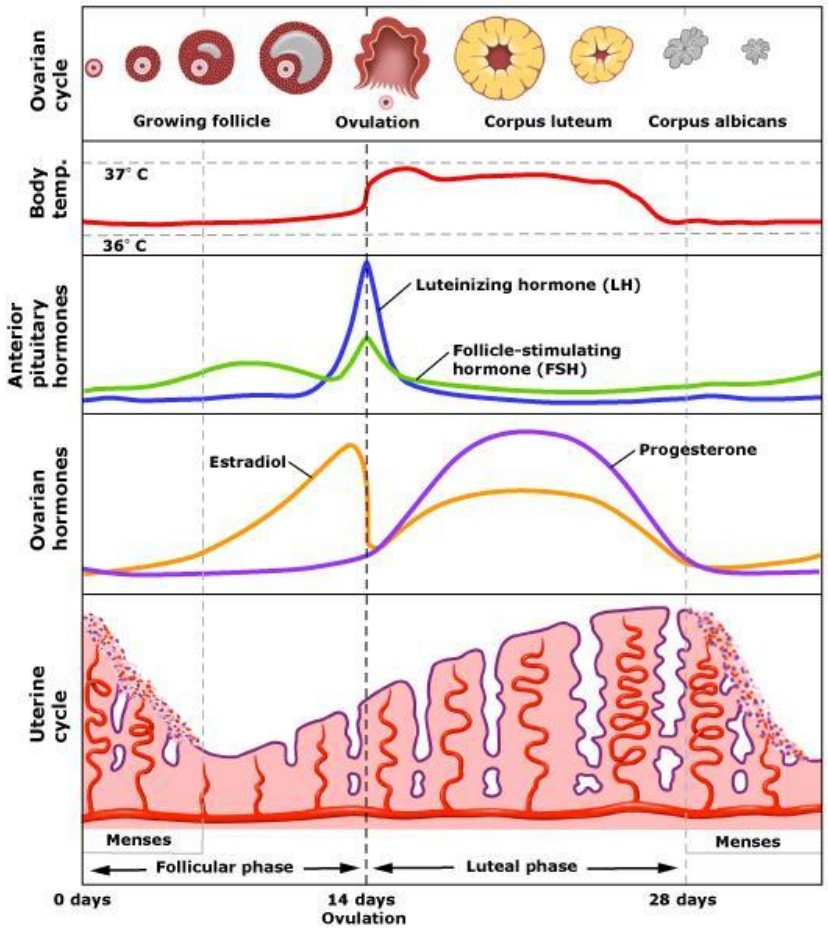

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Figure 3: Coordination among hypothalamic, pituitary, and ovarian hormones, temperature changes together with structural changes in the uterus and the ovaries during a 28-days monthly cycle. (Female hormone cycle. no date. http://www.forresthealth.com/Female-Hormone-Cycle/)

Physiologically, reproduction is considered a costly process because of its significant high energy consumption (Mircea *et al.*, 2007:887-888). According to the energy availability hypothesis, any short-fall in energy requirements of the brain will disrupt the pulsatile secretion of GnRH (Loucks, 2003:146). Specifically, an energy short-fall below the approximate resting energy threshold of 30kcal/kgFFM/d will disrupt LH pulsatility in exercising women (Loucks, 2005:S49). Reductions in rates of bone formation and hormones that promote bone formation have been noted within five days of energy availability falling below 30kcal/kgFFM/d; thus, lending credence to a direct relationship between low energy availability and bone health (Ihle & Loucks, 2004:1239; Barrack *et al.*, 2008:41). Inhibition of reproductive function due to energy

deficit, termed nutritional infertility by Wade and Jones (2004:R1277), points to nutrition and resultant energy availability as one causative factor in menstrual dysfunction.

Theoretically, sequential progression of menstrual function along its continuum in the Triad starts from optimal normal ovulatory cycle or eumenorrhea that lasts between 21 to 35 days (Temme & Hoch, 2013:193). Disorder descends to tenuous disturbances such as asymptomatic

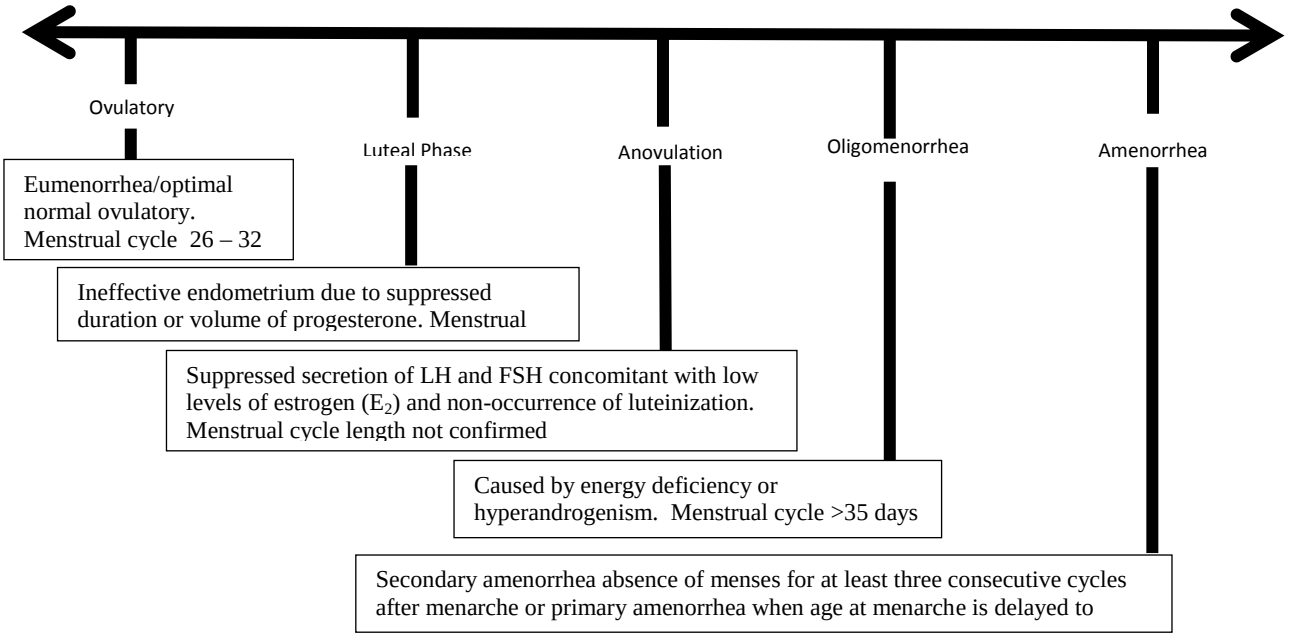

Figure 4: Continuum of Menstrual Function (Adapted from De Souza & Williams, 2004:436)

sub-clinical luteal phase defect (LPD) (Redman & Loucks, 2005:747) and anovulation (Gibbs *et al*., 2013:985). Luteal phase defect, also termed inadequacy or insufficiency, is a reflection of an ineffective endometrium due to suppressed duration or volume of progesterone (De Souza & Williams, 2004:437). The shortening of menstrual cycle in LPD results in more frequent menses (Warren, 1999:1893). An athlete probably experiences LPD during her path from amenorrhoeic recovery (De Souza, 2003:1556). Though anovulation or non-occurrence of an ovulatory event does not conform to any cyclic length, it is the consequence of suppressed secretion of LH and follicle stimulating hormone (FSH) concomitant with low levels of oestrogen (E_2) and non-occurrence of luteinization (De Souza & Williams, 2004:436-437). However, the E_2 level could be just sufficient to allow stimulation of uterine lining and subsequent menses (Redman &

Loucks, 2005:748). Current opinion suggests that the more severe 35-days or longer oligomenorrhoeic cycle (Torstveit & Sundgot-Borgen, 2005a:142) could result either from energy deficiency or hyperandrogenism (Awdishu *et al*., 2009:1066). Amenorrhea, the extreme low energy-related menstrual disorder associated with exercising women, is regarded the most deleterious because of its profound effect on skeletal health (Warren, 1999:1892). Amenorrhea is distinguished either as secondary amenorrhea by absences of menses for at least three consecutive cycles after menarche or as primary amenorrhea when age at menarche is delayed (ACSM, 2007:1869). In cognition of tendency to earlier menarche in modern times, the American Society for Reproductive Medicine (ASRM) recognizes age 15 at menarche as the threshold for primary amenorrhea (ASRM, 2008:S219). Even after menarche, adolescent menstrual bleeding pattern may frequently experience secondary amenorrhea for several months (Dangal, 2005:4). Despite the depiction of menstrual function on a sequential continuum, not all exercising women who present with menstrual dysfunction, progress through the stages (Beals & Meyer, 2007:75). Based on the energy conservation theory, level of dysfunction, as portrayed in the adapted Figure 4, is dependent on intake of sufficient calories relative to energy expenditure (De Souza *et al.,* 2007:974)

2.2.4 Bone Mineral Density

The cumulative effect of energy/nutritional deficiency and subsequent hypoestrogenism suppresses bone formation and increases bone resorption (De Souza & Williams, 2004:443; Scheid *et al.,* 2009:147; Scheid *et al.,* 2011:199), establishing a potential for bone demineralization (De Souza *et al.,* 2008:147). While less severe menstrual dysfunctions are associated with milder E_2 deficiencies, amenorrhoeic suppression of ovarian function presents extreme chronic hypoestrogenism (De Souza & Williams, 2004:436; Scheid *et al.,* 2011:194). The consequences of E_2 deficiency due to amenorrhea, especially during the critical second decade of growth years, could have irreversible deleterious effects on bone formation (Perez-Lopez *et al.,* 2010:451) predisposing athletes to skeletal fragility and osteoporosis in the future (Ducher *et al.,* 2009:766).

Amenorrhea suppresses the protective mechanism of E_2 (Eliakim & Beyth, 2003:204) in stimulating calcium absorption and its deposition into bones (Symanski-Sanders, 2010:5). By adulthood, the continual turn-over in bone tissue involving osteoclast resorption followed by osteoblast bone formation becomes a highly coordinated process. Oestrogen plays its principal

role of suppressing osteoclast activity in ensuring minimal change in overall BMD accumulation (Ihle & Loucks, 2004:1232), and in maintaining skeletal integrity and plasticity (Cromer, 2008: 198).

However, adequate E_2 alone does not fully make up for energy short-comings because it has been reported that despite E_2 treatment and return of menses, disruptions in bone formation continue in a nutritionally-challenged environment (De Souza *et al*., 2008:147). Although genetic disposition, the dominant determinant in the accrual of bone mass cannot be altered, environmental influences that impact on bone can be manipulated to favour optimal bone accrual through healthy eating and lifestyle (Davis *et al.*, 2005:376). Chronic under-nutrition may also mediate through other oestrogen-independent factors in bone impairment (Ihle & Loucks, 2004: 1232). Bone health is not exclusively dependent on the consumption of adequate caloric energy (Barrack & Van Loan, 2011:127). Apart from the influence of energy intake on subsequent hypoestrogenism and bone loss, collective intake of macro- and micronutrients, and mechanical loading play significant roles in maintaining the dual functionality of bone's structure and metabolic integrity (Lorincz *et al*., 2009:253).

Carbohydrates (CHO) are generally the primary macronutrients for energy metabolism that replenish glycogen stores in working muscles (Loucks & Thuma, 2003:309). However, complex CHO from fruits and vegetables are believed to augment bone development by enabling calcium absorption (Lorincz *et al*., 2009:256). Fat, a source of substantial energy, also yields some essential fat-soluble vitamins – A, D, E, and K, and fatty-acids (Barrack & Van Loan, 2011: 127). Reduced daily intake of fat and lowered percentage of total energy from fat accounts for deficiency in fat (Gerlach *et al.,* 2008:5). Proteins, which in the presence of adequate calcium, constitute about 50% volume and approximately one-third mass of the bone matrix, ensure optimal level of insulin growth factor-1 (IGF-1), could also affect calcium excretion and resorption (Heaney & Layman, 2008:1567S). Calcium and vitamin D are two key micronutrients in bone health. The direct influence of parathyroid hormone (PTH) on bone and kidneys and its indirect effect on the intestines reflects a fine interaction among bone, intestines, kidneys and parathyroid glands in regulating and maintaining serum calcium levels within normal range (Henwood & Binkovitz, 2009:5). Deficiency in calcium usually occurs when there is a short-fall in overall energy intake, protein consumption and vitamin D status. Vitamin D could be inadequate from lack of consumption of appropriate foods that contain vitamin D, lack

of exposure to sunlight, and lack of sufficient body fat to store the fat-soluble vitamin D (Lorincz *et al.,* 2009:257; Perez-Lopez *et al*., 2010:5).

Under normal circumstances, peak bone mineral mass is accrued during the critical growth period of adolescence (Ackerman & Misra, 2011:138). In girls, approximately 26% bone mass is accrued within two years straddling peak bone velocity corresponding to Tanner stages 2 – 4 or approximately 11.5 – 13.5 in chronological years (Bailey *et al.,* 2002:2249; MacKelvie *et al*., 2002:254) with the peripubertal years accounting for up to 60% (Perez-Lopez *et al.,* 2010:1). Magnitude and rate of bone mineral accrual during peak growth is positively related to physical activity (Nicholson-Richards *et al.,* 2000:68; Witzke & Snow, 2000:1055; MacKelvie *et al*., 2002:256; Davies *et al*., 2005:375; Christo *et al.,* 2008:1135; McKay *et al.,* 2011:100). The amount of weight-bearing in physical activity determines bone strength index (BSI) (Greene *et al.,* 2005:626). However, rather than being homogeneous, attainment of peak bone mass is site specific (Berenson *et al.,* 2009:1444), as seen in cross-sectional studies of elite male soccer players and distance runners (Fredericson *et al*., 2007:667). Bone accrual is also race/ethnic specific (Harel *et al.,* 2007:52; Wilken *et al.,* 2010:204; Micklesfield *et al.,* 2011:265; Gutiérrez *et al.,* 2010:7); and ethnic differences have been reported in stress fractures (Nattiv, 2000:268).

2.3 ESTABLISHING PRESENCE OF THE TRIAD AND ITS COMPONENTS

2.3.1 The Female Athlete Triad

Most research concerning the female athlete triad has focused on interrelatedness among individual entities rather than the whole TRIAD (Nichols *et al.,* 2006:137; Thein-Nissenbaum & Carr, 2011:109). There has been a dearth in epidemiological research looking into simultaneous occurrence of all three entities constituting the female athlete triad, under both, the former definition of disordered eating, amenorrhea and osteoporosis (Beals & Meyer, 2007:70) and under the revised 2007 ACSM version of energy availability, menstrual function and bone mineral density (Thein-Nissenbaum & Carr 2011:113).

In a systematic review of prevalence of the Triad, Gibbs *et al*. (2013:987) substituted DE for EA where EA had not been evaluated. Based on the DE/ED operational definition, Lauder's (1997:1-35) prospective investigation into prevalence of the female athlete triad in 432 military women (17 – 53 years old) which evaluated ED, menstrual irregularity (MI) and BMD, was the first study to come close to meeting the ACSM 2007 revised definition. Though none of the

participants exhibited simultaneous occurrence of the full Triad, presence of individual components was detected at 8% for ED and at 2.1% amenorrhea in women not on hormonal birth control. DXA scanned comparisons of BMD between participants who presented one or two components of the triad with matched and unmatched controls showed no significant difference.

In a three-part study, Torstveit and Sundgot-Borgen (2005:1449-1459) recruited the entire 13-39 years old elite female athletic population (n=938) and an age matched control group (n=900) in Norway to establish the existence of the female athlete triad in both groups. The criteria for establishing the Triad were presence of ED/DE, menstrual dysfunction and low BMD. To reflect severity of the Triad on a continuum, those with disordered eating, menstrual dysfunction and low BMD (Z-score < 1.0, but $\geq$ -2.0) were classified as having Triad Stage I moderate-severe occurrence. The more severe Triad Stage II included those with clinical eating disorders, menstrual dysfunction and low BMD (Z-score <- 2.0). Eight (4.3%) athletes and five (3.4%) controls satisfied the criteria for all components of the Triad. The eight athletes were distributed equally between Stages I and II, while all the controls fell in Stage I.

The investigation into prevalence of the Triad, based on the former concept of disordered eating, amenorrhea and osteoporosis, among 224 Turkish athletes (16-25 years old) (Vardar *et al.*, 2005:550-555), showed that 16.8% (n=37) had high scores on the EAT-40 questionnaire. Of these, 16.2% (n=6) experienced amenorrhea, while 7.7% (n=14) with normal EAT-40 scores presented amenorrhea. Six athletes presented combination of disordered eating and amenorrhea. Further investigation among these six confirmed two with eating disorders, one as anorexic, one with EDNOS and the remaining four had no conclusive diagnosis. Of the six athletes with disordered eating and amenorrhea, three (1.36%) showed presence of the Triad. However, the BMD interpretation was based on WHO criteria as opposed to ISCD criteria. When comparing combinations of any two components of the Triad, athletes (26.9%) surpassed controls (13.8%) in meeting the criteria for the combination of disordered eating/eating disorders and menstrual dysfunction. On the other hand, controls showed greater tendency (12.4%) than athletes (5.4%) in the menstrual dysfunction and low BMD combination.

To establish the prevalence of the Triad among high school athletes, Nichols *et al*. (2006:137-142) selectively pre-screened 170 high school athletes (13-18 years old) competing in eight different sports. Though the percentage of high school athletes who met criteria for all three

components was low, the authors acknowledged substantial risk for the Triad among high school athletes because 5.9% presented 2 components, and approximately 20% met the criteria for at least one component. When considering individual components in this population, 18.2% and 23.5% met the criteria for eating disorders, and menstrual dysfunction respectively, while 21.8% had low BMD for age based on World Health Organization (WHO) criteria and 4.1% met the International Society for Clinical Densitometry (ISCD) criteria for low bone mass.

Beals and Hill (2006:1-23) examined the prevalence of disordered eating, menstrual dysfunction and bone mineral density, individually and in combinations among 112 (mean age 19.5 years) Division II level athletes representing seven different sports. Using the ISCD recommended *Z*-scores, only one athlete, a cross-country runner in the lean-build category, presented all three components at the criterion *Z*-score below -2.0 for BMD, and another two, also from lean-build category, displayed the full Triad when the criteria was lowered to *Z*-score below -1.0 for BMD. Of the 10 athletes (9%) who presented two disorders, one showed a combination of disordered eating and low BMD, while the others combined disordered eating with menstrual dysfunction. Using the lower criteria of *Z*-score below -1.0, three more athletes presented a combination of two components. Apart from the lean-build category showing a significantly higher prevalence in menstrual dysfunction (32%; n=21) than the non-lean-build (17%; n=8), no other significant differences were found between the two groups either in individual components or in combinations.

Recruiting 15 athletes (35±6 years old) from a triathlon club team, Hock *et al.* (2007:681-682) investigated the prevalence of the Triad among them. Their results indicated that 60% of these athletes were energy deficient, and 40% had either experienced primary or secondary amenorrhea. However, BMD values were all within normal range.

Hock *et al*. (2009:421-428) also investigated prevalence of the female athlete triad in high school athletes. Interestingly, equal distribution of the full Triad between athletes and non-athletes at one each, also reflected almost similar numbers in low energy availability at 36% (n = 29) for athletes and 31% (n=31) for non-athletes and low EAT-26 scores. This suggested that disordered eating may not have been the reason for low energy availability in both groups. In the self-reported menstrual dysfunctions, athletes showed a higher prevalence at 54%, (n=43) generally. Further categorization of the dysfunctions revealed that 6% (n=5) athletes reported primary

amenorrhea, 30% (n=24) secondary amenorrhea, and 15% (n=14) had oligomenorrhoea. On the other hand, the lower 21% general menstrual dysfunction categorization in controls revealed that none had primary amenorrhea, 15% (n=12) presented secondary amenorrhea, and 6% (n=5) suffered oligomenorrhoea. Using ISCD, WHO and ACSM recommended classification based on *Z*-scores, differences in DXA results showed that 16% (n=13) athletes had low BMD compared to 30% (n=28) controls.

Generally, lack of consistency in defining each component, inadequate precision in measuring instruments, differences in competitive levels and heterogeneity of populations, energy and body-type requirements of sports studied, and inadequate or inappropriate controls, reveal wide variations in prevalence of each component (Byrne & McLean, 2001:145; Nichols *et al*., 2006:137-138; George *et al.,* 2011:51-52, Gibbs *et al.*, 2013:986). These discrepancies do not allow true determination of the composite Triad among athletes (Beals & Meyer, 2007:70); making it difficult to generalize, compare or recommend (Hock, *et al.*, 2009:42).

2.3.2 Eating Behavioural Practices and Energy Availability

The revised ACSM Position Stand underscores energy deficiency, whether unintentional, deliberate, or psychopathological, as the underlying factor in reproductive dysfunction and bone health in the Triad (ACSM. 2007:1867). Unhealthy behavioural or psychopathological practices have been implicated as instigators of energy deficiency in the female athlete triad (Tietjen-Smith & Mercer, 2008:2; Thein-Nissenbaum *et al.,* 2011:60). Symanski-Sanders (2010:2) warns that disordered eating that fails to achieve energy balance could be the precursor to more clinically extreme eating disorders of BN and AN. Bulimia nervosa includes spasmodic binge eating, use of laxatives, diuretics, enemas, and vomiting experienced twice a week over a period of three months; while the more serious AN manifests as body weight of 85% or less for age and height, amenorrhea, pathogenic fear of gaining weight or fat, and extreme dissatisfaction with body image or distorted self-image (Beals & Meyer, 2007:72). However, in instances when disordered eating has been present for only a short duration, or when just one or two criteria for the severe clinical eating disorders are present, the athlete is deemed to be experiencing Eating Disorders Not Otherwise Specified (ED-NOS) (Torstveit *et al*., 2008:108). Inconvenient schedules, ignorance about dietary requirements, and even lack of food could be responsible for unintentional energy deficit in athletes (Beals & Meyer, 2007:73).

A systematic review of studies from 11 countries concerning prevalence of disordered eating among female athletes over a period of nine years previous to 2010 found that in majority of the studies both athletes and non-athletes were at similar risks of experiencing disordered eating. (Coelho *et al.,* 2010:386). Eating disorders are more common among those female athletes participating in physical activities that emphasize low body weight or thinness (Byrne & McLean, 2001:87; Sundgot-Borgen & Torstveit, 2004:31). It is possible that because of negative psychological association with unhealthy eating practices, individuals with anorectic tendencies are reluctant to acknowledge unhealthy eating behaviours (Hagmar, 2008:21). It is suspected that the prevalence of disordered eating among adolescent elite athletes (Martinsen *et al.,* 2010:74), and adult elite athletes is under-reported (Torstveit *et al.,* 2008: 116).

Despite shortcomings of self-reports concerning disordered eating, it has been assumed that those with any signs of disordered eating will also experience energy deficit, and hence, such self-reports continue to be used to indirectly establish prevalence of low energy availability (Beals & Meyer, 2007:73; Coelho, 2010:383). By acknowledging existence of validated self-reporting questionnaire tools for determining disordered eating and eating disorders, the IOC implies recommendation of such tools (IOC, 2009:547). Use of the Eating Disorder Inventory (EDI), the Eating Attitudes Tests-26 (EAT-26), the Eating Disorder Examination Questionnaire (EDE-Q), the Bulimia Inventory Test Edinburgh (BITE), and the Three Factor Eating Questionnaire (TFEQ) for examining subclinical disordered eating behaviours in both athletes and non-athletes have been noted (Barrack *et al*., 2008:36). In addition to these, the IOC also refers to the Sick Control One-Stone Fat and Food Questionnaire (SCOFF) a simple four-question tool that can be a prediction guide (Luck *et al.,* 2001:755; Cotton *et al.,* 2003:53).

When determining disordered eating, menstrual dysfunction, and bone mineral density in 18 – 25 years old female runners (n=91), Cobb *et al*. (2003:712) interestingly combined the use of three sub-scales of the EDI with a six month food intake frequency questionnaire. Elevated EDI scores among athletes reflected 19% less daily caloric intake and 25% fewer calories from fat compared to those with normal EDI scores. To establish prevalence of eating disorders, Sundgot-Borgen and Torstveit (2004:27) used the EDI to identify athletes (n=572 women and 687 men) and controls (n=629 women and 574 men) at risk for EDs. It was noted that generally more athletes (13.6%) were identified with diagnostic criteria for subclinical and clinical EDs than controls (4.6%) and more women athletes (20%) showed EDs than their male counterparts (8%).

In the same study, when considering a particular sport and gender, female athletes in endurance sports (n=102) showed that 4% presented AN, 10% BN, 5% athletic amenorrhea (AA) and 5% ED-NOS compared to controls (n=574) who showed significant presentation only in BN (3%) and ED-NOS (6%). The expanded EDI-2 was the tool used by Reinking and Alexander (2005:48) to make comparisons in prevalence of disordered eating behaviours between lean-sports (n=16, age = 19.7± 1.1 years) and non-lean sports female athletes (n=68, age= 19.7± 1.1 years) and non-athletes (n=62, age = 20.2 ± 1.2 years). They found that while 25% (n=4) of lean-sport athletes risked disordered eating, only 2.9% (n=2) of non-lean-sport athletes were at risk.

Using the EDE-Q, Barrack *et al*. (2008: 37) set out to identify associations between DE attitudes and behaviours and BMD among 13 – 18 years high school female runners (N=106) and found that athletes with elevated weight or shape concern, showed higher occurrence of binge eating (15.4%) and athletes who practice food restraint scored higher in self-induced vomiting (20.0%) than those with weight or shape concerns. However, excessive exercise was not practiced by any with weight or shape concerns. Rauh *et al.* (2010:244-246) used the EDE-Q to assess relationships among injury and disordered eating, menstrual dysfunction, and low bone mineral density of high school athletes from eight sports (n=163, 15.7± 1.3 years). Additionally, higher mean scores on all EDE-Q subscales and overall global disordered eating indicated that such athletes were likely to experience more injuries. Another investigation into associations among disordered eating, menstrual function, and musculoskeletal injuries in 311 high school multi-ethnic athletes from various sports also identified eating disorders using the EDE-Q (Thein-Nissenbaum *et al.,* 2011:62). The athletes were categorized into aesthetic if scoring in the sport was based on subjective rating, endurance if the primary energy system was aerobic, or team/anaerobic if in the short term, the activity required intense effort for up to three minutes in which adenosine triphosphate (ATP) and phosphocreatine (PCr) were the primary energy sources. Their results revealed that 41.5% in the aesthetic, 37.1% in the endurance, 33.1% in the team/anaerobic group had disordered eating. Interestingly, results of a cross-nationality study that used EDE-Q to compare prevalence of eating disorders in elite British and Kenyan runners, indicated that Kenyan runners were least likely among athletes and controls from the two countries (8/72 = 11.1%), to present eating psychopathology (Hulley *et al.*,2007:526).

Gibson *et al.* (2004:612) in their review study revealed that the EAT-26 questionnaire, which has been validated for identification of AN, a score of > 30 indicates a high potential for AN, while a score of 15 - 30 suggests presence of subclinical disordered eating habits and anorectic attitudes. Though validated for AN, EAT-26 also seeks to reveal three factors associated with BN - preoccupation with food, preoccupation with need to be thinner, and self-control of eating. The BITE, more specific to BN, assesses binge eating on a scale of severe to normal such that a score of > 20 reflects highly disordered eating patterns with binge eating, scores of 15 – 20 suggest disordered eating habits, while scores of <10 are considered normal.

As advised by Le Grange *et al*. (2004:456), though questionnaires devised in the developed world have been extremely useful in understanding ED, DE and ED-NOS they need to be translated, especially when administered to those whose first language is not English. However, despite the translation, in view of cultural differences in interpreting concepts, language proficiency, acculturation level, and bilingualism, one needs to observe caution when using the same questionnaires outside of western industrialized culture.

2.3.3 Dietary, Nutritional and Energy Intake

Apart from indirect implication of energy deficiency through DE and ED questionnaires, recall records or diaries of dietary/food intake and physical activity in terms of miles covered per week have been used to calculate nutritional and/ caloric intake and energy expenditure respectively (Cobb *et al.*, 2003:712). More direct methods to determine energy intake involved weighing of all food and liquid consumed; thereafter, the computerized version of the National Food Composition Tables of Kenya (Sehmi, 1993) converted weighed food and measured liquid intake over seven consecutive days into dietary and nutritional intake of Kenyan athletes (Onywera *et al*., 2004:712; Beis *et al*., 2011:3) and over two weeks (Christensen *et al*., 2002:712). Software programmes such as the Nutrisurvey for Windows 2007 (Erhardt, 2007) have also been used to determine dietary, nutritional and energy intake (Mala *et al*., 2012:6781).

2.3.4 Measures of Physical Activity and Energy Expenditure

Physical activity ratios (PAR) from detailed record of type, intensity, and duration of all daily living and training-related activities have used the Schofield equation to estimate energy cost (Onywera *et al*., 2004:712). Objective, accurate and reasonably precise monitors are now being used to determine frequency, intensity and duration of physical activity (Colbert & Schoeller,

2011:606). Apart from over-coming inaccuracy, misinterpretation and bias in amount of exercise associated with self-reports, monitors are capable of providing heart rates, and free-living and exercise caloric expenditure (Murphy, 2009:109; Freedson, 2012:S89). Wearable or body-fixed physical activity motion sensors have evolved from simple mechanical foot-impact counters that recorded steps to the more advanced electronic devices that measure accelerations and velocities, and sense changing body orientations (Yang & Hsu, 2010:7773). Recent development in accelerometers includes an integrated circuit (IC) (Murphy, 2009:109) with regularly updated firmware that reduces chances of errors due to loss of calibration (John & Freedson, 2012:S88). Validation against the gold standard doubly labelled water technique has shown reasonable correlation with accelerometers in assessing EE (Plasqui & Westerterp, 2007:2377; Harris *et al*., 2009:1401).

More direct and stringent laboratory measurements used in a study to determine relationship between LPD menstrual cycles and metabolic hormones of thyroid insulin, human growth GH (hGH), leptin and insulin growth factor 1 (IGF-1) confirmed existence of a hypometabolic state (De Souza *et al.,* 2003:339). Reductions in resting metabolic rate, total T_3- a marker of energy balance, leptin, insulin, glucose, and insulin-like growth factor binding protein (IGFBP)-3 have all been used (De Souza & Williams, 2004: 439). Elevations in IGFBP-1, ghrelin, growth hormone and cortisol expose hypometabolic state (Loucks & Thuma, 2003:305; De Souza & Williams, 2004:439).

2.3.5 Determining Menstrual Function

Of the three components in the Triad, menstrual dysfunction is the most obvious and easiest to detect, and as such, its occurrence raises warning signals for possible presence of the Triad (Raymond-Barker *et al*., 2007:2). This perceptibility and ease has been advantageous for self-report menstrual history questionnaires in examining menstrual dysfunction. Many prevalence studies seeking to establish menstrual disorders have used self-report menstrual history questionnaires (Beals & Meyer, 2007:78). Participants have been required to recall age at menarche, frequency, regularity of menstrual cycles, number of menses per annum, and average number of days between cycles over past 12 months (Dusek, 2001:80; Vardar *et al*., 2005:551). Questionnaires included in The Better Eating Safer Training Research Study (B.E.S.T.) used by Raymond-Barker *et al.* (2007:3) and the Healthy Wisconsin High School Female Athlete Survey developed and used by Thein-Nissenbaum *et al.,* (2011:62) added use of contraceptives.

Prevalence of menstrual characteristics and cyclic patterns have also been examined through daily diaries of absence or presence of menstrual bleeding for up to eight cycles (Sternfeld *et al*., 2002:404), alternately, monthly bleeding calendars have been surrogates for ovarian function (Dusek, 2001:80).

While self-report questionnaires are useful screening tools for menstrual disorders, there is risk of underestimating occurrence of dysfunctions because subclinical menstrual disorders that do not have overt symptoms go undetected (Manore *et al.*, 2007:S62; Beals & Meyer, 2007:78). Apart from low energy availability, possibility of other etiological factors, such as organic diseases or genetic disposition in menstrual disorders, suggest diagnoses of menstrual disturbances in athletes and active women by exclusion (Warren, 1999:1892). The serious health consequence of menstrual dysfunction requires more stringent laboratory testing to identify the exact cause of the dysfunction and to determine its management (Manore *et al*., 2007:S62). Endocrine measures of reproductive hormones reveal more precise information concerning dysfunction than recall of menstrual cyclic events (De Souza & Williams, 2004:438).

Laboratory measures have included serum FSH, LH, TSH, prolactin, Free T4, testosterone and dehydroepiandrosterone (DHEA) (Gibson *et al*., 2004:613; Dadgostar *et al*., 2009:3). Ultrasonography study of ovaries has also been included to rule out polycystic ovary syndrome (PCOS) as a causative factor in amenorrhea or oligomenorrhea (Dadgostar *et al*., 2009:3). Rather than collecting sample over just a single cycle, 'gold' standard evaluations recommend daily sample collection over multiple monthly cycles (De Souza & Williams, 2004:439). Analysis of LH pulsatility and accompanying responses of various metabolic substrates and hormones require invasive and cost-prohibitive blood sample collection every 10 minutes for 24 hours (Loucks & Thuma, 2003:299–300). Expert-dependent ultrasound, the most accurate method for detecting ovulation, is also very expensive. Instead, it has been suggested that the less invasive daily urine or saliva samples to establish metabolites of LH, E_2 and progesterone levels could be more feasible in research settings (De Souza & Williams, 2004:439).

Anti-mullerian hormone (AMH), a product of the granulosa cells enveloping ovarian follicles, plays a regulatory role on ovarian function (Hehenkamp *et al*., 2006). Compared to inhibin B, E_2, and FSH, it has shown consistent reliability through the menstrual cycle, higher cycle-to-cycle reproducibility, and ability to provide information from a single measurement (Fanchin *et*

al., 2005:926). These characteristics are gradually giving it recognition as a unique cost-effective and practical alternative endocrine marker for ovarian dysfunction (La Marca *et al*., 2010:464). Serum AMH concentration has been measured using the very popular and sensitive enzyme-linked immunosorbent assay (ELISA) to determine levels throughout normal healthy human menstrual cycles (La Marca *et al*., 2006a:3104) in women with secondary amenorrhea (La Marca *et al*., 2006b:1548), to establish normative values (La Marca *et al*., 2010:464), and in duplicate samples, to examine relationships between AMH, androgens, insulin resistance and follicular status in non-obese sub-fertile women with or without PCOS (Nardo *et al*., 2009:2981).

2.3.6 Determining Bone Mineral Density

The female athlete may be at greater risk of sustaining musculoskeletal injuries, especially stress fracture, because of low energy availability, with or without disordered eating, menstrual dysfunction, and low bone mass (Rauh *et al*., 2010:250). The Nattiv (2000) review concerning bone stress injuries reports that level of disruption in the balance between bone resorption and bone formation is reflected on a bone health continuum that ranges from normal modelling and remodelling, that deteriorates to bone fatigue, finally culminating in a full stress fracture (Nattiv, 2000:269). Current and past menstrual dysfunction and its detrimental potential for bone fragility, suggests need for further investigation (Ducher *et al*., 2009:766). An athlete, who presents with EDs or DE over a total of six months, hypoestrogenism, and/or a history of stress fractures or fractures resulting from minor trauma, must have her BMD evaluated, and re-evaluated 12 months later (ACSM, 2007:1874). This guidance becomes more imperative in view of the lack of signs and symptoms of osteoporosis and its "silent" characteristic until the occurrence of a fracture (Hawkinson *et al*., 2007:320).

Using either ionizing radiation or ultrasound, accuracy and precision vary among the methods that measure BMD centrally in the spine and hip or peripherally in the appendicular skeleton – the extremities. Methods such as very low dose peripheral x-ray densitometry and heel ultrasound may be useful when screening for osteoporosis. However, it is necessary to confirm severity either with dual-energy x-ray absorptiometry (DXA) or quantitative computed tomography (QCT) of the lumbar spine and hip before embarking on treatment and follow-up programme (Hawkinson *et al.*, 2007:320).

Dual-energy x-ray absorptiometry uses differentials in absorption capacity of tissues to distinguish depth and composition of bone, muscle mass, fat and other tissues of the whole body, or regionally of the hip, the spine and the distal radius (Fewtrell, 2003:795). DXA calculates BMD by measuring bone mineral content (BMC) over a specific bone area (BA) and then dividing content by the area scanned as BMC/BA (Fewtrell, 2003:796). Apart from short scan times, DXA delivers relatively low radiation dose, gives better image resolution and improved precision (Hawkinson *et al*., 2009:322).

The International Society for Clinical Densitometry (ISCD) advises against the use of the World Health Organization (WHO) criteria for diagnosing osteopenia and osteoporosis in premenopausal women, men under 50 years and children. Instead it recommends that BMD be expressed as *Z*-score, and compared to age, sex and if possible, population matched. Further, while rejecting use of the term 'osteopenia' in premenopausal women, ISCD categorizes *Z*-scores below -2.0 as low bone density below the expected range for age. It also recommends that osteoporosis in this group be diagnosed only in the presence of clinical risk factors such chronic malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, and previous fractures (ISCD, 2008:1117). Considering the 5 – 15% potentially higher BMD of athletes in weight-bearing sports, ACSM recommends that any athlete showing a BMD of <-1.0 should be investigated further regardless of fracture history (ACSM, 2007:1870).

ISCD prefers DXA for assessing BMC and areal BMD (ISCD, 2008:1119). QCT, a unique three-dimensional volumetric measure for BMD, has ability to measure the more metabolically active trabecular bone in isolation, thus providing greater sensitivity in revealing rates of change in bone disease (Hawkinson *et al.*, 2007: 322). This unambiguous ability to distinguish between effects of bone size and changes in bone mass encouraged Neu *et al*. (2001:227) to use QCT in their investigation of BMD and bone size of participants aged 6 – 40 years. Ducher *et al.* (2009:761) combined QCT and DXA to determine whether history of amenorrhea compromised exercise benefits on cortical and trabecular bone in retired elite gymnasts. QCT was used because of its ability to reflect changes in bone geometry and volumetric density, while DXA determined lean tissue mass and percentage body fat (2009:761). However, there is need for caution because radiation dosage in QCT could be 20 to 40 times higher than in DXA (Hawkinson *et al*., 2007:322).

The Cobb *et al.* (2003:7120) clinical investigation into disordered eating, menstrual irregularity, and bone mineral density of female runners aged about 21 years adjusted the DXA BMD measures for age, body weight, percentage body fat, EDI score and age at menarche (2003:712). Some studies have adhered to the ISDC recommendations in categorizing bone status with reference to age and sex (Barrack *et al.*, 2008:38). When investigating relationships among injury and variables of the Triad in 163 high school athletes from eight different sports, Rauh *et al*. (2010:245) combined the recommended ACSM, WHO, and ISCD criteria for premenopausal women to interpret low bone mass from DXA scores.

In addition to imaging techniques that assess BMD and BMC, sensitive biochemical markers/indicators of bone turnover are also used to assess bone formation and resorption. Gremion *et al*., (2001:16) focused on plasma values of bone turnover markers such as protein, calcium, and osteocalcin to determine bone formation in oligomenorrheic long distance runners. While serum-bone-specific alkaline phosphatase (BAP) and osteocalcin indicate bone formation, urinary N-telopeptide (uNTX) and amino telopeptide cross-linking domain type I collagen are recognized as good bone resorption markers (Harel *et al*., 2007:45; Cristo *et al*., 2008:1129; Barrack *et al*., 2010:653).

2.4 IRON DEFICIENCY ANAEMIA AND HAEMOGLOBIN

Though not a component of the female athlete triad, the status of iron, an essential micronutrient, could have just as serious a negative effect on the health and physical performance of athletes as FAT; and as such, warrants special haemoglobin and ferritin examination in athletes entering elite sports (Fallon, 2008:335). Anaemia has been explained as a decrease in the size and number of red blood cells or decreased haemoglobin in red blood cells; and iron that forms part of haemoglobin, an oxygen-carrying protein in the blood (Ahmadi *et al.*, 2010:93). WHO determined that the estimated 55.1% prevalence among pregnant women and 46.4% among non-pregnant of reproductive age in the general population, anaemia is a severe problem in Kenya (WHO, 2005:27). Suboptimal dietary intake and menstrual bleeding leading to low or negative iron balance and anaemia pose an even greater risk for physically active women with poor iron status (McClung *et al*., 2009:124).

Iron, part of cytochromes found in electron transport system, plays a critical cofactor role in the formation of oxygen-transporting proteins such as haemoglobin in the blood and myoglobin in

the muscles (Akabas & Dolins, 2005:1246S). Iron is stored as ferritin in the body (Rodenberg & Gustafson, 2007:258). In a joint position statement on nutrition and athletic performance, the American Dietetic Association (ADA), Dieticians of Canada (DC) and ACSM advised the critical need for oxygen-carrying in endurance physical activities, especially distance running in which iron requirements could increase by as much as 70%. They also emphasized the role of iron in normal neural, muscular, behavioural and immune functions (ACSM *et al.*, 2009:716). Undue reductions in iron could adversely affect temperature regulation, cognitive abilities, efficiency in energy metabolism, and sports performance (Rowland, 2012:319).

Apart from suboptimal dietary intake of iron-rich foods, vegetarian diet, and menstrual bleeding, deficiency in iron, though not conclusively proven, has also been attributed to increased loss in sweat, faeces and urine, internal intravascular haemolysis or destruction of cells, foot-strike haemolysis, regular blood donation, injury, and inability to absorb iron. Ferritin and haemoglobin could be temporarily diluted if plasma volume increases faster than increase in red blood cell mass (Akabas & Dolins, 2005:1247S; ACSM *et al.*, 2008:717) in a condition known as dilutional or sports anaemia (ACSM *et al.*, 2009:717; Rowland, 2012:321). Distance runners might experience blood loss from transient ischemic microscopic bleeding either in gastrointestinal track or in urine (Rowland, 2012:323). WHO adds parasitic infections such as hook-worms; acute and chronic infections such as malaria and cancer; and other accompanying micronutrient deficiencies such as vitamins A and B12; and copper to the list of causes for anaemia (WHO, 2008:1).

Iron deficiency, occurring in three stages, begins with depletion of stores as reflected by serum ferritin below 12µg/L. In the second stage, iron-deficient erythropoiesis shows increase in transferrin and reduction in transferrin saturation; however, despite depressed serum ferritin concentration, haemoglobin level remains normal; and is therefore, prior to reaching the third stage, termed iron deficiency without anaemia (Rowland, 2012:320). The third and final anaemic stage depicts abnormally small or microcytic hypochromic red cells that stain more deeply with haemoglobin measures at below 12 mg/dL in female and below 13 mg/dL in males (Akabas & Dolins, 2005:1247S). The age and sex based recommended daily 18 mg/d allowance of iron for a menstruating woman on a normal mixed vegetarian/non-vegetarian diet needs to be increased to 33 mg/d if the woman is on a purely vegetarian diet (Rodenberg & Gustafson, 2007:259). Low ferritin concentrations with normal haemoglobin are a lot more common,

especially among female distance runners, than severe iron deficiency with anaemia (Rowland, 2012:326). Though the detrimental impact of iron deficiency anaemia on athletic performance is in no doubt, there are uncertainties about the effect of iron deficiency without anaemia (Rodenberg & Gustafson, 2007:261; Akabas & Dolins, 2010:1247S; Rowland, 2012:326).

The choice for determining iron deficiency falls between the most commonly used serum ferritin marker and the gold standard soluble transferrin receptor (rTfR). Underlying conditions such as inflammation, infection, liver disorder, malignancies and exercise-related haemolysis, all of which are known to raise serum ferritin concentrations, could misrepresent the true status of iron. On the other hand, if muscle growth, the only factor confounding rTfR, is taken into account, rTfR could be a more accurate indicator of iron stores and functional pool of iron (Akabas & Dolins, 2005:1247S).

2.5 CHAPTER SUMMARY

Understanding and definition of the three-component female athlete triad, associated with nutritional/dietary, menstrual/reproductive and skeletal status, which afflicts physically active women, has evolved over the years. The current conceptual model of the Triad, with individual components ranging from optimal energy availability, normal eumenorrheic menstrual function and healthy optimal bone mineral density, that gradually deteriorate to clinical conditions along their respective continuums, was devised by ACSM in 2007. There is no doubt that independent, collective and/or inter-related disruption in the three entities of the Triad presents far reaching deleterious health and performance consequences, especially for those female athletes who strive to achieve a perceived ideal body shape, size, or weight for their sport.

The preceding literature review underscores energy availability as the key instigator in the Triad. The cascade of events in the Triad begins with inadvertent, unintentional or psychopathological restriction in dietary-nutritional intake relative to exercise energy expenditure (EEE) that could result in energy deficit or low energy availability. Chronic energy deficit restrains hypothalamic function and ovulation that manifests as FHA. The combination of dietary-nutritional-energy deficit and consequential hypoestrogenism impairs bone formation while increasing bone resorption. The ensuing bone demineralization lowers bone mineral density and ultimately results in debilitating osteoporosis. Prior to 2007, there was a tremendous amount of information generated under individual, two or all three entities that were thought to comprise the Triad -

EA/DE, amenorrhea, and osteoporosis. As evident in the preceding reviewed literature, research since 2007, though less prolific, has attempted to examine the Triad under the current model of energy availability, menstrual function and bone mineral density along their respective health continuums.

Iron deficiency anaemia, another consequence of dietary-nutritional deficit, also poses grave health consequences for the female athlete. Though not a component of the Triad, it could have a just as serious deleterious impact on the female athlete's health and performance (IOC, 2009:546). WHO determined that anaemia is a severe problem among women of reproductive age in Kenya (WHO, 2005:27:32). The situation is compounded for distance runners whose iron requirements could increase by as much as 70% (ACSM *et al.*, 2009:716).
All of the preceding reviewed literature forms the basis for Chapters 3, 4, 5 and 6.

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CHAPTER 3 Energy availability, menstrual function and bone mineral density in elite Kenyan female middle and long distance runners

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Energy availability, menstrual function and bone mineral density in elite Kenyan female middle and long distance runners

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Energy availability, menstrual function and bone mineral density in elite Kenyan female middle and long distance runners

ABSTRACT

Aim: This study explored energy availability (EA), menstrual function (MF) and bone mineral density (BMD) in elite Kenyan female (middle and long) distance runners and non-athletes.

Methods: Measurements of EA, BMD and MF were undertaken in 39 female participants (Middle distance athletes=12, Long distance athletes=13, Non-athletes=14). EA was calculated from energy intake (EI) noted in a 3-day weighed food record, and exercise energy expenditure (EEE), estimated from an Actigraph GT3X+ accelerometer. Dual energy x-ray absorptiometry (DXA) was used to evaluate fat free mass (FFM) and BMD. A nine-month daily temperature-menstruation diary was used to evaluate menstrual status.

Results: EA among the athletes (28.1±11.5 kcal·kg $FFM^{-1}\cdot d^{-1}$) was significantly lower compared to non-athletes (57.0±21.4 kcal·kg$FFM^{-1}\cdot d^{-1}$) ($p<0.001$). Oligomenorrhea was present in 10 (40%) of the athletes and two (14.3%) non-athletes; and amenorrhea in two (14.3%) non-athletes. Based on ACSM guidelines, low BMD (Z-score <-1) was identified in 19 (76%) athletes and 12 (86%) non-athletes all at the lumbar spine.

Conclusion: These elite runners confirmed earlier reports of low EA in Kenyan athletes. The combination of low EA, the 40% menstrual dysfunction and the high presence of low BMD in the athletes represents a disturbing trend that may place the elite Kenyan athletes 'at risk' for female athlete triad. It is recommended that Kenyan athletes and their coaches be educated regularly about specific dietary and nutritional requirements. Regular clinical screening of the athletes is necessary to ensure early identification of pathologies and implementation of appropriate intervention.

Keyword: Energy availability, menstrual function, bone mineral density, exercise energy expenditure, Kenyan female athletes

INTRODUCTION

Active women are at risk of the female athlete triad (TRIAD), the term ascribed to a syndrome arising from disruptions in energy availability (EA), menstrual function and bone mineral density (BMD). Energy availability, an influential factor in menstrual function and BMD, represents the available energy from dietary energy intake (EI) after deducting energy expended in purposive exercise (EEE), and standardizing the result to fat-free mass (FFM).[1]

Low EA is a hypothesized instigator of menstrual dysfunction and subsequent skeletal health in active women.[2 3] In the human body, functions associated with reproduction receive lowest priority in the hierarchical distribution of energy.[4] The energy deficit associated with low EA has been attributed to deliberate cognitive intent or inadvertent energy restricton.[1-5] The detrimental impact of low EA on menstrual function has been extensively reported in previous investigations.[5-10] EA at ~ 45 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$ is considered balanced in healthy young adult sedentary women.[6-10] EA at ~ 30 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$, corresponding closely to resting metabolic rate, is the threshold below which reproductive dysfunction and subsequent disrupted bone formation has been seen in sedentary women.[1 6 9]

Menstrual dysfunction has a higher prevalence among athletes and active women than the general population.[2] A 28-day cycle, lasting from the first day of bleeding in each cycle, has become the general convenient practice for describing a normal menstrual cycle.[11] Irregularities begin with asymptomatic sub-clinical anovulation and luteal phase deficiency (LPD), descend to the 36 to 90 day oligomenorrhea and culminate in the most serious secondary amenorrhea[12] which has an absence of menstruation for three consecutive months after menarche.[5-10] A stringent review[8] concerning the female athlete triad found that most investigators had used self-report methods to determine menstrual function; and had reported apparent prevalence of 1.0% to 60.0% in secondary amenorrhea , 0% to 56% in primary amenorrhea, and 0.9% to 52.5% in oligomenorrhea. However, in the same review, those that had evaluated sub-clinical irregularities reported LPD prevalence between 5.9% and 43.0%, and anovulation between12.0% to 30.0%.

An energy deficient environment combined with the subsequent menstrual dysfunction mediated through hypoestrogenism has been linked with suppressed bone formation and increased bone resorption, thus establishing a potential for bone demineralization.[13] It has been noted that despite oestrogen treatment and return of menses, disruptions in bone formation continue in a nutritionally-challenged environment among sedentary and active women, suggesting that recovery of oestrogen alone does not fully compensate for low EA.[14] When low BMD has been defined as *z*-score between -1.0 and -2.0, reported prevalence has ranged from 0% to 39.8%

among women participating in physical activity from recreational to international competitive levels.[8]

There is evidence that elite Kenyan male runners often are energy deficient[15] but little evidence is available on the energy status, menstrual function and BMD of elite Kenyan female distance runners. Thus, the purpose of the current study was to establish the status of EA, MF and BMD in elite Kenyan female distance runners (middle and long) and non-athletes. It was hypothesized that Kenyan female runners would present with lower energy availability, more menstrual dysfunction and lower BMD than a group of non-athletes.

METHODS

Study Design and participants

The researchers had no control over the athletes' diet or training programmes, and the results were evaluated retrospectively.

Participants: Thirty two elite national and international Kenyan female athletes (MD=16, LD=16) and 16 non-athletes aged 18 to 30 years, volunteered to participate in the study. Of these, the 25 athletes (MD=12, LD=13) and 14 non-athletes, who completed all the requirements, provided data for the study.

Energy availability was determined by deducting mean exercise energy expenditure (EEE) from mean energy intake (EI) and the result standardized for fat free mass per day ($FFM.d^{-1}$) such that $EA = (EI\text{-}EEE)\cdot kgFFM^{-1.}d^{-1}$.[1 2 5]

Energy intake took cognisance of the lack of variety or change in the Kenyan athlete's diet through the week[16] and was calculated from a 3-consecutive training day weighed food record. Using a digital weighing scale (Aston Meyers, model: 7766) accurate to 1 gram, an individually assigned trained research assistant (RA), who was with her participant from 5.30 am until after the last meal each day, weighed each item of food or drink separately before her participant began eating. After eating, remnant items were separated and their weight deducted to determine actual consumption. Participants were instructed on how to note items consumed in the absence of RA. Such items were weight-matched and recorded against the appropriate day. Weight of every item before and, where applicable, after eating was noted in the food record. Weight of each item actually consumed was entered into the Nutrisurvey for Windows 2007[17] software programme, which computed the average daily energy intake in kilocalories (kcal).

Exercise energy expenditure was assessed using Actigraph GT3X plus (GT3X+) tri-axial

Accelerometer.[18] Except when bathing, the device was worn on the right iliac crest continuously for 72 hours. Actilife, version 5.6 software (Actigraph LLC, Pensacola, FL, USA) was used to initialize, download and analyse data. Participant's weight, measured to the nearest 0.1 kilograms (kg) on a digital scale (A & D Precision Health Scale, Model: UC-322) was added to the energy expenditure template. The Freedson work energy formula[19] incorporating participant weight was used to determine energy expenditure. Though non-athletes did not partake in purposive exercise, their normal daily activities, such as walking to get around, manually cultivating small holdings, collecting firewood and fetching water, could potentially account for substantial energy expenditure. The Actilife output deemed activity counts higher than 1952 counts/minute as being above lifestyle energy expenditure, corresponding to an intensity of 3 to 6 METs. Hence, EEE for all participants was determined by deducting all hourly activity counts above 1952 from total energy expenditure.

Fat free mass (FFM) and Bone mineral density (BMD) were assessed using dual energy x-ray absorptiometry (DXA, Hologic®, Discovery, Hologic APEX software, Version 3.1.2). Measurements were taken at the Aga Khan University Hospital in Nairobi, Kenya, under the direction and supervision of the head of the hospital's nuclear medicine. The system does not perform any scan unless it passes the daily quality control (QC) using a phantom. BMD of the lumbar spine (L1 – L4, CV= 1.0%), left femoral neck (CV=1.0%) and whole body BMD was measured. The guidelines[20] of the International Society of Clinical Densitometry (ISCD) recommending that BMD evaluation for premenopausal women be expressed as *Z*-scores based on comparison to age, sex and population/ethic match, were used to interpret the results. Accordingly, presence of a *Z*-score below -2.0 at any site, evaluated the participant as being below the expected range for age and ethnicity. In view of the physically active daily living among the non-athletes, as recommended by ACSM[2], all participants who presented with *Z*-scores lying below -1 at any measured site were considered 'at risk' for low BMD and warrant further investigation.

Menstrual function evaluation was based on the number of complete menstrual cycles in the nine-month daily menstruation–temperature diary[21] kept by each participant to record her daily temperature (Royal Flexible Waterproof Digital Thermometer, CEO197) and menstruation characteristics. Cycles were classified as eumenorrheic (28 ±7 days) [2 22], oligomenorrheic (36 to 90 days), and amenorrheic (>90 days).[12] In addition, a standardized questionnaire sought information about age at menarche and number of menstrual cycles in the preceding 12 months.

Study procedures

Participants provided signed informed consent after detailed explanation of the background, duration, risks, benefits of the study, and their responsibilities during the study. As part of a larger investigation into the profile of the female athlete triad in elite Kenyan runners, ethical approval, research permit, and general support for the study were given by the Kenyatta University Ethics Review Committee (KU-ERC), the National Council for Science and Technology in Kenya (NCST), and Athletics Kenya, respectively.

Statistical analysis

Data were analysed using SPSS Statistics version 20. Descriptive statistics were computed to establish means and standard deviations for participant characteristics. Calculated frequencies and percentages described MF status. Independent t-tests were conducted to determine whether there were significant differences in the means of EI, EEE, EA, EBP, macronutrient intake between athletes and non-athletes. One-way analysis of variance (ANOVA) was computed to examine whether the means of EA and BMD differed significantly among the middle distance and long distance athletes, and non-athletes. Tukey's post hoc test was used to determine specific inter-group differences when a significant F ratio was found. The α-level of 0.05 or less was taken to determine statistical significance.

RESULTS

Participant characteristics are shown in Table 1. Athletes' age of menarche was significantly later compared to non-athletes. Athletes also presented with lower BMI and percentage body fat than non-athletes.

Table 1 Demographic, anthropometric and energy characteristics of athletes and non-athletes (means ±SD[1])

Characteristic	Athletes (n=25)	Non-Athletes (n=14)	P-value
Age (yrs)	25 ± 3.2	24 ± 8.8	0.921
Age at menarche (yrs)	15.8 ±2.0	14.1 ± 1.0	0.005*
Weight (kg)	49.8 ± 5.5	58.6 ± 5.8	<0.001**
Height (m)	1.62 ± 0.05	1.60 ± 0.04	0.209
BMI (kg/m^2)	18.7 ± 1.3	22.8 ± 2.8	<0.001**

Fat-free mass (kg)	38.6 ± 3.8	38.6 ± 2.6	0.971
Percentage body fat (%)	19.3 ± 3.2	30.5 ± 5.0	<0.001**
Energy Intake (kcal)	1894.04 ± 516.1	2258 ± 799.0	0.091
Exercise Energy Expenditure (kcal)	760.3 ± 222.1	78.14 ± 19.34	<0.001**
Energy availability (kcal/kgFFM^{-1}·d^{-1})	28.1 ± 11.5	57.0 ± 21.4	<0.001**

Notes: p-value computed using independent t-test, *p<0.05 and **p<0.001. [1]SD, standard deviation

Energy Availability

Dietary energy intake tended to be lower among athletes and exercise energy expenditure was significantly (p=<0.001) higher than non-athletes. EA was significantly (p<0.001) lower in athletes (28.1±11.5 kcal·kgFFM^{-1}·d^{-1} [MD=33.26±11.02 kcal·kgFFM^{-1}·d^{-1}, LD=23.27±9.94 kcal·kgFFM^{-1}·d^{-1}]) compared to non-athletes (57.0 ± 21.4 kcal·kgFFM^{-1}·d^{-1}).

Overall 61.5% (n=24) of the participants had EA below 45 kcal/kgFFM^{-1}·d^{-1}. Energy availability below 30 kcal·kgFFM^{-1}·d^{-1} was seen in 56% of the 25 athletes (MD=3, LD=11) and 7% of the 14 non-athletes (n=1). Energy availability between 30 and 45 kcal·kgFFM^{-1}·d^{-1} was seen in 36% athletes (MD=8, LD=1) and 21% non-athletes (n=3) (Figure 1). Significantly more athletes (t=-=.533; p=<0.001) fell below the 45 kcal·kgFFM^{-1}·d^{-1}.

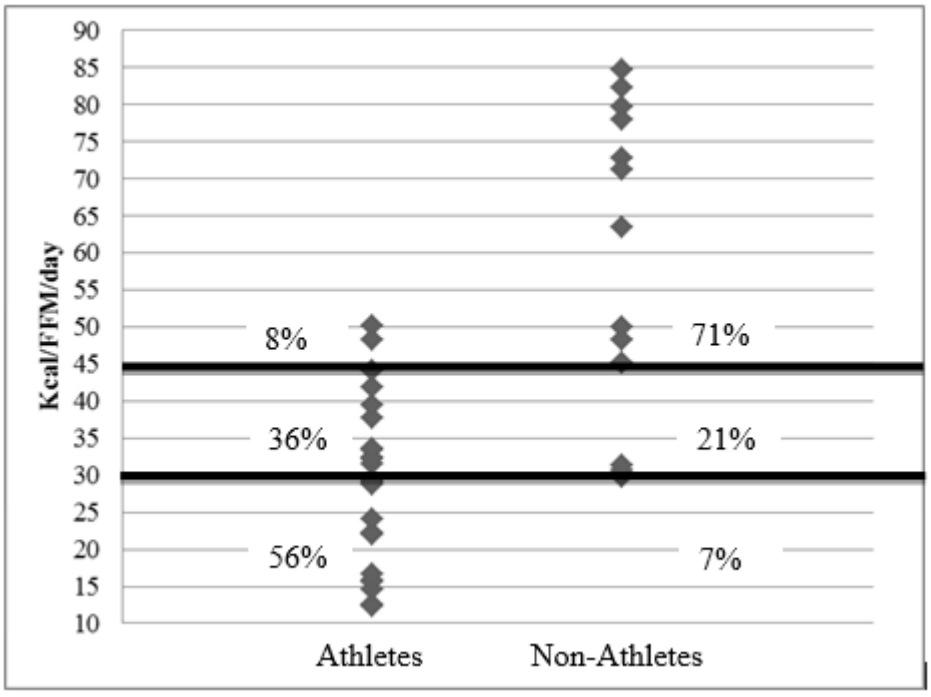

Figure 1 Energy availability of athletes (n=25) non-athletes (n=14)

Menstrual Function

No differences were found between athletes and non-athletes for reported menstrual function. Eumenorrhea was reported by 60% (MD=8, LD=7) athletes and oligomenorrhea by 40% (MD=4, LD=6, Figure 2). Most non-athletes reported eumenorrhea (71.4%), 14.3% oligomenorrhea, and 14.3% secondary amenorrhea. The primary amenorrhea noted in 44% (n=17) of participants identified 88.2% as athletes (MD=7, LD=8) and 11.76% (n=2) as non-athletes. Of these primary amenorrheics, 47% (LD=4, MD=4) presented current oligomenorrheic dysfunction, 62.5% (LD=2, MD=3) started training prior to onset of menarche.

Bone Mineral Density

Overall BMD evaluation based on ACSM guidelines showed 80% presentation of low BMD among the participants that was distributed as 76% athletes (MD=10, LD=9) and 86% (n=12) non-athletes.

Table 2 Bone mineral density (BMD) characteristics of athletes and non-athletes (ACSM guidelines)

Characteristics	**Athletes (*n*=25)**	**Non-Athletes (n=14)**	***p*-value**
Lumbar Spine (L1-L4) (g/cm^2)	.945 ± .021	0.968 ± 0.3	.102
Neck of Left Proximal Femur (g/cm^2)	.945 ± .0731	.983 ± .0995.	.181
Total Body BMD (g/cm^2)	1.045 ± .584	1.016± .0710	.176
BMD *Z*-score (g/cm^2)	1.24 ± 0.44	1.14 ± 0.40	.484

Note: p-value computed using independent t-test

Further breakdown of low BMD distribution by region showed that apart from one non-athlete (8%), who had low BMD at femoral neck, all other low BMD presentations were at the lumbar spine among both, the 76% athletes (MD=10, LD=9) and the remaining 92% non-athletes. Though two athletes and two non-athletes showed the neck of left femur as their weakest BMD site measured, and one athlete had the same *Z*-score at the lumbar spine and neck of left femur, their *Z*-scores at respective sites were within the healthy normal limit by ACSM guidelines.

Among those with low BMD at the lumbar spine, 36% of the athletes (MD=5, LD=4), and 21% of the non-athletes (n=3) had *Z*-scores <-2.0. The t-test did not reveal significant difference between the athletes' BMD mean Z-score of 1.24 ± 0.44 g/cm^2 and the non-athletes' BMD mean Z-score of 1.14 ± 0.44 g/cm^2 (p=<0.436).

DISCUSSION

The elite Kenyan female athletes showed significantly lower EA compared to the non-athletes. However, they did not show similar significant differences either, in menstrual dysfunction nor in low BMD compared to the non-athletes.

Energy Availability

Considering that EA at ~30 kcal/kgFFM^{-1}·d^1 is deemed the critical threshold below which menstrual and skeletal health are potentially adversely affected,[1] low EA poses risk for the elite Kenyan distance runners. Results from both groups of runners support earlier findings that elite Kenyan runners are often in negative or borderline energy balance.[15] Specifically, individual athletes, who were well below the EA threshold, could be at greater risk for both menstrual dysfunction and low BMD.[22] Since bone formation can be suppressed under less severe EA, those above the threshold for low EA but below the 45 kcal/kgFFM^{-1}·d^{-1} healthy criteria are at considerable 'risk' for bone degenation.[13]

Disordered eating and eating disorders have been identified as contributory factors to low EA[1-2] especially in athletes participating in physical activities that emphasize leanness.[12] However, comparison of eating disorders and psychopathological eating behaviours between elite female distance runners and controls from the United Kingdom and Kenya[24] found that elite Kenyan female runners were least likely to present with eating disorder psychopathology. It is therefore more likely that low to borderline EA in the current Kenyan runners and those previously reported[15] is a result of inadvertent under-eating or ignorance on maintaining healthy energy balance.[2] Perhaps the predominant carbohydrate-dense ugali in the Kenyan diet[15] acted as a satiety enhancer forcing inadvertent reduction in energy intake.[24] This combined with the suggestion that exercise could also act as a satiety enhancer[10], might be a reason for low energy intake and resultant energy deficiency. The non-athletes in the present study shared the same cultural, dietary, and social characteristics with the athletes. Features that differentiated non-athletes from athletes were their slightly higher EI and their non-participation in purposive exercise. Despite the non-athletes' physically active rural lifestyle, they had higher EA than

athletes. Therefore, high energy expenditure through purposive intensive and/prolonged training could be another factor that denied the elite Kenyan athlete a substantial portion of energy consumed for other metabolic processes.[10] This suggests a serious need for education about energy, dietary and nutritional intake for all Kenyan female athletes and their coaches on eating enough food to meet their energy needs. It also suggests a need for more research into possible causes of insufficient energy intake among elite Kenyan female athletes.

Menstrual Function

The higher prevalence of outwardly apparent eumenorrhea among elite Kenyan runners and non-athletes concurs with earlier findings of more favourable menstrual function among Kenyans compared to the British athletes' showing of lowest eumenorrheic proportion.[24] A comprehensive review[8] of stringently selected studies reported up to 60 % prevalence of secondary amenorrhea, up to 56% primary amenorrhea, and 0.9% to 52.5% in oligomenorrhea among physically active women. Notwithstanding the diversity of sports and ages in the review, the 60% outwardly apparent eumenorrheic status among elite Kenyan athletes seems to be slightly better than those reported in the review. Despite the possible ethnic differences, the outwardly apparent 40% oligomenorrheic presence in Kenyan athletes, just 2% higher than elite British athletes,[26] warrants further investigation to determine its underlying causes.

It must, however, be emphasized that menstrual status was based on the presence and regularity of menses. Abnormal and subtle sub-clinical menstrual disturbances could not be confirmed in such apparently eumenorrheic athletes without appropriate hormonal and ovarian steroidal investigations.[9] A delicate balancing act between energy availability and exercise energy expenditure determines the amount of EA and subsequent menstrual dysfunction in a low to borderline EA environment. Elite Kenyan athletes could therefore be caught in the pendulum swing from ovulation to subtle sub-clinical menstrual dysfunctions of luteal phase deficiency and anovulation corresponding to intermittent changes in exercise energy expenditure and subsequent EA.[27] It is also important to note that oligomenorrheic cycles can be ovulatory or anovulatory,[12] and could be associated with factors other than EA.[28]

Bone Mineral Density

While some the participants were within normal BMD limits, the predominance of those below the expected limit represents a worrying inclination towards premature osteoporosis and irreversible bone loss. Of greater concern are 52% of the apparently eumenorrheic athletes with

low BMD. All showed low to borderline EA, a known causative factor in low BMD[15]. It has been reported that impairment in bone formation can start under less severe EA than 30 kcal.kg/FFM^{-1}·d^{-1}. [13] Therefore, the 40% designation of these Kenyan athletes to low BMD under the ACSM guidelines should raise grave concern. The lone energetically healthy, but oligomenorrheic athlete designated to low BMD may possibly be a victim of primary amenorrhoea that could have denied her full exposure to oestrogen during the critical peak bone accrual adolescent period.[5] This, combined with her current oligomenorrheic status that is usually associated with depressed oestrogen, [12] suggests need for further hormonal investigation.

Notwithstanding the ethnic differences, compared to the current Kenyan athletes' 36% osteoporotic (*Z*-score <-2.0) and 40% low BMD *(Z*-score <-1.0) presence all at the lumbar spine, Danish-Norwegian athletes revealed 19% osteoporotic and 46.7% respective presence but did not indicate the site.[29] Whereas the British athletes[26] showed 4.9% prevalence of low BMD in the total body, 34.2% at the lumbar spine, and 13.8% at the femoral neck, the current Kenyan athletes reflected 36% for the total body, 32% at the lumbar region, but none at the femoral neck. Comparative osteoporotic (*Z*=<-2.0) prevalence between them, showed British athletes presenting just 7.3% at the lumbar spine, compared to 36% of the Kenyan athletes. Such high prevalence of low BMD at lumbar spine is not unique to Kenyan runners. Despite the ethnic differences, low lumber spine BMD is a common presentation in both male and female endurance runners from elsewhere.[30]

This is the first study in Kenya to investigate energy availability, menstrual function and BMD in elite female distance runners and a control group. A novel outcome is that both, athletes and non-athletes showed similar prevalence in low BMD. Furthermore, it seems likely that low EA was due to a mismatch between energy intake and increased energy expenditure. In conclusion, elite Kenyan female athletes predominantly presented with less energy availability which was significantly lower than that of the non-athletes. Though 40% of athletes presented with menstrual dysfunction, no differences were found between the athletes and non-athletes. While presence of low bone mineral density was high among the athletes, the non-athletes showed a slightly higher prevalence which did not translate to significance. As such the observed low bone mineral density among athletes warrants further attention to prevent long-term health consequences which may hamper sustainability in performance.[31]

The study was limited by the lack of direct hormonal and steroidal assessment which prevented identification of subtle sub-clinical menstrual dysfunctions.[9] Consequently, the relatively

apparent healthy menstrual status among these Kenyan participants may be an overestimation of the true picture. Further, although physical activity was assessed objectively with accelerometry, the conversion of activity counts to measures of energy expenditure introduces some error into the analysis.

The tendency for low to borderline EA among Kenyan athletes may require bone-relevant nutrient supplementation to enhance their BMD. It may be necessary for elite Kenyan athletes to eat deliberately rather than depending on appetite.[10] Improvement in energy status could be further enhanced through research to determine possible causes of insufficient energy intake and to formulate recommendations for meeting an athlete's specific energy needs. It is recommended that Kenyan endurance runners and their coaches be educated about specific energy requirements for their events. Regular clinical screening should become routine practice to ensure early identification of pathology and implementation of appropriate intervention.

New Findings:

- Significant difference was noted in energy availability between athletes and non-athletes.
- Menstrual function did not differ between athletes and non-athletes
- Low bone mineral density showed high prevalence among both athletes and non-athletes.
- All low bone mineral density presentations were at the lumbar spine.

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CHAPTER 4 Association between energy availability and menstrual function in elite Kenyan runners and non- athletes

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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^{-1}·d^{-1} was seen in 61.53% of the participants (athletes: 28.07 ±11.45 kcal·kgFFM^{-1}·d^{-1}, non-athletes:56.97 ±21.38 kcal·kgFFM·d^{-1}). Results of menstrual dysfunction were as follows: oligomenorrhea (athletes: 40%; non-athletes: 14.3%) and amenorrhea (non-athletes: 14.3%). None of the athletes were amenorrheic. The analysis 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.

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 pituitary 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^{-1}·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^{-1}·d^{-1} (Manore et al., 2007).

Increasing energy expenditure through exercise is one of the deliberate causative factors in low energy availability (Nattiv, Loucks, Manore, Sandborn, Sundgot-Borgen & Warren, 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, Lee, VanHeest, Scheid, West & Williams, 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, Westerterp, Kiplamai, Onywera, Boit, Kayser, 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 MF (ii) explore the association between EA and MF in the elite Kenyan female long and middle distance runners, and non-athletes. 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) EA and MF 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 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 (Long distance runners=13, and Middle distance runners=12, Non-athletes=14) who completed the requirements, provided data for the study.

Measuring instruments

Energy availability (EA) was determined after deducting EEE from EI, and the remnant energy normalized for their fat free mass per day ($FFM^{-1}\cdot d^{-1}$). Hence, EA = (EI-EEE)/$FFM^{-1}\cdot d^{-1}$ and was expressed as $kcal\cdot kgFFM^{-1}\cdot d^{-1}$(Manore et al., 2007). Energy availability <45 $kcal\cdot kgFFM\cdot d^{-1}$ was defined as low availability and EA >45 $kcal\cdot kgFFM^{-1}\cdot d^{-1}$ 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, Kiplamai, Tuitoek, Boit & Pitsiladis, 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 every dinner time. Compliance at the participants' training centres was enhanced by random presence of the principal Investigator during any food weighing session. The individually assigned 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 for recording 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 (ADA, 2009 35), Dieticians of Canada, and the American College of Sports Medicine 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 (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, herding, 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 (Fairburn & Beglin, 2008) Eating Disorder Examination Questionnaire (EDE-Q). It 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) 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 for age, sex and population/ethnic 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 lying 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, 2014). 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) (Sloane, 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 of EI, EEE and EBPs. Independent samples T-test was used to determine significant difference in EI, EEE and EA between athletes and non-athletes. Further, an analysis of variance (ANOVA) was used to determine 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 honestly significant difference (HSD) to establish specific inter-group differences. Descriptive statistics were used to establish and describe menstrual status among the athletes and non-athletic cohorts. The three categories of menstrual function 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. The Pearson correlation coefficient (*r*) was also conducted to determine level of statistical significant contributory association between EBPs and EA. The 0.05 α level 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 the participants

Characteristic	**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 indicated 4648.10$kcal/kg \cdot d^{-1}$ for the athletes and 2409.1$kcal/kg \cdot d^{-1}$ for non-athletes.

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

Energy characteristics	Athletes (*n*=25)	Non-Athletes (*n*=14)	*p* value
Estimated energy requirements ($kcals \cdot d^{-1}$)	4648.10	2406.10	0.001*
Energy intake ($kcal \cdot d^{-1}$)	1893.60 ±516	2258.82±799.1	0.091
Exercise energy expenditure ($kcal \cdot d^{-1}$)	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 $kcal \cdot kg \cdot d^{-1}$ had lower intake than the non-athletic cohorts' mean of 2270.01±766.38 $kcal \cdot kg \cdot d^{-1}$. 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 separated by distance (LD and MD) category 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 translated 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

	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 long distance, middle distance 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±11.45 kcal·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 middle distance athletes with a mean of 33.27 (±11.02) kcal·kgFFM·d^{-1}, long distance athletes with a mean of 23.31 (±10.10) kcal·kgFFM·d^{-1} and non-athletes mean of 56.97 (±21.38). 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 follow-up post-hoc Tukey's HSD 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 middle distance (33.27 ± 11.02) and long distance (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) (table 2) revealed a global mean of 0.62 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 mean low EA mean of 38.44 (±20.89) kcal·kgFFM·d^{-1}(data not shown).

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 5% 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) were 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.

The Spearman's rho analysis conducted to explore association between EA and MF did not show significant correlation coefficient between them (*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 long distance and middle distance 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. Energy availability among the non-athletes was in the above 45 kcal/kgFFM·d^{-1} healthy range.

Fudge et al. (2006) examined energy availability 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 Kenyan runners still operating under borderline sub-optimal to very low EA.

The Hoch et al.'s, (2009) investigation of 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), overall 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. In contrast, 29% of the non-athletes presented EA≤30 kcal·kgFFM·d.

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 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 energy availability on menstrual function, reproduction and fertility (Williams, Helmreich, David, Parfitt, Caston-Balderrama & Cameron, 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 outwardly 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 by Burke et al. (2007) and 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 presence of the subtle sub-clinical conditions such as luteal phase deficiency (LDP) and anovulation. Using daily hormone measures, De Souza et al. (De Souza, Toombs, Scheid, O'Donnell, West & Williams, 2010) confirmed presence of LPD and anovulation in cycles that were apparently within normal eumenorrheic range. It has been reported (De Souza & Williams, 2004) 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. 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 so many Kenyan athletes exhibiting eumenorrheic status.

It is very likely that despite regularity in cycles within normal range, the outwardly apparent 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 lay midway in the borderline suboptimal category. Apart from need to manipulate cycles for competition convenience and avoid pregnancy, no other reason was given for HC use. It is most likely that their 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 through 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/bias associated with some methods when assessing EA (Gibbs et al., 2013). This 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 the 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 did not allow estimation of ovulatory cycles. Unfortunately, this failure combined with the lack of either direct hormonal and steroidal assessments or their biomarkers, highlights a weakness in 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 outwardly apparent eumenorrheic menstrual scenario 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 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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CHAPTER 5 Relationship of energy availability and menstrual function to bone mineral density in elite Kenyan female athletes and non-athletes

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Abstract

The influence of energy availability (EA) on bone health, and its indirect role through optimal menstrual function (MF), and subsequent oestrogen (E_2) production have been extensively reported outside Kenya. This study explored relationship of EA and MF to bone mineral density (BMD) among elite Kenyan female runners. Data were provided by 25 elite Kenyan female runners (long distance [LD] *n*=13, middle distance [MD] *n*=12) and 14 non-athletes. EA was computed using the formula EA= Energy Intake (EI) – Exercise Energy Expenditure (EEE)/Fat Free Mass (FFM). Weight of all food and drink consumed during three consecutive days determined EI. Energy expended in physical activity above lifestyle level was deducted from the Actigraph. GT3X+ accelerometer measured total energy expended. DXA scanning determined composition of hard and soft tissue in the participants. A daily nine-month temperature-menstrual log determined menstrual function, and a standardized menstrual history questionnaire sought information about menarche and physical activity in daily living. Spearman's correlation coefficient analysis showed a significant relationship between MF and BMD among the athletes as a combined group (*rho*=0.497; *p*=.011), and among middle distance athletes on their own (*rho*=0.632; *p*=.027). The independent t-test showed significant difference in EEE (*p*<.001) and EA (*p*<.001) between athletes and non-athletes. ANOVA and Tukey's HSD comparing LD, MD and NA confirmed that the values of non-athletes as significantly different (*p*<.001) from LD and

MD. The high prevalence of low EA and low BMD raises concern for the reproductive and bone health of the athletes in the future.

Keywords: Energy availability, menstrual function, bone mineral density

Introduction

Energy availability (EA), the energy remaining from initial energy intake (EI) after deducting energy expended in purposive exercise (EEE) and normalizing to fat free mass (FFM) [1], supports all other physiological functions, including those of reproduction and endocrine [2]. EA above 45kcal·kgFFM·d^{-1} in young adult women is regarded as healthy. Energy availability at 30kcal·kgFFM·d^{-1} is considered the threshold below which disruptions in menstrual function suppress production of oestrogen (E_2), the bone-protecting reproductive hormone [3]. Osteocalcin (OC), the most abundant protein in bone, is abruptly suppressed between 20 – 30 kcal·kgFFM·d^{-1} (OC) [4]. Energy deficiency in athletes could occur through deliberate or unintentional restriction in dietary energy intake relative to energy expenditure [5].

Higher prevalence of menstrual dysfunction has been reported among physically active women than in the general population [5]. For convenience, it has become general practice to describe a 28-day menstrual cycle, beginning from the first day of bleeding in each cycle, as normal [6]. However, in reality, a normal ovulatory cycle or eumenorrhea lasts 28±7 days [7], that descends from the 35 to 90 day oligomenorrhoeic dysfunction [5] to the most serious (more than 90-day) menstrual dysfunction [8]. Though signs of menstruation may be present, asymptomatic sub-clinical anovulation and luteal phase deficiency (LPD) disorders could be present in an energetically challenged environment [9]. These can only be detected through appropriate hormonal and steroidal investigations [10]. Despite the inherent problem of honesty, recall accuracy and participant bias, menstrual history questionnaires that require respondents to recall information such as age at menarche and regularity or number of cycles in the past 12 months, have been the most commonly used tool for determining menstrual function [11].

Optimal pulsatile secretion of gonadotropin releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that encourages E_2 and progesterone (P) production is dependent on the extremely hypometabolic-sensitive hypothalamus [12]. Oestrogen plays its principal role of suppressing osteoclastic activity in ensuring minimal change in overall

BMD accumulation by stimulating calcium absorption and its depositions in bones [4]. The cumulative effect of energy/nutritional deficiency, menstrual dysfunction and subsequent hypoestrogenism establishes the potential for bone demineralization by suppressing bone formation and increasing bone resorption [2], manifesting as the female athlete triad (FAT/the TRIAD) in exercising women [5]. Despite E_2 treatment and return of menses, disruptions in bone formation continue in a nutritionally-challenged environment [2]. This suggests involvement of other oestrogen-independent factors associated with low EA in bone impairment [4]. As suggested by the American Dietetic Association (ADA) [13], one such dimension could be poor nutrient intake, which is the expected outcome under chronically low energy intake. Collective intake of macro- and micronutrients plays a significant role in maintaining the dual functionality of bone's structure and metabolic integrity [14]. The combination of this preamble with the report that elite Kenyan endurance runners are often in negative energy balance [15], warrants investigation into factors that could affect the performance and health of elite Kenyan female runners. The purpose of this study was to examine the relationship of EA and menstrual function (MF) to bone mineral density (BMD) in elite Kenyan female athletes. It was hypothesized that both, EA and MF would show positive relationship to BMD among the participants.

Methods

Study design

This report was a part of a larger investigation into the profile of the female athlete triad in elite Kenyan runners. The study was based on a quasi-experimental design because the researchers did not manipulate any variable nor was there random assignment. Since the researchers had no control over the participants' diet, training programmes and daily activities, and the results were evaluated retrospectively, the study specifically used the ex post facto/causal-comparative design.

Ethical approval and permission

As part of a larger investigation into the profile of the female athlete triad in elite Kenyan runners, ethical approval, research permit, and support for the study were obtained from the Kenyatta University Ethics Review Committee (KU-ERC), the National Council for Science and Technology (NCST), and Athletics Kenya (AK) respectively. Prior to signing the informed

consent, each participant was given written and verbal explanation in English and *Swahili* about the requirements of the study and her expected role in it. In addition, the non-athletes were certified as fit for participation by respective Provincial Medical Officers before signing the informed consent.

Participants

Initially, thirty two currently active elite female long and middle distance (LD=16, MD=16) runners, and 16 non-athletes (NA) aged between 18 and 30 years, four in each age-group cluster of 18 – 20, 21 – 23, 24 – 26, and 27 – 30 years for parity, were selected for participation in the study. The athletes were required to have competed at national championships level and above. The non-athletes were recruited from the same areas as the training centres through the assistance of the coaches. Data were provided by 39 participants (LD=13, MD=12, NA=14) who completed all the requirements of the study.

Establishing Energy Availability

Energy availability was established by deducting exercise energy expenditure (EEE) from energy intake (EI) and normalizing the result to fat-free mass per day ($FFM^{-1}{\cdot}d^{-1}$) [EA = (EI-EEE)/$kgFFM^{-1}{\cdot}d^{-1}$]. EA status was classified into two categories of EA<45kcal/$kgFFM{\cdot}d^{-1}$=Low, and >45 kcal/$kgFFM^{-1}{\cdot}d^{-1}$ healthy [12].

Energy and nutrient intake

With consideration for the lack of variety in the Kenyan athlete's diet through the week [16], and of measurement of energy intake over three consecutive days eliciting reliable data [17], EI was measured during three consecutive days. From the initial 48 female trainee research assistants trained by the principal investigator in the food-weighing and questionnaire administration protocols, 25 who showed proficiency and had attended four of the six training sessions, were selected as research assistants. Except when the athlete was training, the research assistant was with the participant through the day from 5.30 am until after the last meal at night. Compliance was enhanced by impromptu random attendance by the principal investigator during any meal time when the participants' food was being weighed. Participants were instructed on how to use a cup, spoon or bowl to note any item consumed in the absence of the research assistant. At a convenient time the next morning, the research assistant weight-matched and recorded its weight

against the appropriate day. The food weighing protocol required that each item of food be weighed separately prior to eating. At the end of eating, the 'left-over' food items on the plate were separated and their weight noted in the food diary. The trained research assistant used a digital weighing scale (Aston Meyers, model: 7766) accurate to 1 gramme (g) to separately weigh each item of food and drink consumed by her assigned participant. Weight of "left over" item was noted and deducted from the original weight to arrive at actual amount consumed. Weight of each item actually consumed was entered into the Nutrisurvey for Windows software programme [18] which analysed the average daily energy intake in kilocalories (kcals) and nutrient intake in grammes (gms). Appropriate dietary guideline formulae [13] were used to estimate the minimum EI requirements.

Exercise energy expenditure

Exercise energy expenditure was assessed using the Actigraph GT3X plus (GT3X+) tri-axial accelerometer [19] which was worn against the right iliac crest continuously for 72 hours except when bathing. It recorded all physical activity during the same three consecutive days as when energy intake was measured. Initializing, downloading and analysis of energy expenditure were done using the Actilife, version 5.6, software (Actigraph LLC, Pensacola, FL, USA). Initialization at 1 second epoch length allowed the accelerometer to record even the shortest activity counts at various intensities and durations. Despite the lack of purposive exercise, the non-athletes' daily living activities of walking, cultivating their small holding, herding life-stock, fetching firewood and water, had the potential for expending substantial energy. Activity counts higher than 1952 counts/minute that correspond to an intensity of 3 to 6 Mets are categorized as above lifestyle energy expenditure by the Actilife output. Therefore, for both, the athletes and non-athletes, all hourly activity counts above 1952 counts/minutes were identified and their corresponding kcal energy expenditure values deducted from total energy expenditure to determine EEE. The Freedson work energy combination formula, incorporating participant weight [20], was used to determine total energy expenditure. Weight of each participant, measured on a digital scale (A & D Precision Health Scale, Model: UC-322) accurate to nearest to 0.1 kilogramme (kg), was entered into the Actilife analysis template.

Eating behaviour practices (EBP)

Psychopathological eating behaviour practices such as dietary restraint, eating concern, shape concern and weight concern that could influence EA were explored using the validated Fairburn and Beglin [21] Eating Disorder Examination Questionnaire (EDE-Q). The seven point (0-6) scoring scale, places each eating behaviour subscale on a healthy (score 0) to extreme eating disorder (score 6) continuum. Either the English or the *Swahili* version of the questionnaire was completed by each participant in the presence of the investigator if she needed further clarification.

Fat free mass (FFM) and bone mineral density (BMD)

Dual energy x-ray absorptiometry (DXA) scanning with Hologic®, Discovery (USA) was done at the Aga Khan University Hospital in Nairobi, Kenya, under the direction and supervision of the head of the hospital's Nuclear Medicine to assess FFM and BMD. The system had to pass the daily quality control (QC) using a phantom before a scan was performed. In addition to assessing total body BMD, standard Hologic positioning protocols for lumbar spine (L1- L4, CV=1.0%), and left proximal femur (CV=1.0%) determined BMD at these regions and were expressed as grammes per centimetre squared (g/cm^2). Initial evaluation for these premenopausal participants was done according to the guidelines provided by the International Society of Clinical Densitometry (ISCD) [22] which require comparison of BMD against expected norms for age, sex and population/ethnic match, and expression 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, 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 American College of Sports Medicine (ACSM) [5], all participants with *Z*-scores lying below -1were categorized as having low BMD.

Menstrual Function

Each participant used an oral thermometer (Royal Flexible Waterproof Digital thermometer, CEO 197) to record her temperature, and signs and symptoms associated with menstruation in a

log every morning on waking up for nine continuous months starting on the morning after she signed her informed consent form. A complete menstrual cycle in the menstrual log was counted from the first day of appearance of menstruation and ended on the day previous to the appearance of the next menstruation. Complete cycles of 21-35 days were classified as eumenorrheic, 35-90 days as oligomenorrheic and >90 days as amenorrheic [5]. In addition, a standardized questionnaire was used to seek information about the participants' menstrual history, current regularity of menstrual cycles and physical activity in daily living.

Statistical analysis

The IBM SPSS Statistics version 20 was used to carry out statistical analysis. Means and standard deviations of energy and nutrient intake and global EBP were calculated. Independent samples t-test were conducted to determine if there were significant differences in means of EI, EEE, EA, micronutrient intake of carbohydrate, protein and fat, and BMD between athletes and non-athletes. Analysis of variance (ANOVA) was conducted to determine if there were significant differences among LD, MD and NA in the same variables. Frequencies and percentages were used to establish menstrual status. The Pearson product moment correlation coefficient (*r*) was used to determine the relationship between EBP and EA, and EA and BMD. The Spearman rank correlation coefficient (Spearman's rho) was computed to determine relationship between MF and BMD. Binary logistic regression was used to explore the odds of a participant with primary amenorrhea presenting with menstrual dysfunction in future, and to explore whether MF would predict BMD.

Results

As shown in Table 1, though the non-athletes met the estimated energy requirements, the athletes fell short by 2754.1 kcals which translates to less than 59% of the estimated energy requirements (ADA, 2007). Independent t-test results showed significant difference in EEE ($p<0.001$) and EA ($p<0.001$) between the athletes and non-athletes. When considered separately (Table 2), the ANOVA revealed significant differences in EEE among LD, MD and NA ($p<0.001$). The Tukey's test further revealed that the source of the difference were the non-athletes, who had lower EEE than the athletes. Overall EA of less than 45 $kcal \cdot kgFFM \cdot d^{-1}$ was present in 61.5% (n= 24) of the participants. Further breakdown indicated that out of these, 56% of the athletes (LD=11, MD=3) and 7% non-athletes (n=1) had EA <30 $kcal \cdot kgFFM^{-1}d^{-1}$, while 36% (LD=1,

MD=8) and 21% (NA=3) showed EA of between 30 and 40 kcal·kgFFM^{-1}·d^{-1}. Results of the ANOVA showed significant differences in EA among the participants. Tukey's HSD revealed that the source of difference in EA were the non-athletes.

Detailed breakdown of the macronutrient showed that 73% (±7%) of the athletes energy intake constituted carbohydrates (CHO) which translated to 12.91(±4.02) g/body weight in kg [BW], 11% (±2%) was protein (PRO) which translated to 1.69 (±0.59) g/BW, and 17% (±1%) was fat which reflected 1.43 (±0.70) g/BW. The non-athletes' intake showed that 75% (± 6%) was CHO providing 12.92 (±4.02) g/BW, 9% (±2%) was PRO yielding 1.61 (±0.56) g/BW, and 16% (±5%) was fat that amounted to 1.18 (±.32) g/BW. The independent t-tests revealed protein as the only micronutrient that showed significant difference (t=2.11; p=<0.041) between athletes (10.72±1.92) and non-athletes (9.35±1.94). Additionally, while the athletes' calcium intake was 723.3 ± 438.8 mg·d, the non-athletes' calcium intake was 792.1 ±325.5 mg·d (t=.468; p=0.643).

Table 1 Characteristics related to Energy and BMD in Athletes and Non-Athletes.

Characteristics	**Athletes (n=25)**	**Non-Athletes (n=14)**	***p*-value**
Demographic			
Age (years)	25 ± 3.2	25 ± 3.2	.921
Age at Menarche (years)	15.8 ±2.0	14.1 ± 1.0	.005
Weight (kg)	49.8 ± 5.5	58.6 ± 5.8	<.001
Height(m)	1.62 ± 0.05	1.60 ± 0.04	.209
BMI (kg/m^2)	18.7 ± 1.3	22.8 ± 2.8	<.001
Fat Free Mass (FFM)(kg)	38.6 ± 3.8	38.6 ± 2.6	.971
Energy/Dietary			
Estimated Energy Requirements (EER) (kcal)	4648.10 ± 224.63	2406.85 ±58.88	<.001
Energy Intake (kcal)	1894.0 ± 516.1	2258 ± 799.0	.091
Exercise Energy Expenditure (kcal·kgFFM^{-1}·d^{-1})	760.3 ± 222.1	78.14 ± 19.34	<.001
Energy Availability (kcal·kgFFM^{-1}·d^{-1})	28.1 ± 11.5	57.0 ± 21.4	<.001
Global EBP	.62±.56	.57±.66	.813
Bone Mineral Density (BMD)			

Lumbar Spine (L1-L4) (g/cm^2)	.945 ± .021	0.968 ± 0.3	.102
Neck of Left Proximal Femur (g/cm^2)	.945 ± .0731	.983 ± .0995.	.181
Total Body BMD (g/cm^2)	1.045 ± .584	1.016± .0710	.176
Z-score (g/cm^2)	1.24 ± 0.44	1.14 ± 0.40	.484

Note: p-value computed using independent t-test

The mean of global psychopathological EBP was 0.68 in the group as a whole. When considered separately there was no significant difference between athletes (0.62±.56) and non-athletes (0.57±.66) in EBP. Eating behavioural practices did not show significant relationship with EA, MF and BMD.

Table 2 Characteristics related to Energy and BMD in LD and MD runners, and NA

Characteristic	**Long Distance (*n*=13)**	**Middle Distance (*n*=12)**	**Non-athletes (*n*=14)**	***p*-value**
Energy Related				
Estimated Energy Requirements (EER)	4587.35 ± 213.02	4714.1 ± 227.3	2406.85 ± 58.88	<0.001
Energy Intake (kcal)	1815.62 ± 155.1	1978.1 ± 473.44	2258.83 ± 799.0	0.199
Exercise Energy Expenditure (kcal)	717.75 ± 187.1	804.78 ± 254.90	78.14 ±19.33	<0.001
Energy Availability ($kcal \cdot kgFFM^{-1} \cdot d^{-1}$)	23.27 ±9.94	33.26 ±11.02	57.0 ± 21.4	<0.001
Bone Mineral Density (BMD)				
Lumbar Spine (L1-L4) ($g/cm^{2)}$	0.950 ± 0.92	0.939 ± 0.11	1.00 ± 0.11	0.258
Neck of Proximal Femur (g/cm^2)	0.954 ± 0.08	0.935 ± .073	0.983 ± 0.11	0.350
Total Body BMD ($g/cm^{2)}$	1.040 ± 0.05	1.050 ± 0.07	1.016 ± 0.71	0.374
Z-score (g/cm^2)	-1.646 ± .08	-1.708 ± 0.62	-1.329 ± 0.60	0.301

Note: *p*-value computed using independent t-test

Table 3 Frequency distribution and percentages of menstrual function among the participants

	Athletes	Non-athletes	*p*-value
Menstrual function			
Eumenorrhea	60% (MD=8, LD=7)	71.4% (n=10)	.058
Oligomenorrhea	40% (MD=4, LD=6)	14.3% (n=2)	.058
Amenorrhea	0	14.3%(n=2)	.058
Primary Amenorrhea			
Primary Amenorrhea	60% (MD=7, LD=8)	13.3%(n=2)	.006

Note: *p*-value computed using chi square

Overall distribution of menstrual function (Table 3) among the 39 participants showed that 5% were amenorrhoeic, 31% Oligomenorrheic and 64% eumenorrheic. Distribution by participant category (Table 4) revealed that 60% of the athletes (LD=7, MD=8) were eumenorrheic and 40% (LD=6, MD=4) were oligomenorrheic. Among the 14 non-athletes, 71.4% (*n*=10) were eumenorrheic, while 14.3% (*n*=2) were oligomenorrheic and the remaining 14.3% (*n*=2) were amenorrheic. The presence of primary amenorrhea reported among 44% (*n*=17) of the participants was distributed as 88.24% (LD=8, MD=7) among the athletes and 11.76% (*n*=2) among non-athletes. Out of these, 47% (LD=4, MD=4) were currently oligomenorrheic.

Table 4 Frequency distribution and percentages of menstrual function among LD and MD runners, and NA.

	LD	MD	NA	*p*-value
Menstrual Function				
Eumenorrhea	7 (54%)	8 (67%)	10 (71.4%)	.186
Oligomenorrhea	6 (46%)	4 (33%)	2 (14.3%)	.186
Amenorrhea	0	0	3 (14.3%)	.186
Primary Amenorrhea				
Primary Amenorrhea	8 (62%)	7 (58%)	2 (14.3%)	.022

Note: *p*-value computed using chi square

Bone mineral density results showed 80% low BMD presentation among the participants as a group. Further break down showed that 76% of the athletes (LD=9, MD=10), and 86% of non-athletes (*n*=12) had low BMD. Low BMD distribution by region showed that while one non-

athlete (3%) had low BMD at the neck of femur, all remaining 97% of low BMD presentations were at the lumbar spine, and this 97% distributed as 63% among athletes (LD=9, MD=10) and 37 % (NA=12) among non-athletes. With respect to the weakest region by MF among the 25 athletes, one oligomenorrheic, and one eumenorrheic showed neck of femur as the weakest region. One oligomenorrheic had equal weakness at neck of femur and the lumbar spine. The remaining eight oligomenorrheics and 14 eumenorrheics showed lumbar spine as their weakest regions. Among the non-athletes, eight eumenorrheics, two oligomenorrheics and two amenorrheics showed lumbar spine as the weakest region, while two eumenorrheics showed the neck of femur as the weakest region. The t-test results did not reveal any significant difference between the athletes' mean BMD of 1.24 ± 0.44 g/cm^2 and non-athletes mean BMD of 1.14 ± 0.44 g/cm^2 (t=0.707; p=.484).

Relationship of EA and MF to BMD

Only significant relationships of EA and MF to BMD have been presented in Table 5.

Table 5 Relationships of energy availability and menstrual function to bone mineral density

Participant	n	Relationship	Correlation coefficient	p-value	Correlation type
LD	13	Total body BMD and EA	0.560*	0.046	*r*
Athletes	25	Total body BMD and MF	0.623**	0.001	*rho*
MD	12	Total body BMD and MF	0.819**	0.001	*rho*
Athletes	25	BMD *Z*-score and MF	0.497**	0.001	*rho*
MD	12	BMD *Z*-score and MF	0.632*	0.001	*rho*

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation coefficient analyses showed no significant relationship between EA and BMD *Z*-scores in all the participants (p>.05). However, when considering relationship between EA and BMD measurements by region, a significant relationship (Table 5) was seen between EA and total body BMD among the LD athletes (r=0.560; p=.046) on their own. The Spearman's correlation coefficient results showed that there was significant relationship (Table 5) between MF and BMD *Z*-scores in the athletes (rho=0.497, p=.011). This relationship was further

highlighted with respect to MD (*rho*=0.632, *p*=.027). The only regional BMD measurement to show relationship with MF was total BMD in all athletes *(rho*=0.623; *p*=.001), and in MD athletes (*rho*=0.819; *p*=.001). Although not statistically significant, the binary logistic regression result showed that the odds of those with primary amenorrhea having menstrual dysfunction in the future were 2.4 (95% CI, 0.6 – 9.0, *p*=.206) times. The binary logistic regression revealed that MF did not predict BMD (OR=4.07, 95% CI, 0.8 -20.7, *p*=.091).

Discussion

Relationship between EA and BMD

Impairment in bone formation has been reported under less severe restrictions than the 30 $kcal \cdot kgFFM \cdot d^{-1}$ threshold below which, both menstrual function and bone health are abruptly disrupted [4]. Overall, Kenyan participants showed 61.5% prevalence of EA < 45 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$. Closer scrutiny of 61.5% of participants presenting low EA revealed that whereas 36% of athletes and 21% non-athletes had suboptimal 30 to 45 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$ EA, 56% of the athletes and 7% non-athletes had EA < 30 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$, the critical threshold affecting menstrual and bone health. In contrast, one of the few studies to have evaluated EA reported 36% prevalence of low EA among high school athletes and 39% among their sedentary counterparts. Only 6% of these athletes and 4% of the non-athletes presented EA below the 30 $kcal \cdot kgFFM^{-1} \cdot d^{-1}$ threshold [23].

Though no significant relationship was seen between EA and BMD *Z*-scores among the current participants as a group, significant relationship was seen between EA and total body BMD in the LD and MD athletes separately. When officially recognizing EA as an instigator of menstrual dysfunction and low BMD in 2007, ACSM also replaced disordered eating with EA [5]. Unfortunately, few studies have attempted to evaluate EA or its relationship/association to the other components in the female athlete triad since then [24]. This makes it difficult to compare findings in the current study. Despite the dearth of observations specific to EA, physiologic biomarkers of energy deficiency and behaviour practices associated with energy deficiency revealed elevated bone resorption in adolescent female endurance runners [25]. Elsewhere, regardless of oestrogen status, neither bone formation nor resorption were apparently agitated in an energy adequate environment in premenopausal exercising women. However, reduced bone formation and elevated bone resorption that resulted from simultaneous presentation of energy

and oestrogen deficiency in the same women, created an environment for potential bone degeneration [2]. High cognitive dietary restraint (CDR), an eating disorder resulting in energy deficiency, was associated with lowered lumbar spine and total BMD among physically active premenopausal women [26]. Interestingly, regardless of whether the *Z*-score was within expected normal limit or in the low BMD classification, the lumbar spine was the weakest region among current Kenyan participants, and total body BMD was the only dimension to show significant relationship with EA.

Apart from the direct impact of low EA on hypoestrogenism and subsequent bone loss, initial low EI relative to EEE usually results in low EA concomitant with deficiency in micronutrients such as calcium [13]. Calcium plays a significant role in maintaining the dual functionality of bone structure and metabolic integrity [14]. Though the recommended daily allowance of calcium for this age group is 1000 $mg \cdot d^{-1}$., [27], athletes, especially those presenting low EA and hypoestrogenism, could be at risk of early osteoporosis. Therefore, such athletes are advised to consume 1500 $mg \cdot d^{-1}$ [13]. It was clear that the elite Kenyan and British female athletes [28] were comparable to each other in their low EI, and in failing to meet the recommended 1000$mg \cdot d^{-1}$ of calcium. Though Kenyan athletes did not report any injuries during the preceding 12 months, the same group of British athletes reported 27% history of stress fractures. In view of the similarities of low EI and low calcium intake, Kenyan athletes could be at risk for early osteoporosis and stress fractures, and hence need to ensure balanced and adequate diet to meet specific energy and nutritional needs [13]. Since approximately 50% of bone volume and a third of bone mass constitutes PRO, its adequacy and in turn, protein's constructive interaction with calcium is important to bone health [29]. With the athletes showing 1.69 g $PRO \cdot kg\ BW^{-1} \cdot d^{-1}$ and the non-athletes 1.61 g $PRO \cdot kg\ BW^{-1} \cdot d^{-1}$, both groups met the recommended target of between 1.5 and 1.7 g $PRO \cdot kg\ BW^{-1} \cdot d^{-1}$ set by Stellingwerff et al. [30] for endurance runners whose training loads are large and intense.

Relationship between MF and BMD

The Z-score at the weakest region determined each participants BMD status. Whereas the results did not show significant relationship between MF and BMD *Z*-scores among the participants as a group, the athletes' MF showed significant relationship with BMD Z-scores. Closer scrutiny of relationship between MF and BMD by distance category among the athletes revealed that it was

the middle distance category that highlighted the relationship further. The combined group of athletes' total BMD also showed significant relationship with MF.

Low EA, the catalyst in the cascade of disruptive events of the female athlete triad in exercising women, initiates menstrual dysfunction that subsequently leads to low BMD [5]. Despite the low to sub-optimal EA among elite Kenyan athletes, 60% of whom showed apparent eumenorrhea, they tended towards the healthier end of the MF health continuum. In comparison, the non-athletes had EA within the healthy range with 71.4% showing eumenorrheic status. In this apparently healthy menstrual environment, 76% of the athletes and 21% of the non-athletes presented with low BMD. A possible explanation could be that while the low to sub-optimal EA among the athletes was sufficient in supporting an outwardly apparent healthy menstrual regularity, the cycles probably experienced subtle sub-clinical anovulation [9]. Perhaps, athletes who did not achieve the normal limit for *Z*-score, fell victim to the simultaneous presence of energy deficiency and hypoestrogenism that cumulatively favour osteoclast activity and suppresses osteoblast activity [10].

Presentation with primary amenorrhea was seen in the delayed menarche among 60% of these athletes in the current study; and athletes accounted for 26% menstrual dysfunction in the whole group. Whereas the Cobb et al. [31] findings indicated that delayed menarche could be a strong predictor of menstrual dysfunction in the future, the odds of primary amenorrhea predicting future menstrual dysfunction among the Kenyan athletes was not as strong. In the context of MF and BMD, 40% of the primary amenorrheics currently presented with menstrual dysfunction and low BMD. Here again, the binary logistic regression did not show possibility of MF predicting BMD. Though hormonal investigations were not part of the current study, findings of Scheid et al. [32] indicate that E_2 concentrations, an objective evaluation of MF, were strong predictors of lumbar spine BMD among exercising women, are instructive and cannot be ignored, especially when they speculate that the lumbar spine could be more susceptible to changes in circulating estragon concentrations. Perhaps, the over-representation of lumbar spine as the weakest region among Kenyan athletes needs hormonal assessment to rule out subtle sub-clinical menstrual disorders. Whereas British athletes [33] did not show any significant relationship between MF and *Z*-scores, significant association between MF and BMD *Z*-scores was seen in the current elite Kenyan athletes as a group; and in MD athletes when the LD, MD and NA participants were considered separately.

Low EA among Kenyan athletes, instead of cascading into wholesale menstrual cyclic dysfunction, presented 40% with apparent signs of dysfunction. Subsequent analysis revealed intriguing interactions between MF and regional BMD. On closer inspection, one oligomenorrheic and one eumenorrheic Kenyan athlete showed neck of femur as the weakest region, and one oligomenorrheic had the same *Z*-score of -0.5 at both, the neck of femur and the lumbar spine. However, none of these sites fell in the low BMD category. The remaining eight oligomenorrheic and 14 eumenorrheic athletes showed lumbar spine as the weakest region. Regional weakness distribution by MF among the non-athletes was present in eight eumenorrheics and two oligomenorrheics and two amenorrheics with lumbar spine as the weakest region, and two eumenorrheics with neck of femur as the weakest region. The predominant prevalence of weakness at the lumbar spine confirms findings of low BMD at the lumber spine in both male and female endurance runners [34].

In the paucity of information, these are novel and important findings concerning elite Kenyan female athletes. However, lack of assessment of metabolic hormones, and direct hormonal and steroidal assessment to establish the full spectrum of MF, are limitations of this study. Further, it must also be recognized that despite the objectivity of accelerometry in assessing physical activity, conversion of activity counts inherently introduces some errors in analysis.

In the short term, to alleviate inadvertent low EI immediately, there is need to up-date and educate athletes, coaches and their significant others about personalized energy, dietary and nutritional requirements specific to respective distances. In the long term, the relevant institutions should make it a matter of policy for all athletes to undergo regular comprehensive clinical screening.

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CHAPTER 6 Profile of the female athlete triad in elite Kenyan endurance athletes and in non-athletes

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Profile of the female athlete triad in elite Kenyan endurance athletes and in non-athletes

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Abstract

Women participating in endurance sports are at risk of presenting with low energy availability (EA), menstrual dysfunction (MD), and low bone mineral density (BMD), collectively termed the female athlete triad (FAT or TRIAD). Therefore, the purpose of the study was to determine the profile of the TRIAD among elite Kenyan female athletes and among non-athletes. There were 39 participants (athletes: 25, non-athletes:14) who provided the data for this study. Exercise energy expenditure (EEE) was deducted from energy intake (EI), and the remnant energy normalized to fat free mass (FFM) to determine energy availability (EA). Weight of all food and liquid consumed during three consecutive days determined EI. EEE was determined after isolating and deducting energy expended in exercise or physical activity above lifestyle from the total energy expenditure output as measured by Actigraph GT3X+. Dual energy x-ray absorptiometry (DXA) determined both, FFM and BMD. Menstrual function was determined from a daily temperature-menstrual log kept by each participant for nine continuous months. Low EA (<45 kcal·kgFFM^{-1}·d^{-1}) was evident in 61.53% of the participants (athletes: 28.07 ±11.45 kcal/kgFFM·d-1, non-athletes: 56.97 ±21.38 kcal·kgFFM^{-1}·d^{-1}). The overall 36% MD seen among all participants was distributed as 40% among the athletes, and 29% among the non-athletes. None of the athletes were amenorrheic. Low BMD was seen in 79% of the participants (athletes: 76%, non-athletes:86%). Overall, 10% of the participants (athletes: 4, non-athletes: 0) showed simultaneous presence of all three components of the TRIAD. The Independent sample t-test showed significant difference (t=5.860; p<.001) in prevalence of the TRIAD between athletes and non-athletes. The hypothesized higher prevalence of the TRIAD among athletes compared to non-athletes was partially accepted. To alleviate conditions arising from low EA, both athletes and their coaches need regular education on how to ensure they adequately meet specific dietary and nutritional requirements for their competition events.

Keywords: Energy availability, menstrual dysfunction, bone mineral density, exercise energy expenditure

Introduction

Understanding the female athlete triad (FAT) or the TRIAD, its recognition, prevention, and management has evolved with ever-increasing research (Gibbs, Williams, & De Souza, 2013). Prior to 2007, an active woman was deemed to be experiencing the TRIAD if she presented with all three interrelated entities of disordered eating (DE), amenorrhea and osteoporosis (Nattiv,

Loucks, Manore, Sanborn, Sundgot-Borgen & Warren, 2007). Currently, the TRIAD is conceptualized as a complex syndrome arising from relationships among the three components of energy availability (EA), menstrual function (MF), and bone mineral density (BMD). Redefinition and consideration of each entity along a continuum from health to disease state (Manore, Kam, & Loucks, 2007:S61; Pantano, 2009), from the previous strict criteria of disordered eating, amenorrhea and osteoporosis, has allowed wider scope for inclusion of those with less severe conditions (Thein-Nissebaum & Carr, 2011). The essence of interrelatedness among the three basic entities of TRIAD associated with dimensions of eating behaviour and food, menstrual function and bone health has been reaffirmed in the new ACSM 2007 Position Stand (De Souza & Williams, 2010).

Disturbances in each component have varying possible causes. Low EA could result from intentional or unintentional/inadvertent eating behavioural practices or from expending more energy relative to intake (Thein-Nissenbaum, 2013). Though anatomical anomalies in the uterus or vagina, or disruptions in endocrine signals, have been identified as causative factors in menstrual disorders (Redman & Loucks, 2005), low EA has been recognized as a leading instigator in menstrual dysfunction (Nattiv, et al., 2007). Any one factor or more from among genetics, nutrition, hormones, weight-bearing exercise, alcohol consumption, and cortisol levels could be implicated in low BMD (Papanek, 2003). The cumulative effect of energy/nutritional deficiency and subsequent hypoestrogenism is to suppress bone formation and increase bone resorption, effectively establishing potential for bone demineralization (De Souza & Williams, 2004). In the context of the composite TRIAD, there appears to be a sequential pattern in the avalanche of events instigated by inadvertent or intentional low EA that subsequently impairs menstrual/reproductive and skeletal health (Nattiv, et al., 2007). Though these conditions could occur independently of each other, it is possible that an athlete experiencing deterioration in one component may also have problems in the other components (De Souza & Williams, 2010).

Most research concerning the TRIAD has focused on interrelatedness among individual entities rather than the whole TRIAD (Nichols *et al.*, 2006; Thein-Nissenbaum & Carr, 2011). There has been a dearth of epidemiological research looking into simultaneous occurrence of all three entities constituting the TRIAD, under both the former definition of disordered eating, amenorrhea and osteoporosis (Beals & Meyer, 2007) and the revised 2007 ACSM version of EA, MF and BMD (Thein-Nissenbaum & Carr, 2011). A recent stringent review to evaluate prevalence of the TRIAD between 1971 and 2011 reported that among the nine studies (n=991)

that investigated prevalence of all three entities, 0% to 15.9% athletes presented with all three conditions (Gibbs, et al., 2013).

Increasing number of girls and women in Kenya are taking up competitive running to alleviate poverty (Onywera et al., 2006). Despite phenomenal success achieved by Kenyan female athletes since their first international appearance in 1965, very few studies have investigated factors that could affect Kenyan female athletes' health and performance. Evidently, elite Kenyan male runners often function under an energetically-challenged environment (Fudge et al, 2006). In view of the instigation of low EA in sequential disruption of MF and subsequent bone health, such evidence among male athletes raises concern for the Kenyan female athlete. No study that looked at the simultaneous occurrence of EA, MF and BMD in elite Kenyan athletes could be found. Therefore, the purpose of this study was to determine the female athlete triad profile among elite Kenyan female athletes compared to non-athletes. It was hypothesized that elite Kenyan female athletes would show significantly higher profile of the female athlete triad than non-athletes.

Methodology

Study design

This investigation was part of a larger study that sought to establish status of EA, MF and BMD, associations/relationships between these variables of the TRIAD, and to determine the profile of female athlete triad in elite Kenyan runners. 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

The Kenyatta University Ethics Review Committee (KU-ERC); Athletics Kenya (AK) and the National Council for Science and Technology (NCST) in Kenya gave ethical approval, relevant permissions, and research permits respectively. The participant signed the informed consent form after receiving verbal and written information about her expected role in the study, both in *Swahili* (the Kenyan athlete's native language) and English. The respective Provincial Medical Officer certified non-athletes as medically fit for participation in the study.

Participants

Initially, 16 long distance and 16 middle distance actively training elite Kenyan female runners, and 16 age-matched non-athletic controls volunteered to participate in the study. For parity, these were four in each age-group cluster of 18-20, 21-23, 24-26, and 27-30 years (N = 48). Out of these, the 39 participants (Long distance=13, Middle distance=12, Non-athletes=14) who completed all the requirements, provided data for the study.

Measuring instruments

Energy availability (EA) was determined by deducting exercise energy expenditure (EEE) from EI, then standardizing the remaining energy to fat free mass (FFM), so that EA = (EI-EEE)/FFM$^{-1.}$d^{-1}, and was expressed as kcal·kgFFM$^{-1.}$d^{-1}(Manore et al., 2007). The threshold for healthy energy availability was set at >45 kcal·kgFFM$^{-1.}$d^{-1}. Therefore, any value below that was deemed low EA (Nattiv et al., 2007).

Energy and nutrient intake (EI) evaluations took cognisance of 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, the principal investigator trained 48 female trainee research assistants in food weighing and in the administration of a standardized questionnaire during six training sessions. Out of these, only the 25 who showed proficiency and had attended at least four sessions were recruited as research assistants. The food-weighing and questionnaire exercise was repeated every evening at the training venue, starting on the day of arrival at the athlete's training centre. The principal investigator randomly attended any meal-time food-weighing session. Except when the athlete was training, the individually assigned trained research assistant was with the participant from 5.30am till after the last meal each evening during the three consecutive days when EI was assessed. The Research assistant (RA) used a digital scale (Aston Meyers, model: 7766), accurate to the nearest 0.1kg, to weigh all food and liquid consumed by her participant during these hours. 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. The Research assistant also instructed her participant on how to use a spoon, cup or bowl to note portions consumed after the departure of the RA each evening. The next morning, the RA weighed these items and recorded them against the appropriate date.

Subsequent to entering weight of actual food and liquid consumed into the programme, the Nutrisurvey for Windows 2007 (Erhardt, 2007) software programme was used to determine the daily average energy and nutrients intake in kilocalories (kcals). The estimated energy requirements (EER) of the participants for their activity levels was based on the joint guidelines of the American Dietetic Association (ADA, 2009 35), Dieticians of Canada, and the American College of Sports Medicine which 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 (ADA, 2009).

Exercise energy expenditure (EEE) was assessed using the Actigraph GT3X plus (GT3X+) tri-axial accelerometer (Actigraph, 2011). The device, worn upright and flat on the right iliac crest for 72 hours continuously during the same period coinciding with the measurement of energy intake, was only removed when bathing. Initialization, downloading and final analysis was done using Actilife, version 5.6 software (Actigraph LLC, Pensacola, FL, USA). The Freedson work energy combination formula incorporating participant weight was used to determine energy expenditure (Freedson, Melanson & Sirard, 1998). By setting initialization at 1 s epoch length, the monitor was able to record the shortest activity counts at various intensities and their durations during wear time. Each participant's weight, accurate to nearest to 0.1 kilogrammes (kg) on a digital scale (A & D Precision Health Scale, Model: UC-322), as required by the Freedson formula, was entered into the analysis template as required to determine her total energy expenditure. According to Actilife output, activity counts higher than 1952 correspond to 3-6 Mets intensity, a level at which energy expenditure is considered higher than lifestyle energy expenditure. The manual-intensive daily living activities could account for substantial energy expenditure among the non-athletes. Therefore, all hourly activity counts above 1952 were identified and their corresponding kcal values were deducted from total energy expenditure to account for both, the athletes' and non-athletes', exercise energy expenditure.

Eating behaviour practices (EBP), considered contributory factors to EA, were explored using the validated Fairburn and Beglin (Fairburn & Beglin, 2008) Eating Disorder Examination Questionnaire (EDE-Q). The questionnaire assesses eating psychopathologic behaviour in the sub-scales of dietary restraint, eating concern, shape concern and weight concern on a seven point (0 – 6) severity score. 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 that feature. The questionnaire was administered during the same period when EI and EEE were being measured. Though the questionnaire was available in both, English and *Swahili,* the participant completed the questionnaire she found more comprehensible. This was done in the presence of the principal investigator who, as a native speaker of *Swahili,* was able to provide further clarification/explanation when necessary.

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) 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%). The International Society of Clinical Densitometry (ISCD) (Lewiecki et al., 2008) guidelines, requiring comparison of BMD against expected norms for age, sex and population/ethnic match, and their expression as *Z*-scores, were used for initial evaluations. In the ISCD guideline, a *Z*-score of less than -2.0 is considered the threshold below the expected limit for age, sex and population. However, since runners are expected to have better BMD than the general population, the American College of Sports Medicine (Nattiv, et al., 2007) cautions that athletes with slightly better BMD values than the ISCD threshold could be at risk of fracture and/osteoporosis in the future. Similarly, 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 lying below -1 were categorized further as having low BMD.

Menstrual function (MF) was based on a nine-month daily temperature-menstruation log (US Department of Health and Human Services, 2014) kept by the participant. 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 on waking up 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 form. 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 that denoted start of the next cycle. Only complete cycles within the nine months of actual recording were considered for establishing menstrual functional status. The status was

categorized as eumenorrheic (21 – 35 days), oligomenorrheic (35 – 90 days) and amenorrheic (>90days) (Nattiv et al., 2007). When determining menstrual function in profile of the TRIAD, oligomenorrhea and amenorrhea were grouped together as menstrual dysfunction (MD).

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. Analysis was done by participant category as athletes and non-athletes; and by distance category as long and middle distance athletes and non-athletes. Means and standard deviations were computed for EI, EEE, EBPs, carbohydrate (CHO) and calcium intakes. The Pearson correlation coefficient was conducted to determine level of statistical significant contributory association between EBPs and EA. Independent samples T-tests were used to determine significant difference in means of EI, EEE, EA, CHO and calcium intakes. An analysis of variance (ANOVA) was used to determine significant differences in the means of EI, EEE, and EA by distance categories of long and middle distance and non-athletes. A significant *F* value warranted use of Tukey's honestly significant difference (HSD) to establish specific inter-group differences. Frequencies and percentages of EA, MF and BMD were also determined. Though the three categories of menstrual function that emerged were ranked from eumenorrheic/normal status that descended to the lesser oligomenorrheic dysfunction to the most serious dysfunction of amenorrhea, oligomenorrhea and amenorrhea were grouped together as menstrual dysfunction in the TRIAD profile. The 0.05 α level was set to determine statistical significance.

Results

Demographic, anthropometric, and energy characteristics presented in Table 1 revealed significant differences between athletes and non-athletes in the demographic factors of weight (p<.001) and BMI (p<.001). As is evident from Table 1, whereas the non-athletes met the EER, the athletes with an EI of 2754.1 kcal were 59% short of the EER. The mean global psychopathological EBP score for the whole group stood at the 0.68. The Pearson correlation coefficient between EBPs and EA (r=0.142) was not significant (p=0.390), suggesting that psychopathological EBPs were not a contributory factor to the participants' low EA of 38.44 (±20.89) $kcal \cdot kgFFM^{-1} \cdot d^{-1}$. Overall prevalence of low EA was 69% (Athletes=23, Non-

athletes=4). Further scrutiny revealed that 62% of these had EA <45 kcal·kgFFM^{-1}·d^{-1}, 36% had EA between 30 and 45 kcal·kgFFM^{-1}·d^{-1}, and 56% had EA <30 kcal·kgFFM^{-1}·d^{-1}. The breakdown of CHO percentage constituting overall EI revealed no significant difference between the athletes' 73% (± 7%) and the non-athletes' 75% (±6%). The athletes' calcium intake was 723.3 (±438.8 mg·d), while the non-athletes' calcium was 792.1 (± 325.5) mg·d (t=.468;p=0.643). Frequencies and percentage distribution of the three components of the TRIAD by participant category and by distance category are presented in Table 2.

Table 1 Demographic, anthropometric, and energy characteristics of athletes and non-athletes

Characteristics	Athletes (n=25)	Non-Athletes (n=14)	p-value
Demographic			
Age (years)	25 ± 3.2	25 ± 3.2	.921
Age at Menarche (years)	15.8 ±2.0	14.1 ± 1.0	.005
Weight (kg)	49.8 ± 5.5	58.6 ± 5.8	<.001
Height(m)	1.62 ± 0.05	1.60 ± 0.04	.209
BMI (kg/m^2)	18.7 ± 1.3	22.8 ± 2.8	<.001
Fat Free Mass (FFM)(kg)	38.6 ± 3.8	38.6 ± 2.6	.971
Energy Related			
Estimated Energy Requirements (EER) (kcal)	4648.10 ± 224.63	2406.85 ±58.88	<.001
Energy Intake (kcal)	1894.0 ± 516.1	2258 ± 799.0	.091
Exercise Energy Expenditure (kcal·kgFFM^{-1}·d^{-1})	760.3 ± 222.1	78.14 ± 19.34	<.001
Energy Availability (kcal·kgFFM^{-1}·d^{-1})	28.1 ± 11.5	57.0 ± 21.4	<.001
Global EBP	.62±.56	.57±.66	.813

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

Menstrual function (Table 2) among the 39 participants as a group showed that 5% (non-athletes=2), were amenorrhoeic 31% (long distance=6, middle distance=4, non-athletes=2) were oligomenorrheic, and 64% (long distance=7, middle distance=8, non-athletes=10) were eumenorrheic. Out of the 44% (long distance=8, middle distance=7, non-athletes=2) who had reported primary amenorrhea, 21% (long distance=4, middle distance=4) revealed current oligomenorrheic status.

Results of BMD (Table 2) revealed that 80% (long distance=10, middle distance=9, non-athletes=12) of the 39 participants presented with low BMD. Characteristic of BMD by region (Table 3) did not show significant difference between athletes and non-athletes. Further breakdown of low BMD distribution by region showed that while there was 3% (non-athletes=1) presentation with low BMD at the neck of femur, the lumbar spine accounted for remaining the 97% low BMD and was distributed as 63% among the athletes (long distance=9, middle distance=10), and 37% (*n*=11) among non-athletes.

Table 2 Frequencies and percentage distribution of energy availability, menstrual function and bone mineral density (BMD) by participant category and by distance category

Characteristic	**Athletes (n=25)**	**Non-athletes (n=14)**	**Long Distance Athletes (n=13)**	**Middle Distance Athletes (n=12)**
Energy Availability				
≤30 kcal·kgFFM^{-1}·d^{-1}	14 (56%)	1 (7%)	11 (85%)	3 (25%)
>30 and ≤45 kcal·kgFFM^{-1}·d^{-1}	9 (36%)	3(21%)	1(8%)	8 (67%)
>45 kcal·kgFFM^{-1}·d^{-1}	2 (8%)	10 (71%)	1 (8%)	1 (8%)
Menstrual Function				
Eumenorrhea	15 (60%)	10 (71.4%0	7 (54%)	8 (67%)
Oligomenorrhea	10 (40%)	2 (14.3%)	6 (46%)	4 (33%)
Amenorrhea	0	2 (14.3%)	0	0
Primary Amenorrhea	15 (60%)	2 (14.3%)	8 (62%)	7 (58%)
Bone Mineral Density				
Low BMD	19 (76%)	12 (86%)	10 (7)	9 (75%)
Normal BMD	6 (24%)	2 (14.3%)	3 (23%)	3 (25%)

Table 3 BMD characteristics among athletes and non-athletes.

BMD Characteristic	Athletes (*n*=25)	Non-Athletes (*n*=14)	*p*-value
Lumbar Spine (L1-L4) (g/cm^2)	.945 ± .021	0.968 ± 0.3	.102
Neck of Left Proximal Femur (g/cm^2)	.945 ± .0731	.983 ± .0995.	.181
Total Body BMD (g/cm^2)	1.045 ± .584	1.016± .0710	.176
Z-score (g/cm^2)	1.24 ± 0.44	1.14 ± 0.40	.484

Note: p-value computed using independent t-test

Dysfunctions among the three components of the female athlete triad in dual combinations, and as the combined trio in the female athlete triad are presented in Table 4. Combination of low energy availability and menstrual dysfunction were seen in 15% (*n*=5) of the participants (long distance=3, middle=2, non-athletes=1). Low energy availability and low BMD revealed an overall 56% (*n*=14) presence, all among the athletes (long distance=6, middle distance=8). The combination of menstrual dysfunction and low BMD an 8% (*n*=3) overall presence (middle distance=1, non-athletes=2). Simultaneous dysfunctional presence of the three entities was seen in 10% (n=4) of the participants (long distance=3, middle distance=1). Out of the 39 participants, 4% (long distance=1) had healthy EA, MF and BMD.

Table 4 Prevalence of components in dual combinations and as the trio in the female athlete triad

Prevalence by components of the female athlete triad	Athletes (*n*=25)	Non-Athletes (*n*=14)	Long Distance Athletes (*n*=13)	Middle Distance Athletes (*n*=12)
Low energy availability	23 (92%)	4 (29%)	12 (92%)	11 (92%)
Menstrual dysfunction	10 (40%)	4 (29%)	6 (46%)	4 (33%)
Low bone mineral density	19 (76%)	3 (21%)	9 (69%)	10 (83%)
Low energy availability and menstrual dysfunction	5 (20%)	1 (7%)	3 (23%)	2 (17%)

Low energy availability and low bone mineral density	14 (56%)	0	6 (46%)	8 (67%)
Menstrual dysfunction and Low bone mineral density	1 (4%)	2 (14%)	0	1 (8%)
Low energy availability, menstrual dysfunction and low bone mineral density	4 (16%)	0	3 (24%)	1 (8%)
None	1 (4%)	6 (42%)	1 (8%)	0

The results of the t-test comparing the prevalence of the TRIAD components between athletes and non-athletes showed significant difference (t=5.860; p<.001). The one way ANOVA comparing long and middle distance athletes and non-athletes also indicated significant difference (F=16.708; p<.001). The Tukey's post-hoc HSD test identified non-athletes as being significantly different from the long and middle distance athletes. The hypothesized higher profile of the female athlete triad among athletes compared to non-athletes was accepted.

Discussion

Prevalence of the female athlete triad, based on both the previous entities of disordered eating, amenorrhea and osteoporosis, and the revised entities as low energy availability, menstrual dysfunction and low bone mineral density (Nattiv et al., 2007), has been reported as being between 0% and 16% (Gibbs et al., 2013). While the profile of the female athlete triad at 16% prevalence among the current elite Kenyan female athletes appears to concur with this report, the Kenyan athletes seem to be at the higher end of prevalence range. None of the Kenyan non-athletes experienced all three components simultaneously. It must however, be noted, that menstrual function in the current study did not include hormonal and steroidal assessment that prevented identification of subtle and sub-clinical menstrual dysfunctions. Consequently, the 16% prevalence of low EA and MD in the current participants may not be a true reflection of actual presence. Hoch et al. (2009) were among the few studies to have examined the TRIAD using the revised concept identified one (1%) soccer player and one (1%) sedentary control out of 80 high school athletes, and 80 sedentary controls with simultaneous presence of all three components (Hoch et al., 2009). However, participants in the Hoch study, drawn from varied

sports, were younger than those in the current study by about nine years. Elite Kenyan runners' showing of 16% simultaneous presence of all three components compares well with elite British endurance runners' showing of 15.9% presence (Pollock, Grogan, Perry, Pedlar, Cooke, Morrissey & Dimitriou, 2010). However, the dimension of energy in the British study was examined based on disordered eating. Torstveit and Sundgot-Borgen (2005) drew 186 athletes from the entire mixed-sports population of elite female athletes in Norway, aged between 13 and 39 years, and an age matched control group of 145 in Norway to determine existence of the TRIAD. They reported that both elite athletes (4%) and controls (4%) met criteria for all three components of the TRIAD. Beals and Hill (2006) also explored prevalence of disordered, menstrual dysfunction and low BMD varsity students aged about 19 years, and reported simultaneous presence of the three components in one cross-country runner and among two athletes in lean-build sports. The variance in population ages, mix of sports, and differences in definitions among the few studies that have examined prevalence of the TRIAD makes it difficult to compare the present study with others. Of concern should be that the over representation of chronic low EA among the elite Kenyan female athletes could lead to more serious health consequences such as endothelial dysfunction and unfavourable lipid profile (Rickenlund, Eriksson, Schenck-Gustfsson & Hirschberg., 2005) and osteoporosis (Nattiv et al., 2007).

It is possible that the three conditions of low EA, MD and low BMD of the TRIAD may not be present simultaneously at the extreme ends along respective health continuums. However, De Souza and Williams (2010) cautioned that because of the interrelatedness among the disorders, an athlete presenting with one condition, may be experiencing minor or subtle signs and symptoms in the other conditions. Low EA has been recognized as an instigator in MD (De Souza et al., 2008); and directly and indirectly in low BMD (Ihle & Loucks, 2004). The elite Kenyan athletes showed predominance of low EA, but an apparent menstrual status on the healthier side of the continuum. In this low EA and apparently healthy MF environment, elite Kenyan athletes showed 20% and non-athletes 23% prevalence in the combined components of low EA and MD, compared to the Hoch et al. (2009) study that reported 14% simultaneous presence of EA and MD among varied sports athletes, and 10% among sedentary controls. Torsveit and Sundgot-Borgen (2005) reported 26.9% in athletes and 13.8% prevalence in disordered eating/eating disorders and MD. Like Torsveit, Sundgot-Borgen, Quah, Poh, Ng. and Noor (2009) used disordered eating and reported that out of all the combinations explored by

them, at 24.1%, eating disorder and MD presented the highest occurrence. In contrast to the latter studies, results of the EBPs revealed that Kenyan participants were least likely to present with eating disorders. The negative difference between EER and EI among Kenyan athletes is probably more to do with inadvertent restriction or ignorance about ensuring adequate balance IE and energy expenditure (Pantano, 2009). Whereas independently low EA and low BMD showed prevalence of 92% and 76% among the athletes and 29% and 21% among the non-athletes, athletes were the only ones to show concurrent presence of 56% in low EA and low BMD. Comparatively, the much younger high school athletes (4%) and sedentary controls (5%) showed a lower simultaneous occurrence of low EA and low BMD (Hoch et al., 2009). Similarly, elite Malaysian athletes from various sports showed a much lower 9.4% presence in the same combination (Quah, 2009) compared to Kenyan athletes. Regardless of the difference in the energy dimensions evaluated, the considerably higher showing among Kenyan athletes is cause for concern for their bone health in the future. While oestrogen remains a key element in bone health, as cautioned by Ihle and Loucks (2004), chronic under-nutrition may affect bone impairment through factors that are independent of oestrogen. Though Kenyan non-athletes' EI met the EER, Kenyan athletes' EI fell short of the EER. Low EI relative to EEE usually results in low EA and concurrent deficiency in micronutrients such as calcium (American Dietetic Association [ADA], 2009). Calcium's significant role in bones structure and metabolic integrity cannot be underscored (Lorincz et al, 2009). Athletes with low EA and hypoestrogenism are advised to consume 1500mg·d^{-1} (ADA, 2009). It was clear that at 723.3 (±438.8 mg·d^{-1}) Kenyan athletes consumed approximately 48% less than the recommended amount.

Whereas simultaneous occurrence of MD and low BMD was seen at 4% in the athletes, at 14%, Kenyan non-athletes showed a higher occurrence of this combination. In exploring prevalence of the female athlete triad among elite Malaysian athletes, Quah et al. (2009) also considered simultaneous occurrence in the same combination of MD and low BMD. Compared to the current Kenyan athletes, elite Malaysian athletes showed an even lower occurrence of simultaneous presence of MD and low BMD (2009). The high school athletes, however, showed a slightly higher of 8% occurrence among athletes and a 4% lower showing among sedentary controls (2009). Neither study provides sufficient grounds for comparison. Though exact numbers for each sport were not indicated, the athletes were drawn from aesthetic sports, martial arts, fencing, archery, shooting, field hockey and squash. Apart from being much younger than the Kenyan participants, the high school athletes were also drawn from a wide range of sports,

making it difficult to compare appropriate findings. However, in the paucity of relevant comparisons, it is worth noting that hormonal and steroidal investigation reported lowered BMD especially at the lumbar spine in oligoamenorrheic endurance runners with deficient dietary intake (Gremion, Rizzoli, Slosman, Theintz & Bonjour, 2001). In view of the dominance of the lumbar spine as the weakest region among athletes and non-athletes in Kenya, there is need for further investigation of their hormonal and steroidal profiles.

Strengths and Limitations

Contributory factors to the strength of the study were the presence of an individually assigned trained RA from 5.30am till after the last meal to do the direct weighing of EI, measurement of EEE using objective accelerometry, and assessment of FFM using the gold standard DXA scanning. Apart from the principal investigator visiting the training centres at least once a month to meet with each participant individually, each RA also had bi-weekly mobile phone communication with her assigned participant. While motivating the participants, this communication also enhanced compliance.

However, conversion of activity counts per minute to estimate energy expenditure using accelerometry could be accompanied by errors in analysis. Though enthusiasm and compliance enhanced accuracy in determining start and finish of each menstrual cycle in the temperature-menstrual log, mistakes in temperature reading did not allow estimation of ovulatory cycles. This, combined with the lack of hormonal and steroidal assessments could be considered limitations in the study.

In conclusion, the hypothesized difference of a higher profile among athletes as compared to non-athletes was realized. Apart from the combination of MD and low BMD, athletes showed a higher presence among all other combinations. Despite the dominant low EA presentation, these elite Kenyan athletes showed an apparently healthy eumenorrheic menstrual status. In the absence of hormonal and steroidal evaluations, subtle sub-clinical dysfunctions of anovulation and LPD, usually associated low EA, were not identified. Therefore, this apparent healthy menstrual status may be an overestimation. The over representation of chronic low EA among the elite Kenyan female athletes could lead to more serious health consequences such as endothelial dysfunction and unfavourable lipid profile (Rickenlund, Eriksson, Schenck-Gustfsson & Hirschberg., 2005) and osteoporosis (Nattiv et al., 2007). While the results showed

almost negligible presence of psychopathological EBPs, Kenyan athletes should be encouraged to increase IE forcefully rather than waiting until hungry (Loucks, 2005). It is recommended that both athletes and their coaches be regularly educated about dietary and nutritional requirements specific to training and competition distance. Regular health screening should become a matter of policy for early identification of pathologies and timely implementation of appropriate intervention.

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CHAPTER 7 SUMMARY, CONCLUSIONS, LIMITATIONS, RECOMMENDATIONS AND FUTURE RESEARCH

7.1 SUMMARY

Chapter 1 provided the outline of the problem statement that expounded on the aims and objectives, and the hypothesis of this study. The objectives of this study that were set in chapter 1 were to: (1) determine the status of energy availability, menstrual function, bone mineral density in elite Kenyan runners; and; (2) determine the association between energy availability and menstrual functions among elite Kenyan athletes and non-athletes; (3) investigate relationship of energy availability and menstrual function to bone mineral density; and (4) determine the profile of the female athlete triad and in elite Kenyan athletes and non-athletes.

Chapter 2 reviewed relevant literature concerning the issues raised in the problem statement. The preceding literature review underscores energy availability as the key instigator in the Triad. The cascade of events in the Triad begins with inadvertent, unintentional or psychopathological restriction in dietary-nutritional intake relative to exercise energy expenditure (EEE) that could result in energy deficit or low energy availability. Chronic energy deficit restrains hypothalamic function and ovulation that manifests as FHA. The combination of dietary-nutritional-energy deficit and consequential hypoestrogenism impairs bone formation while increasing bone resorption. The ensuing bone demineralization lowers bone mineral density and ultimately results in debilitating osteoporosis. Prior to 2007, there was a tremendous amount of information generated under individual, two or all three entities that were thought to comprise the Triad - EA/DE, amenorrhea, and osteoporosis. As evident in the preceding reviewed literature, research since 2007, though less prolific, has attempted to examine the Triad under the current model of energy availability, menstrual function and bone mineral density along their respective health

continuums. The terms used throughout the present study are defined and clarified in Chapter 2 and also in the introduction of each article in the thesis. Relevant data were identified for use in respective representative articles in Chapters 3, 4, 5 and 6.

The thesis is submitted in article format, as approved by the Senate of the North-West University, and therefore consists of four articles (Chapters 3, 4, 5 and 6 respectively), each of which has been submitted for publication in peer-reviewed, accredited journals.

The investigation of the article representing Chapter 3 sought to establish the status of energy availability, menstrual function and bone mineral density, the three components that comprise the female athlete triad, in elite Kenyan female athletes, and in Kenyan non-athletes. The article was submitted for publication to the *British Journal of Sports Medicine (BJSM)*. There was no significant difference in EA between the long and middle distance runners, but significant differences were found between each athletic category and the non-athletes (Middle distance=33.3; Long Distance=23.3; Non-athletes=57.0 kcal/kgFFM^{-1}·d^{-1}, $p<.05$). Oligomenorrhea was present in 10 (40%) of the athletes and two (14.3%) non-athletes; and amenorrhea in two (14.3%) non-athletes. Low BMD was found in 19 (76%) of the athletes, whereas 12 (86%) non-athletes were identified as having low BMD.

The Chapter 4 article, seeking to determine association between energy availability and menstrual function in elite Kenyan runners, and non-athletes is published in the *African Journal for Physical, Health Education, Recreation and Dance (AJPHERD, June 2014, 20(2:1): 291-307)*. Results of menstrual dysfunction showed 40% oligomenorrhea presence in athletes and 14.3% in non-athletes; and 14.3% amenorrheic in non-athletes. None of the athletes were amenorrheic. The analysis 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.

The article representing Chapter 5 investigated relationships of both, energy availability and menstrual function to bone mineral density among elite Kenyan female athletes and among non-athletes; and was submitted to *BMC Public Health*. Spearman's correlation coefficient analysis showed a significant relationship between MF and BMD among the athletes as a combined group (*rho*=0.497; *p*=.011, and among middle distance athletes on their own (*rho*=0.632; *p*=.027). The

independent t-test showed significant difference in EEE (p<.001) and EA (p<.001) between athletes and non-athletes. ANOVA and Tukey's HSD comparing LD, MD and NA confirmed that the values of non-athletes are significantly different (p<.001) from LD and MD.

The article representing Chapter 6 explored the differences between the athletes and the non-athletes in simultaneous presence of the components of the female athlete triad. This article is published in the *African Journal for Physical, Health Education, Recreation and Dance (AJPHERD, June 2014, 20(2:2):610-625)*. Low EA (<45 kcal·kgFFM^{-1}·d^{-}1) was evident in 61.53% of the participants (athletes: 28.07 ±11.45 kcal·kgFFM^{-1}·d^{-}1, non-athletes: 56.97 ±21.38 kcal·kgFFM^{-1}·d^{-1}). The overall 36% MD seen among all participants was distributed as 40% among the athletes, and 29% among the non-athletes. None of the athletes were amenorrheic. Low BMD was seen in 79% of the participants (athletes: 76%, non-athletes:86%). Overall, 10% of the participants (athletes: 4, non-athletes: 0) showed simultaneous presence of all three components of the TRIAD. The Independent sample t-test showed significant difference (t=5.860; p<.001) in prevalence of the TRIAD between athletes and non-athletes.

7.2 CONCLUSIONS

The conclusions that are drawn from this thesis are presented in accordance with the hypotheses presented in Chapter 1.

7.2.1 Hypothesis 1 (Chapter 3): The status of energy availability, menstruation function, and bone mineral density would differ significantly between elite Kenyan female and non-athletes.

Hypothesis 1 is partially accepted based on the findings that no significant difference in energy availability between the long and middle distance runners, but significant differences were found between each athletic category and the non-athletes (Middle distance=33.3; Long Distance=23.3; Non-athletes=57.0 kcal·kgFFM^{-1}·d^{-1}, p<.05). Oligomenorrhea was present in 10 (40%) of the athletes and two (14.3%) non-athletes; and amenorrhea in two (14.3%) non-athletes. Low BMD was found in 19 (76%) of the athletes, whereas 12 (86%) non-athletes were identified as having low BMD.

7.2.2 Hypothesis 2 (Chapter 4): Significant association between energy availability and menstrual function would be found among elite Kenyan female athletes and non-athletes.

Hypothesis 2 is not accepted based on the research findings that neither the elite athletes nor the non-athletes showed any significant association between energy availability and menstrual function.

7.2.3 Hypothesis 3 (Chapter 5): Significant relationship of energy availability and menstrual function to bone mineral density and high prevalence of the TRIAD in elite Kenyan distance runners would be found.

Hypothesis 3 is partially accepted based on the following findings. The only significant relationship that could be found was between energy availability and bone mineral density; and between energy availability and the dimension of total bone mineral density among the athletes as long and middle distance runners. The athletes showed significant relationship between menstrual function and BMD Z-scores. In the case of non-athletes, the results did not reveal a significant relationship between menstrual function and bone mineral density, and hence, the hypothesis could not be accepted for them. The significant relationship between menstrual function and total body BMD among the combined group of athletes allowed the hypothesis to be accepted. The binary logistic regression revealed that in these Kenyan athletes and non-athletes menstrual function did not predict BMD.

7.2.4 Hypothesis 4 (Chapter 6): Kenyan female athletes would show significantly higher profile of the female athlete triad than the non-athletes.

Hypothesis 4 is partially accepted based on the research findings that overall, 10% of the participants (athletes: 4, non-athletes: 0) showed simultaneous presence of all three components of the TRIAD. The Independent sample t-test showed significant difference (t=5.860; p<.001) in prevalence of the TRIAD between athletes and non-athletes. Additionally, overall 36% MD seen among all participants was distributed as 40% among the athletes, and 29% among the non-athletes. None of the athletes were amenorrheic. Low BMD was seen in 79% of the participants (athletes: 76%, non-athletes:86%).

7.3 Limitations

The most glaring weakness of the study was the lack of direct hormonal and steroidal assessment that would have identified subtle sub-clinical menstrual dysfunctions such as luteal phase deficiency and anovulation. These assessments would have allowed confirmation of the true menstrual profile of the elite Kenyan athlete. Recording of daily temperature and characteristics associated with menstruation through a cycle enhanced motivation, enthusiasm, compliance and relative accuracy in documenting start and finish of each cycle. However, mistakes and inconsistencies in reading the thermometer also denied an opportunity for estimating ovulation. In the absence of both of these, the healthy menstrual profile among elite Kenyan runners may be an over-estimation. Although physical activity was assessed objectively with accelerometry, the conversion of activity counts to measure energy expenditure introduces some error in the analysis.

Despite these limitations this study provided important findings, both for the enhancement of theoretical knowledge on this topic especially from Kenyan female athletes and for practical implications.

7.4 Recommendation and future research

Chronic low energy availability that is potentially a high health risk factor for elite Kenyan female athletes in the long term could be addressed in the short term. To overcome the problem of high prevalence of low energy availability among elite Kenyan female runners, there is urgent need for athletes, coaches and athletes' significant others to be educated on individualized energy and nutritional intake requirements based on running distance. Since psychopathological eating behaviour was not a culprit in the high prevalence of low energy availability among elite Kenyan female athletes, it is recommended that Kenyan athletes increase energy intake by force instead of depending on appetite. This will increase energy availability which, in turn, would increase chances of ensuring ovulatory menstrual cycles. While carbohydrate intake by the athletes seems adequate, in view of the high prevalence of low bone mineral density, it is recommended that athletes increase intake of calcium rich foods to enhance their bone mineral density.

In the long term, it is recommended that comprehensive physical and clinical screenings become routine practice for all Kenyan athletes. This will allow identification of any under-lying pathology that could affect the health and performance of an athlete; and for implementation of intervention strategies in good time. Further, athletes and their coaches need to be regularly educated on meeting the dietary, nutritional and energy requirements specific to competition distance.

As one of the first studies concerning Kenyan female athletes, issues arising from this study warrant further investigation. It is recommended that future research includes direct hormonal and steroidal assessment when determining menstrual function. Further investigations are also recommended to determine the exact cause of low energy intake among athletes. The investigations should include Kenyan females from pre-pubescent to menopausal ages, participating competitively and recreationally in a variety of sports and physical activity. In view of the increasing number of Kenyan girls and women taking up physical activity, especially sports to alleviate poverty, there is an even greater need for comprehensive determination of energy availability and nutritional adequacy to meet their respective demands.

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APPENDICES

APPENDIX A

Guidelines for Authors

(a) British Journal of Sports Medicine

(b) African Journal for Physical, Health Education, Recreation and Dance

(c) BMC Public Health

(a) British Journal of Sports Medicine.

http://bjsm.bmj.com/site/about/guidelines.xhtml#original_reports.

Instructions for Authors

For guidelines on policy and submission across our journals, please click on the links below:
Manuscript preparation
Editorial policies
Patient consent forms
Licence forms
Peer review
Submission and production processes

Editorial policy

The British Journal of Sports Medicine (BJSM) aims to highlight clinically-relevant orginal research, editorials and commentary that will be of interest to the field of sport and exercise medicine. The journal is aimed at physicians, physiotherapists, exercise scientists and those involved in public policy.

Please note that references will be published online only; references should be provided as a separate data supplement.

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Article types and word counts

- Original reports
- Review articles
- Editorials
- Occasional piece / Analysis
- Education reviews
- Fillers
- Head to Head
- Preferred reviewers
- I-test - Sports medicine radiology/imaging
- Pictorial essay
- Supplements
- Plagiarism detection

The word count excludes the title page, abstract, tables, acknowledgements and contributions and the references.

Please note: Maximum word counts are strictly enforced and manuscripts that exceed these guidelines are usually rejected.

If you are not a native English speaker and would like assistance with your paper there is a professional editing service available.

BMJ's pre-submission checklist

Original reports

Papers should be a maximum of 3000 words in length (not including abstract, figure/table legends, references).

Abstracts should be a maximum of 250 words in length and structured as follows:

- Background/Aim
- Methods
- Results
- Conclusions

Please include a summary box summarising in 3-4 bullet points 'what are the new findings'. Peer reviewed by 2 external reviewers.

Review articles

Review articles should provide concise in-depth reviews of both established and new areas in sports medicine.

Systematic reviews
Systematic reviews provide level 1 evidence; they form a critical part of the literature. Here we provide some ground rules for SRs of interest in this journal. These guidelines are meant to inform authors but are not absolute.

Is the review of interest to our core readership?
BJSM is a clinical journal so the topic must have relevance and some application to clinical practice. Ask the key question "will the findings change what practitioners do?"

The scope of the question and review
Very specific questions and very broad questions may both have limited appeal. Those that ask and answer 'meaty' questions that reflect clinical issues have greater interest to BJSM readers.

Is the review worth the journal space?
Succinct and focussed reviews are always of more interest. Questions that are topical, novel or controversial that will attract readers and researchers to the journal will be more likely to be accepted.

Do the authors have broad knowledge in the topic area?
We are looking for experts to synthesise the literature and to comment on the outcomes of the review in a meaningful and clinically relevant way. The conclusion that 'more research is needed' does not add value for readers - it is uninformative.

So, after you consider these questions, please send in your SRs. We are open to amendments to these guidelines - contact us with your suggestions.
Please include a summary box summarising in 3-4 bullet points 'what are the new findings'.

Please provide 5 multiple choice questions (MCQs) each with 4-5 possible answers (only 1 correct answer), so the reader can test his or her understanding of the article. These MCQs will be published online only in the form of an E-learning module.

How to easily create multiple choice questions:

- Make the questions a positive single choice with only one correct answer
- Provide 4-5 answer options for each question
- The reader should be able to answer the questions need from the material provided in the article
- Problem orientated questions in form of a short case description are best

- Make sure that each question focuses only on one problem
- The answers you offer should be homogeneous: for example 5 diagnostic procedures, 5 therapeutic interventions
- Avoid options that contain vague terms such as "common," "often", "rare," "sometimes," and absolute statements such as "never" or "always"
- Avoid "all of the above" of none of the above
- Please give us an answer key for your questions! The correct answer with a short explanation for each answer
- Please check all your questions and answers carefully - do this with a colleague.

Word count: up to 4000 words (not including figure/table legends, references).
Peer reviewed by 2 external reviewers.

Editorials

These are written or commissioned by the editors, but suggestions for possible topics and authors are welcome.
Word count: a maximum of 1000 words (not including figure/table legends, references).
References: up to 10.
Peer reviewed by 2 reviewers who may be external or by the Editorial Board.

Occasional piece / Analysis

Contributions with a medical and sporting interest are welcomed. Papers should be a maximum of 2000 words in length (not including figure/table legends, references).

Education reviews

These are written or commissioned by the editors and should follow the proforma guidelines that will be supplied by the editorial office.
Peer reviewed by 2 external reviewers.

Fillers

We try to make the best use of every page of the printed BJSM, so we use small gaps to publish fillers. Most fillers have the added advantage of entertaining readers and making them think. If the filler refers to an identifiable person we will need written consent to publication from that person or a relative. We welcome articles of up to 400 words (we also like and need much shorter ones) on topics such as:

- Any other story conveying instruction, pathos or humour.

Head to Head

"Head to Head" provides BJSM readers with both sides of a clinically relevant "hot and topic". The case for each side should be made inside two pages (1400 words (not including figure/table legends, references), with a maxiumum of 10 references). "Head to Head" articles are solicited

but authors are also encouraged to submit ideas with 200 word outlines for each side to the editorial office

Preferred reviewers

Please suggest up to four reviewers who the editors can approach to review if needed. First name, last name, institution and email are required. You are required to suggest at least two reviewers, and preferably, at least half of the nominated reviewers should be from a country other than your own. Reviewer nominees from the same institution as any of the authors are not permitted.

I-test - Sports medicine radiology/imaging

I-tests aim to provide readers with a succinct imaging-based educational opportunity in a clinical context familiar to a sports medicine readership. The main thrust of the article is the diagnosis of the condition through imaging; however, the clinical presentation should be addressed as well as basic aspects of treatment (surgical or otherwise). The specific role of imaging in the diagnosis and management of the condition should be highlighted.

The "question" part of the I-test should comprise a short description of the clinical presentation (< 200 words) accompanied by up to 3 images; the "answer" should include a discussion of the clinical, imaging and management issues (< 1200 words), supplemented by up to 3 additional images and 8 references.

The "question" and "answer" parts should be submitted online as a single article following the standard formats.

Pictorial essay

Pictorial essays are educational articles that are extensively illustrated (radiographs, ultrasound, CT, MRI, etc) with limited text. The teaching points and educational goals should be given as bullet points at the beginning of the article. Articles must be accompanied by five multiple choice questions, which can be answered by reading the article and supported by the cited references. Video images (eg, AVI files of dynamic ultrasound examinations) are encouraged to enhance the article on-line.

The article should be submitted online as a single article following the standard formats. The multiple choice questions and answers should be submitted online as a supplementary file.

Article format

- Educational goals/teaching points (bullet points)
- Introduction < 250 words describing the clinical context of pictorial essay
- Main text < 1000 words
- Up to 30 figure parts and supporting legends
- Up to 15 references

Please provide 5 multiple choice questions (MCQs) each with 4-5 possible answers (only 1 correct answer), so the reader can test his or her understanding of the article. These MCQs will be published online only in the form of an E-learning module.

How to easily create multiple choice questions:

- Make the questions a positive single choice with only one correct answer
- Provide 4-5 answer options for each question
- The reader should be able to answer the questions need from the material provided in the article
- Problem orientated questions in form of a short case description are best
- Make sure that each question focuses only on one problem
- The answers you offer should be homogeneous: for example 5 diagnostic procedures, 5 therapeutic interventions
- Avoid options that contain vague terms such as "common," "often", "rare," "sometimes," and absolute statements such as "never" or "always"
- Avoid "all of the above" of none of the above
- Please give us an answer key for your questions! The correct answer with a short explanation for each answer
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(b) African Journal for Physical, Health Education, Recreation and Dance

http://reference.sabinet.co.za/documents/journal_documents/guidelines/11174315.pdf

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iii) create an awareness in the rest of the world about the professional practice in the disciplines in Africa.

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- Authors' contributions
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- Acknowledgements
- Endnotes
- References

- Illustrations and figures (if any)
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The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

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Three to ten keywords representing the main content of the article.

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The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

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The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

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The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

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According to ICMJE guidelines, An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; 3) have given final approval of the version to be published; and 4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
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Examples of the *BMC Public Health* reference style are shown below. Please ensure that the reference style is followed precisely; if the references are not in the correct style they may have to be retyped and carefully proofread.

All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, in the following format: **The Mouse Tumor Biology Database** [http://tumor.informatics.jax.org/mtbwi/index.do]. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Examples of the *BMC Public Health* reference style

Article within a journal

Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996,**13:**266-267.

Article within a journal supplement

Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999,**43**(Suppl 3):149-170.

In press article

Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract

Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings

Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference*

on Porous Sieves: 27-30 June 1996; Baltimore. Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book

Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal

Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings

Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book

Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series

Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.*Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology*, vol 1.]

Book with institutional author

Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis

Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL

The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)

Corpas M: **The Crowdfunding Genome Project: a personal genomics community with open source values** [http://blogs.biomedcentral.com/bmcblog/2012/07/16/the-crowdfunding-genome-project-a-personal-genomics-community-with-open-source-values/]

Dataset with persistent identifier

Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): **Genome data from sweet and grain sorghum (Sorghum bicolor)**.*GigaScience*. http://dx.doi.org/10.5524/100012.

Clinical trial registration record with persistent identifier
Mendelow, AD (2006): **Surgical Trial in Lobar Intracerebral Haemorrhage**. Current Controlled Trials. http://dx.doi.org/10.1186/ISRCTN22153967

Preparing illustrations and figures

Illustrations should be provided as separate files, not embedded in the text file. Each figure should include a single illustration and should fit on a single page in portrait format. If a figure consists of separate parts, it is important that a single composite illustration file be submitted which contains all parts of the figure. There is no charge for the use of color figures.

Please read our figure preparation guidelines for detailed instructions on maximising the quality of your figures.

Formats

The following file formats can be accepted:

- PDF (preferred format for diagrams)
- DOCX/DOC (single page only)
- PPTX/PPT (single slide only)
- EPS
- PNG (preferred format for photos or images)
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- JPEG
- BMP

Figure legends

The legends should be included in the main manuscript text file at the end of the document, rather than being a part of the figure file. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals - i.e. Figure 1, 2, 3 etc); short title of figure (maximum 15 words); detailed legend, up to 300 words.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures or tables that have previously been published elsewhere.

Preparing tables

Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order.

Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell display as black lines. Commas should not be used to indicate numerical values. Color and shading may not be used; parts of the table can be highlighted using symbols or bold text, the meaning of which should be explained in a table legend. Tables should not be embedded as figures or spreadsheet files.

Larger datasets or tables too wide for a portrait page can be uploaded separately as additional files. Additional files will not be displayed in the final, laid-out PDF of the article, but a link will be provided to the files as supplied by the author.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). As with all files, please use the standard file extensions.

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Although *BMC Public Health* does not restrict the length and quantity of data included in an article, we encourage authors to provide datasets, tables, movies, or other information as additional files.
Please note: All Additional files **will be published** along with the article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files should be sent by email to editorial@biomedcentral.com, quoting the Manuscript ID number.
Results that would otherwise be indicated as "data not shown" can and should be included as additional files. Since many weblinks and URLs rapidly become broken, *BMC Public Health* requires that supporting data are included as additional files, or deposited in a recognized

repository. Please do not link to data on a personal/departmental website. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.
Additional files can be in any format, and will be downloadable from the final published article as supplied by the author. We recommend CSV rather than PDF for tabular data.

Certain supported files formats are recognized and can be displayed to the user in the browser. These include most movie formats (for users with the Quicktime plugin), mini-websites prepared according to our guidelines, chemical structure files (MOL, PDB), geographic data files (KML).

If additional material is provided, please list the following information in a separate section of the manuscript text:

- File name (e.g. Additional file 1)
- File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

Additional file formats

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

- Additional documentation
- PDF (Adode Acrobat)
- Animations
- SWF (Shockwave Flash)
- Movies
- MP4 (MPEG 4)
- MOV (Quicktime)
- Tabular data
- XLS, XLSX (Excel Spreadsheet)
- CSV (Comma separated values)

As with figure files, files should be given the standard file extensions.

Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root.
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders.
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

Style and language

General

Currently, *BMC Public Health* can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise.

BMC Public Health will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Language editing

For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz. BioMed Central has arranged a 10% discount to the fee charged to BioMed Central authors by Edanz. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication. Please contact Edanz directly to make arrangements for editing, and for pricing and payment details.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on Writing titles and abstracts for scientific articles.

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. American Scientist also provides a list of resources for science writing. For more detailed guidance on preparing a manuscript and writing in English, please visit the BioMed Central author academy.

Abbreviations

Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All lines and pages should be numbered. Authors are asked to ensure that line numbering is included in the main text file of their manuscript at the time of submission to facilitate peer-review. Once a manuscript has been accepted, line numbering should be removed from the manuscript before publication. For authors submitting their manuscript in Microsoft Word please do not insert page breaks in your manuscript to ensure page numbering is consistent between your text file and the PDF generated from your submission and used in the review process.
- Use the *BMC Public Health* reference format.
- Footnotes are not allowed, but endnotes are permitted.
- Please do not format the text in multiple columns.
- Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. **Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.**

Units

SI units should be used throughout (liter and molar are permitted, however).

APPENDIX B:

LETTERS AND COMMUNICATIONS

(a) Ethical Approval

(b) Research Authorisation

(c) Athletics Kenya

(d) National Olympic Committee, Kenya

(a)

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12

Tel: 8710901/12

Our Ref: KU/R/COMM/51/33

Date: April 18th, 2012

Yasmin Goodwin,
Dpt. of Physical and Health Education,
Kenyatta University.

Dear Yasmin,

APPLICATION NUMBER PKU010/E02 OF 2011 - 'THE FEMALE ATHLETE TRIAD PROFILE OF ELITE KENYAN RUNNERS AND ITS FUTURE HEALTH IMPLICATIONS'. VERSION 2.

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic 'THE FEMALE ATHLETE TRIAD PROFILE OF ELITE KENYAN RUNNERS AND ITS FUTURE HEALTH IMPLICATIONS', version 2, dated 29th March 2012.

2. APPLICANT

Yasmin Goodwin,
Dpt. of Physical and Health Education,
Kenyatta University.

3. SITE

Athletics training camps around Kenya and Nairobi.

4. DECISION REACHED.

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

(i) Scientific design and conduct of study,

(ii) Recruitment of research participant,

(iii) Care and protection of research participants,

(iv) Protection of research participant's confidentiality,

(v) Informed consent process,

vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 18th April, 2012.

Note: This approval supersedes the one dated October 12th, 2011

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Clearance must be obtained for transportation of any biological material out of the country i.e. Kenya.

iv. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

v. Submit to KU-ERC in writing the dosage of radiation to be exposed to participants.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I YASMIN GOODWIN accept the advice given and will fulfill the conditions therein.

Signature Goodwin Dated this day of 19TH APRIL 2012.

cc. Vice-Chancellor
Director: Institute for Research Science and Technology

(b)

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams:"SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123.
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RRI/12/1/SS011/1187**

Date: **26th August, 2011**

Goodwin Yasmin
Kenyatta University
P.O BOX 43844
NAIROBI

RE:RESEARCH AUTHORIZATION

Following your application for authority to carry out research on; **The female athlete triad profile of elite Kenyan Runners and its future health implications,** I am pleased to inform you that you have been authorized to undertake research in **all Districts,** Kenya for a period ending **30th August 2013**

You are advised to report to **The District Commissioner, The District Education Officer & The District Medical Officer of Health,** before embarking on the research project.

On completion of your research project you are advised to submit **one hard copy and one soft copy** of your thesis/ project to this office.

P.N NYAKUNDI
FOR: SECRETARY/CEO
Copy to:

The District Commissioners

The District Education Officer

The District Medical Officer of Health

(c)

Affiliated to I.A.A.F, A.A.C., N.O.C.K, K.N.S.C.
Riadha House, Aerodrome Road, Nairobi West, P.O. Box 46722-00100 G.P.O. Nairobi, Kenya
Tel: +254 (20) 6005021 Fax: +254 (20) 6005021, 0736 747217
Email: athleticskenya@wananchi.com

ATHLETICS KENYA

AK/ADM/VOL.16/2011 Monday, May 23, 2011

Mrs. Yasmin Goodwin
P.O. Box 16754-00620
NAIROBI

Dear Yasmin,

RE: SUPPORT FOR STUDY ON KENYAN FEMALE ATHLETES

This is in reference to your letter dated 17th March, 2011 addressed to Mr. Isaiah Kiplagat, Chairman, Athletics Kenya on the matter.

After consultation with Mr. Kiplagat, we have agreed that you can go ahead with the research after which we expect you to furnish us with the findings on same.

We advise that you get in touch with Mrs. Mary Chege, the Chairlady of the Athletics Kenya Women's Sub Committee who will assist you in the mobilization and co-ordination of the women athletes you may need. Her mobile contact is 0722513834.

However and due to financial constraints, we regret to inform you that Athletics Kenya will not be in a position to offer any financial support towards this project.

We wish you success in your research.

Yours Sincerely,

DAVID S. OKEYO, HSC
SECRETARY GENERAL

C.c.
Mr. Isaiah Kiplagat
Mrs. Mary Chege – Please assist when called upon.

(d)

National Olympic Committee - Kenya

Olympic House
Kenya Road, Upper Hill
P.O. Box 46888, 00100, G.P.O
Nairobi, Kenya

Tel: +254-020- 2712347
+254-020-2738109
Fax: +254 -020 -2712343
Email: nock@iconnect.co.ke
Website: www.olympickenya.org

June 8, 2011

Mrs. Yasmin Goodwin
Mobile Plaza, Muthaiga
P.O. Box 16754 – 00620
NAIROBI
Mobile: 0733 935 594
E-mail: jygoodwin@gmail.com

Dear Yasmin,

We were delighted to make acquaintance with you and are especially pleased by your interest in the health of our female athletes. Your research is promising and has potential to benefit their well being. I would also like to acknowledge receipt of your letter of introduction.

As discussed during our brief encounter, NOC-K is unfortunately not in a position to lender financial support towards your research. However, we would be happy to offer any other assistance you may require and is within our means.

We wish you all the best in this endeavor,

Kind Regards

Francis K. Paul
Secretary General.

APPENDIX: C

(a) INFORMATION LEAFLET

(b) INFORMED CONSENT FORM

(a) INFORMATION LEAFLET

PROJECT TITLE: THE FEMALE ATHLETE TRIAD PROFILE OF ELITE KENYAN RUNNERS AND ITS FUTURE HEALTH IMPLICATIONS

Primary Investigator: Mrs. Yasmin Goodwin, M.Sc. (Physical Education)

Study Leader: Prof. A Monyeki, Ph.D.

Co-supervisor: Prof. Hans de Ridder, Ph.D

Co-supervisor: Prof. A. L. Toriola, Ph.D

Co-supervisor: Prof. Michael K Boit, Ph.D

Dear Athlete,

You are invited to participate in a research study that forms part of my formal Ph.D. studies. This information leaflet will help you to decide it you would like to participate. Before you agree to take part, you should fully understand what is involved. You should not agree to take part unless you are completely satisfied with all aspects of the study.

WHAT IS THE STUDY ALL ABOUT?

You and other Kenyan female runners have achieved phenomenal success over the past few years. To continue this successful trend, it is important to establish the status of factors that might pose a threat to your health, performance, and ultimately, your ability to continue earning a living from running.

Women, who are physically active, could suffer from a condition called the female athlete triad (FAT). Lack of energy, disturbance in monthly periods, and weak or brittle bones are the three components that make up the triad. In addition to this, female athletes could also suffer from lack of iron in the blood.

Very little information concerning problems faced by Kenyan female runners has been gathered. Because of possible far reaching health and performance consequences of the Triad and lack of iron on female runners, this study intends to establish their status and to determine whether this status might affect your health in the future.

WHAT WILL YOU BE REQUIRED TO DO IN THE STUDY?

If you decide to take part in the study, you will be required to do the following:

- To sign this informed consent form

- To complete a personal information questionnaire which will ask about your date of birth, the age when you had your first monthly period, the schools that you have attended, how far were the schools from where you lived or were you a boarder at the school, the type of physical activity that you did at school and at home as a school girl, the age when you started training specifically for running competitions, regularity of your menses over the past 12 months, if you have experienced any injuries during the past 12 months and whether you use hormonal contraceptives. The questionnaire will be done any time over the three days when the research team will be at your training camp to collect most of the information. This will take about five to ten minutes to complete.

- To keep a daily record of your menstrual characteristics and a record of your monthly menstrual cycles on a chart for nine consecutive months. This will include measuring and recording your temperature every morning on waking. You will be provided with a thermometer for this purpose. This should take you about five minutes each morning.

- To complete a food diary after weighing all food items, confectionaries, and fluids every time you consume them over three consecutive days. This will be done at your training centre or at your home with the assistance of research assistants, who will be especially trained for this study. She will have access to a small digital scale to weigh and record every item you eat or drink. At the end of eating, every left-over item will have to be weighed and recorded to calculate your actual consumption. You will also be required to keep records in the same way of all items which you consume when your assigned research assistant is not with you, and she will weight-match these items for recording against your intake for the appropriate day.The procedure for weighing the items is described below:

  <u>Procedure for Weighing the Items</u>

  1. Zero the scale, put your empty plate, cup or bowl on the scale and zero the scale again.
  2. Record each item on a new line and give a full description of each item:
     e.g. Ugali (maize meal boiled)
     Sukuma wiki (shallow fried with Elianto)
     Goat Meat (stewed)
     White milk bread
     Chapati (made with Kimbo)
     Egg (fried in Chipsi)
  3. Add the first item to the plate and record the weight. Zero the scale, add the second item to the plate, and record the weight of the second item. Continue like this until all the items have been weighed and recorded.
  4. Remember to record all fluids consumed indicating type of fluid and the amount.
  5. As many pages as needed may be used to keep these records; **however,** please that each day starts on a new page.

This process will take about 5 minutes at each meal time over the three days.

- To wear a light-weight non-interfering activity monitoring device for three and a half days. On the first half day, you will be familiarized on how to attach and detach the device on your preferred hip and thereafter, you will wear the device for three continuous days and nights (except when showering/bathing or if swimming). It is important that you keep it on continuously, snugly against your body throughout the three days and nights. After the last night of wear, it will be collected for downloading.
- To complete a questionnaire about your eating practices. This questionnaire is a screening tool recommended by the International Olympic Committee to determine eating practices that might lead to energy deficiency. This will be done at your training camp any time over the three days when the research team will be at your training camp. It will take about 20 minutes to complete.

- To have your height and weight measured. This will be done during the same three days when the research team will be with you at the training camp collecting information about energy intake, energy expenditure, and eating behavioural practices. To do these measurements, you will be required to present yourself in bare feet and dressed in your running shorts and 'T' shirt for the measurement of your standing height and weight. The measurement will be done in a secluded room by a trained researcher. This will take about five minutes.

- To make your own travel arrangements to come to Nairobi for blood analysis and the DXA scan that will measure your lean body mass, fat mass and bone mineral density. However, you will be compensated as explained later in this leaflet.

- To provide the researcher with a selection of dates when it will be convenient for you to come to Nairobi. This will be done after completing your height, weight, skinfold, food intake and energy expenditure measurements and eating and dietary habits questionnaires. Based on the selection of dates provided by you, the researcher will make an appointment for you at the Aga Khan University Hospital for the DXA scan on one of these dates. **It is very important to keep the date and time.** Arrangements will be made for you to stay at a hotel for two nights. After a night's rest, transport will be provided for you to go to the Aga Khan University Hospital and the PathCare Kenya laboratory in Nairobi.

- To provide one blood sample at the Pathcare Kenya Laboratory. The blood sample of 2 to 4 ml for haemoglobin concentration will be taken via venipunctures from the vein in the fold at the elbow.

  To have your body composition assessed using the dual energy X-ray absorptiometry (DXA) at the Aga Khan University Hospital in Nairobi under the direction and supervision of the head of the hospital's nuclear medicine. On the day of the scan, you can eat normally; but do not take any calcium supplement for at least 24 hours before the test. Be dressed in comfortable clothing which does not have any zips, belts or buttons made of metal; and neither should you have any other metallic objects like keys, jewellery, eye glasses, dental braces, money, or purses with buckles on you. **It is important that you inform the doctor and the technologist carrying out the scan if you suspect or know that you are pregnant. This should be done immediately on your arrival at the Nuclear Medicine Centre.** During the scan, you will be required to lie on a padded table. An x-ray generator will be located below your hip region, and an imaging device will be positioned above. To assess the lumbar spine region, your legs will be supported on a padded box to flatten the pelvis and lumbar spine. To assess the hip, your foot will be placed in a brace that rotates the hip inwards. In both cases, the imaging device will be slowly passed over the area. You will be required to stay very still; and you may be asked to hold your breath for a few seconds every time an x-ray picture is taken. Do not get concerned if the technician walks behind a wall or goes into the next room to activate the machine. The test may take anywhere between 10 to 30 minutes.

ARE THERE ANY CONDITIONS THAT MAY EXCLUDE YOU FROM THE STUDY?

You will not be eligible to participate in the study if you are younger than 18 years, older than 30 years or if you are pregnant. You will not be eligible to continue participating in the study if you become pregnant during the study. In addition to these, non-athletes will also be excluded if the medical report indicates that she is not medically fit to participate in the study.

CAN ANY OF THE STUDY PROCEDURES RESULT IN PERSONAL RISK, DISCONFORT OR INVONVENIENCE?

Questionnaires: Due to the personal and intimate nature of some of the questions, you may experience some embarrassment or shyness.

Height, weight and skinfold measurements: Though you will be minimally dressed, the research assistants involved have been trained to ensure preservation of your modesty.

Venipunctures: The main risk involved with performing venipunctures is bruising (hematoma) and/or infection at the site where the needle is inserted. Standard aseptic techniques will be used at all times to avoid the risk of infection. You may feel a small pin prick or discomfort. Blood will be drawn by a trained laboratory technician (phlebotomist).

DXA scans: The amount of radiation dosage poses no risk. However, as indicated before, should you suspect or know that you are pregnant, you must inform the doctor and the technician. If this is your first time to have an x-ray, you may find the machines and loneliness a little intimidating.

WHAT ARE THE POTENTIAL BENEFITS THAT MAY COME FROM THE STUDY?

The benefits of participating in the study are:

- You will make a contribution towards establishing a profile of the female athlete triad of elite Kenyan runners
- Your contribution will help you, your coach and those involved in your training in making decisions about your training based on information acquired through scientific methods
- You will receive written results and evaluations of your status based on the tests
- You will make valuable contribution towards filling some information-gaps concerning Kenyan female runners.

WILL YOU RECEIVE ANY FINANCIAL COMPENSATION OR INCENTIVE FOR PARTICIPATING IN THE STUDY?

Please note that though you will not be paid for participating in the study, you will receive a small token of appreciation when you have completed **all** the requirements in the study. However, based on current fares on public transport and upon production of an official receipt from the transport company, you will receive compensation for transport expenses to Nairobi and back home when you come for the DXA scan at the Aga Khan University Hospital and blood analysis at PathCare Kenya. If you use your own transport, you will get the same amount as you would have spent on public transport. You will be given a small allowance for your meals/refreshments during the journey. You will also get full-board accommodation for the two-night's stay in Nairobi.

WHAT ARE YOUR RIGHTS AS A PARTICIPANT IN THIS STUDY?

Your participation in the study will be entirely on voluntary basis. You will have the right to withdraw from the study at any stage without any penalty or loss of favour without disclosing your reasons for withdrawing. However, you may be asked to withdraw from the study if you fail to adhere to the requirements of the study.

HOW WILL CONFIDNETIALITY AND ANONYMITY BE ENSURED IN THE STUDY?

You are assured that all information regarding you gathered during the study will be handled in strictest confidence. Your identity will not be revealed during or after the study, nor when the study is published or used in any format. All data sheets that will be collected will be stored in a secure place. All identification on any data sheet will be removed or masked. All information relating to you will be coded.

IS THE RESEARCHER QUALIFIED TO CARRY OUT THE STUDY?

As a holder of Master of Science degree (Springfield College, Massachussetts, USA) and having been a lecturer in the Department of Physical and Health Education (formerly Exercise, Recreation and Sports Science) at Kenyatta University, Nairobi, Kenya, the researcher is adequately trained to carry out the study. The research assistants in the areas that do not require invasive or laboratory expertise will be female students, tutorial fellows and lecturers from the Departments of Physical Education and Health, and Recreation Management and Exercise Science at Kenyatta University. Qualified and experienced laboratory technicians from Pathcare Kenya Limited, a fully accredited diagnostic laboratory by the South African National Accreditation System (SANAS) will do all blood analysis; while the body composition will be evaluated by the head of Nuclear Medicine at the Aga Khan University Hospital, Nairobi, Kenya.

HAS THE STUDY RECEIVED ETHICAL APPROVAL?

Yes. All the relevant departments at North-West University in South Africa have approved the formal study proposal. The Ministry of Higher Education (Kenya) has granted a research permit. Ethical approval has been obtained from the Ethics Boards in Kenya through Kenyatta University and through North-West University in South Africa.

WHO CAN YOU CONTACT FOR ADDITIONAL INFORMATION REGARDING THE STUDY?

Should you require any further information regarding this study, you can contact any of the following:

Primary Investigator:	Mrs. Yasmin Goodwin	Mobile Contact: 0733 935 594
	Prof. Prof. A. Monyeki, Ph.D	Mobile Contact: +278 264 44342
	Prof. Hans de Ridder, Ph.D	Email: hans.deRidder@nwu.ac.za
	Prof. A.L.Toriola, Ph.D	Mobile Contact: +278 297 45836
	Prof. M.K.Boit, Ph.D	Mobile Contact: 0722794167
Ethics:	Prof. N.K.Gikonyo, Ph.D	Telephone: Nairobi 8710901/12

DECLARATION: CONFLICT OF INTEREST

There is no conflict of interest that may influence procedures, data collection, data analysis and publication of results of this study.

A FINAL WORD

Your co-operation and participation in the study will be greatly appreciated. Please sign at the end of informed consent form below if you agree to participation in the study. In such a case, you will receive a copy of the signed informed consent from the researcher.

(B) INFORMED CONSENT FORM

I hereby confirm that I have been adequately informed by the researcher about the nature, conduct, benefits and risks of the study. I have also received, read and understood the above written information. I am aware that the results of the study will be anonymously processed into a research report. I understand that my participation is voluntary and that I may, at any stage, without prejudice, withdraw my consent and participation in the study. I had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Research participant's name:_____(Please print)

Research participant's signature:_____

Date:_____

Researcher's name:_____(Please print)

Researcher's signature:_____

Date:_____

VERBAL CONSENT

I hereby declare that I have read and explained the contents of the information sheet to the research participant. The nature and purpose of the study were explained, as well as the possible risks and benefits of the study. The research participant has clearly indicated that she is aware of the right to withdraw from the study at any time, for any reason and without jeopardizing her relationship with the research team. I hereby certify that the research participant has verbally agreed to participate in this study.

Research participant's name:_____(Please print)

Researcher's name:_____(Please print)

Researcher's signature:_____

Date:_____

APPENDIX D

DATA FORMS

(a) Personal Information

(b) Menstrual Characteristics' and Patterns' Questionnaire

(c) Temperature-Menstrual Cycle Log/Diary

(d) Three-day Food Diary

(e) GT3X+ Daily Energy Expenditure Output

(f) GT3X+ Graphic Daily Energy Expenditure Output

(g) Eating Disorder Examination Questionnaire (EDE-Q)

(h) Anthropometric Measurements and Dual Energy X-ray Absorptiometry

(a) Personal Information

Name:……Study Code:……Phone Contact……

Date of Birth……..

How long did it take you to get to primary school?.....Minutes

How did you get to the school?

.....

How long did it take you to get to secondary school?.....Minutes

How did you get to school?

…….

When did you start participating in sports? Year……..

When did you start training for running competitions? Year…….

As a student, apart from sports, what other physical activities did you do at home or in the community? (e.g. washing clothes, fetching water, herding life stock)……...……
……

(b) Menstrual Characteristics and Menstrual Patterns Questionnaire

How old were you when you got your first monthly period (menarche)? ……. Which year?.....

During the last 12 months, how many times have you had a monthly period?.....

On average, how often do you menstruate? (Number of days between each cycle)…….

Outline any signs or symptoms that you experience before, during or after your menstrual period.
Before:……During:…… After:……..

Do you have any children?.....How many?.....

Do you use any contraceptive?.....

If yes, please indicate: Type (e.g. oral/pill, injection):……...

Outline any injuries that have prevented you from training or competing in the past 12 months.……..……

(c) Temperature-Menstrual Cycle Log/Diary

Code:……..

Day	Month	Temperature	SYMPTOMS	Month	Temperature	SYMPTOMS
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						

(d) Three-day Food Diary

Procedure for Weighing the Items

1. Zero the scale, put your empty plate, cup or bowl on the scale and zero the scale again.
2. Record each item on a new line and give a full description of each item:
   e.g. Ugali (maize meal boiled)
   Sukuma wiki (shallow fried with Elianto)
   Goat Meat (stewed)
   White milk bread
   Chapati (made with Kimbo)
   Egg (fried in Kimbo)
   Fruit (banana, pineapple)
3. Add the first item to the plate and record the weight. Zero the scale, add the second item to the plate, and record the weight of the second item. You continue like this until all the items have been weighed and recorded.
4. Remember to record all fluids consumed indicating type of fluid and amount.
5. You may use as many pages as needed to keep these records; however, please start a new day on a new page.

Daily Food Diary

Participant's Code……

Date:……..

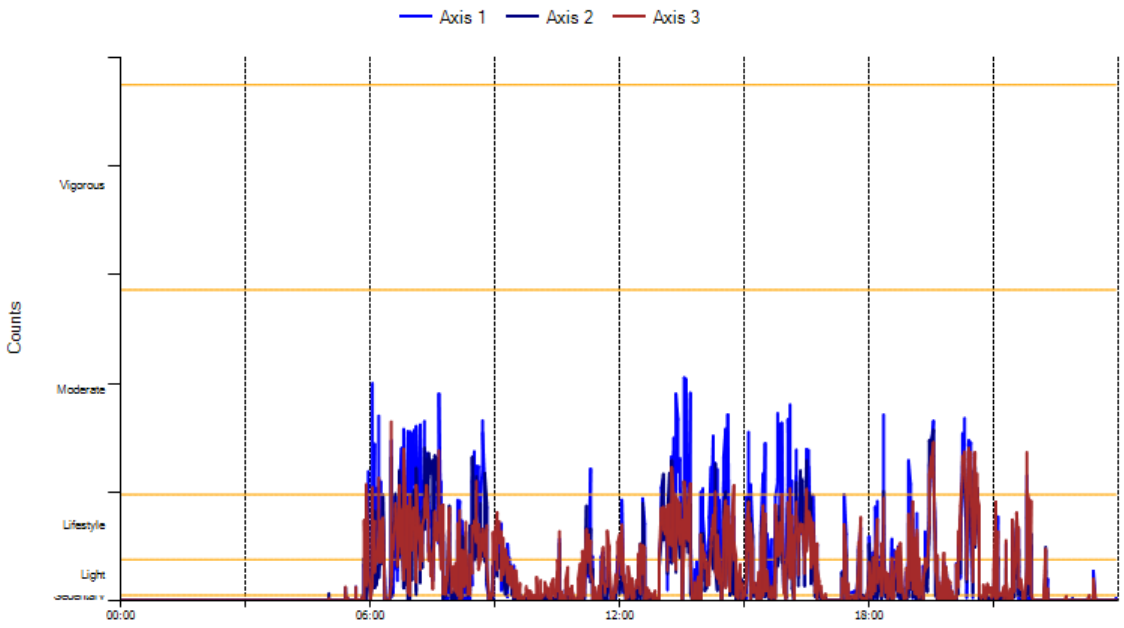
TIME	DESCRIPTION OF FOOD OR FLUID	WEIGHT	LEFT OVER WEIGHT	LEAVE BLANK

(e) GT3X+ Daily Energy Expenditure Data Output

Date	Hour	Activity kCals	Total Axis 1 Counts	Total Axis 2 Counts	Total Axis 3 Counts	Total Vector Magnitude Counts	Total Steps
23/10/2012	5:00	9.159861	7849	5551	8083	12560.07	244
23/10/2012	6:00	90.08617	77194	52034	58131	109752.7	1346
23/10/2012	7:00	108.5821	93043	83673	65794	141375.5	2340
23/10/2012	8:00	71.68826	61429	44298	44774	87980.37	1290
23/10/2012	9:00	21.79391	18675	16514	26499	36382.2	774
23/10/2012	10:00	5.354242	4588	3501	10591	12061.34	325
23/10/2012	11:00	22.29106	19101	14169	25112	34586.43	668
23/10/2012	12:00	17.73272	15195	11522	20026	27652.94	692
23/10/2012	13:00	107.2727	91921	59372	64000	126769.5	2156
23/10/2012	14:00	75.94084	65073	39399	49062	90519.92	1533
23/10/2012	15:00	71.53771	61300	32782	35732	78160.89	1463
23/10/2012	16:00	77.31908	66254	51400	51162	98229.85	2065
23/10/2012	17:00	12.50451	10715	8236	9121	16304.46	270
23/10/2012	18:00	37.89982	32476	17653	29481	47280.53	836
23/10/2012	19:00	49.64461	42540	35103	42835	69833.44	1394
23/10/2012	20:00	57.25001	49057	48375	61672	92467.1	1813
23/10/2012	21:00	24.26914	20796	19847	30563	41958	474
23/10/2012	22:00	2.269834	1945	1810	2269	3493.921	76
23/10/2012	23:00	0.786565	674	169	505	858.9889	8

(f) GT3X+ Graphic Daily Energy Expenditure Output

23/10/2012



(g) Eating Disorder Examination Questionnaire (EDE-Q)

Instructions: The following questions are concerned with the past four weeks (28 days) only. Please read each question carefully. Please answer all the questions. Thank you.

Questions 1 to 12: Please circle the appropriate number on the right. Remember that the questions refer to the past four weeks (28 days) only.

	On how many of the past 28 days…….	No days	1 – 5 days	6 -12 days	13 -15 days	16 – 22 days	23 – 27 days	Every day
1	Have you been deliberately trying to limit the amount of food you eat to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
2	Have you gone long periods of time (8 waking hours or more) without eating anything at all in order to influence your shape or weight?	0	1	2	3	4	5	6
3	Have you tried to exclude from your diet any foods that you like in order to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
4	Have you tried to follow definite rules regarding your eating (for example, a calorie limit) in order to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
5	Have you had a definite desire to have an empty stomach with the aim of influencing your shape or weight?	0	1	2	3	4	5	6
6	Have a definite desire to have a totally flat stomach?	0	1	2	3	4	5	6
7	Has thinking about food, eating or calories made it very difficult to concentrate on things you are interested in (for example working, following a conversation, or reading)?	0	1	2	3	4	5	6
8	Has thinking about shape or weight made it very difficult to concentrate on things you are interested in (for example, working, following a conversation, or reading)?	0	1	2	3	4	5	6
9	Have you had a definite fear of losing control over eating?	0	1	2	3	4	5	6
10	Have you had a definite fear that you might gain weight?	0	1	2	3	4	5	6
11	Have you felt fat	0	1	2	3	4	5	6
12	Have you had a strong desire to lose weight?	0	1	2	3	4	5	6

Questions 13 to 18: Please fill the appropriate number in the boxes on the right.
Remember that the questions only refer to the past four weeks (28 days).
Over the past four weeks (28 days)……

13 How many times have you eaten what other people would regard as an unusually large

amount of food (given the circumstances)? ……

14 On how many of these times did you have a sense of having lost control over your eating (at the time you were eating)?……

15 On how many **DAYS** have such episodes of overeating occurred (i.e. you have eaten an unusually large amount of food and have had a sense of loss of control at the time)?

……

16 How many times have you made yourself sick (vomit) as a means of controlling your shape or weight? ……

17 How many times have taken laxatives as a means of controlling your shape or weight?……..

18 How many times have you exercised in a "driven" or "compulsive" way as a means of controlling your weight, shape or amount of fat or to burn off calories?……

Questions 19 to 21: Please circle the appropriate number. Please note that for these questions the term "binge eating" means eating what others would regard as an unusually large amount of food for the circumstances, accompanied by a sense of having lost control over eating

		No days	1 – 5 days	6 -12 days	13 -15 days	16 – 22 days	23 – 27 days	Every day
19	Over the past 28 days, on how many days have eaten in secret (i.e. furtively)? ……..Do not count episodes of binge eating	0	1	2	3	4	5	6

20	On what proportion of the times that you have eaten have you felt guilty (felt that you've done wrong) because of its effect on your shape or weight?	None of these times 0	A few of the times 1	Less than half 2	Half the times 3	More than half 4	Most of the time 5	Every day 6
21	Over the past 28 days, how concerned have been about other people seeing you eat? ……Do not count episodes of binge eatin	Not at all 0	1	Slightly 2	3	Moderately 4	5	Markedly 6

Questions 22 to 28: Please circle the appropriate number on the right. Remember that the questions only refer to the past four weeks (28 days).

	Over the past 28 days…….	Not at all Slightly Moderately Markedly
22	Has your weight influenced how you think about (judge) yourself as a person?	0 1 2 3 4 5 6
23	Has your shape influenced how you think about (judge) yourself as a person?	0 1 2 3 4 5 6
24	How much would it upset you if you had been asked to weigh yourself once a week (no more, or less, often) for the next four weeks?	0 1 2 3 4 5 6
25	How dissatisfied have you been with your weight?	0 1 2 3 4 5 6
26	How dissatisfied have you been with your shape?	0 1 2 3 4 5 6
27	How uncomfortable have you felt seeing your body (for example, seeing your shape in the mirror, in a shop window reflection, while undressing or taking a bath or shower)?	0 1 2 3 4 5 6
28	How uncomfortable have you felt about others seeing your shape or figure (for example, in communal changing rooms, when swimming, or tight clothes)?	0 1 2 3 4 5 6

Adopted from: Fairburn, C.G. & Beglin, S. (2008: 309-313)

What is your weight at present? (Please give your best estimate) ……kg
What is your height? (Please give your best estimate) …… cms
Over the past three-to-four months have you missed any menstrual periods?.....
If so, how many?.....
Have you been taking the "pill"?.....

(h) Anthropometric Measurements and Dual Energy X-ray Absorptiometry

Code:…… Date of Birth:……

Date:…… Time:…… Ambient Temperature:……

Mass/Weight (kg):…… Height (cm):……

BMI:……

Body Composition – Dual Energy X-ray Absorptiometry (DXA)

Lean Body Mass:…….

Fat Mass:…….

Bone Mineral Desity (BMD) by Region	Lumbar Vertebrae (L1 – L4)	
	Proximal Femur	
	Total Body	
	Z-score	