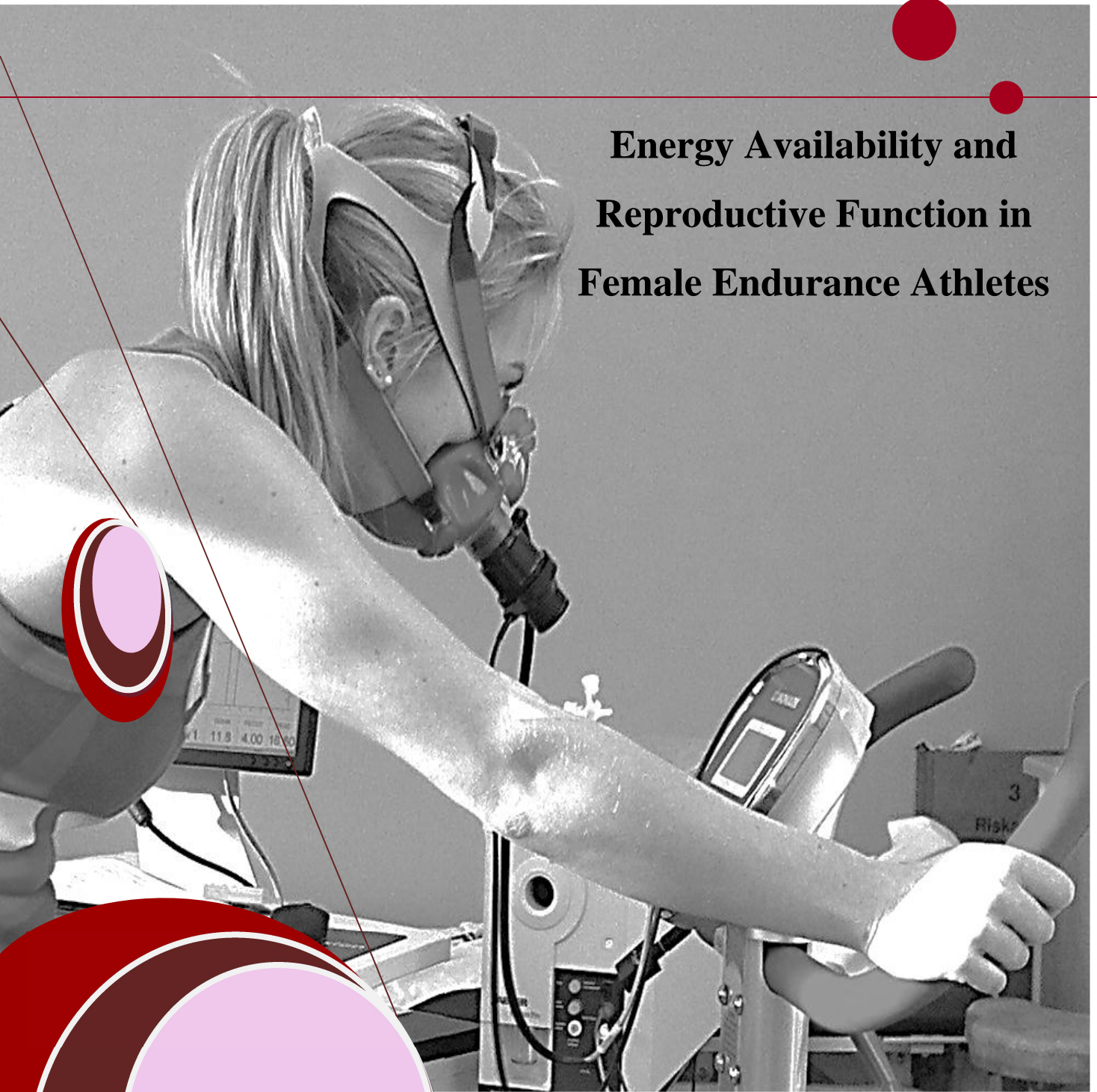# Energy Availability and Reproductive Function in Female Endurance Athletes

PhD Thesis 2014 Anna Melin

PhD thesis

Energy Availability and Reproductive Function in Female Endurance Athletes

Anna Melin, RD, MSc

Department of Nutrition, Exercise and Sports

University of Copenhagen

Energy Availability and Reproductive Function in Female Endurance Athletes

PhD Thesis by Anna Melin, RD, MSc, submitted 20 October 2014

Academic advisors

Anders Sjödin
Department of Nutrition, Exercise and Sports
University of Copenhagen, Denmark

Åsa B. Tornberg
Department of Health Sciences, Division of Physiotherapy
Lund University, Sweden

Sven Skouby
Endocrinological and Reproductive Unit, Department of Obstetrics/Gynaecology, Herlev Hospital
Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Jorunn Sundgot-Borgen
Norwegian School of Sport Sciences, Denmark

Evaluation committee

Christian Mølgaard
University of Copenhagen, Denmark

Dan Benardot
Georgia State University, USA

Angelica Lindén Hirschberg
Karolinska Institutet, Sweden

Contact
Anna Melin, aot@nexs.ku.dk
Department of Nutrition, Exercise and Sports
Faculty of Science, University of Copenhagen
Rolighedsvej 26, 1958 Frederiksberg, Denmark.

CONTENTS

PREFACE

The purpose of this PhD project was to investigate the association between current energy availability (EA), as well as reproductive function in female endurance athletes, and energy metabolism. The aim was also to assess dietary characteristics associated with current low EA and oligomenorrhea/functional hypothalamic amenorrhea (FHA) in female athletes without eating disorders (ED) or disordered eating behaviour (DE). Furthermore, the purpose was to investigate the prevalence of the Female Athlete Triad (Triad), which links low EA, oligomenorrhea/FHA, and impaired bone health and related physiological aspects in Danish and Swedish female endurance athletes. Finally, its purpose was to develop and validate a self-administered screening tool called the Low EA in Females Questionnaire (LEAF-Q), designed to identify female athletes at risk for the Female Athlete Triad (Triad) and to complement existing validated DE screening instruments in order to enable early detection and intervention of the Triad.

The clinical examinations and tests were carried out at the Department of Health Sciences, Division of Physiotherapy, at Lund University in Sweden, and at the Endocrinological and Reproductive Unit, Department of Obstetrics/Gynaecology, Herlev Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

The study, which we called the LEA study, was multidisciplinary, incorporating aspects of nutrition, the spectrum of DE, energy and bone metabolism, endocrinology, reproductive function, cardiovascular factors, immunology, function of the autonomic nervous system, neuromuscular performance, and the ability to recover between training bouts.

This thesis is based on three scientific manuscripts that used data from the LEA study:

1. *Energy Availability and the Female Athlete Triad in Elite Endurance Athletes.* **Melin A.**, Tornberg ÅB., Skouby S., Møller SS., Sundgot-Borgen J., Faber J., Siedelmann J., Aziz M., Sjödin A. Scand J Med Sci Sports 2014 May 30. doi: 10.1111/sms.12261.

2. *Lower Energy Density and High Fibre Intake are Dietary Concerns in Female Endurance Athletes.* **Melin A.**, Tornberg ÅB., Skouby S., Møller SS., Faber J., Sundgot-Borgen J., Sjödin A., in review.

3. The LEAF Questionnaire – A Screening Tool for Identification of Female Athletes with Increased Risk for the Female Athlete Triad. **Melin A.**, Tornberg ÅB., Skouby S., Faber J., Ritz C., Sjödin A., Sundgot-Borgen J. Br J Sports Med 2014;48:540–545.

In addition to these three papers, I have written one review on ED/DE in aquatic sports and contributed to one article on neuromuscular performance. I have also provided three conference abstracts. The following papers and abstracts are not included in this thesis:

Papers
1. *Disordered Eating behaviour and Eating Disorders in Aquatic Sports.* **Melin A.**, Torstveit MK., Burke L., Marks S., Sundgot-Borgen J. Int J Sport Nutr Exerc Metab 2014;24(4):450-9.

2. Decreased Neuromuscular Performance in Female Endurance Athletes with Secondary Functional Hypothalamic Amenorrhea. Tornberg ÅB., **Melin A.**, Skouby S., Koivola F., Johansson A., Faber J., Sjödin A. In review

Conference Abstracts
1. Energy Availability in Female Endurance Athletes – Impact on Energy and Bone Metabolism as well as Health and Performance – Preliminary Results from an on-going Study. **Melin A.**, Tornberg ÅB., Skouby S., Sundgot-Borgen J., Sjödin A. Abstract presented orally at the Scandinavian Congress of Medicine and Science in Sports, Malmö 2012, and awarded as the best oral presentation.
2. Energy Availability in Female Endurance Athletes and the Impact on Energy and Bone metabolism, Health and Recovery after Exercise. Preliminary Results from an on-going Study. **Melin A.**, Tornberg ÅB., Skouby S., Sundgot-Borgen J., Sjödin A. Inter J Sports Nutr Exerc Metab, 2013:3(23);3. Abstract presented orally at the International Sport and Exercise Nutrition Conference, Newcastle, 2012.

3. Energy Availability and Reproductive, Bone and Cardiovascular Health in Female Endurance Athletes: Preliminary Results from an on-going Study. **Melin A.**, Tornberg ÅB., Skouby S., Sundgot-Borgen J., Sjödin A. Med Sci Sports Exerc 2013:45(5);S434. Abstract and poster presented orally at a thematic poster presentation at the annual conference of American College of Sports Medicine, Indianapolis.

Together with Monica Klungland Torstveit and Marianne Martinsen and with Professor Jorunn Sundgot-Borgen as chair, I had the opportunity to present the clinical lecture 'How to Win the Battle Against the Triad' at the American College of Sports Medicine in Indianapolis in 2013. Furthermore I presented in the workshop titled 'Eating Disorders and Relative Energy Deficiency in Sports (RED-S)' at the Nordic Eating Disorder Society Conference in Stockholm in September 2014, together with Solfrid Bratland-Sanda, Therese F. Mathiesen, Monica Klungland Torstveit, and Jorunn Sundgot-Borgen.

I was also invited to:

- The FINA sport nutrition expert meeting in London in December 2013, as the expert and presenter of the topic: 'Eating Disorders and Disordered Eating Behaviour and Associated Triad Conditions in Aquatic Sports'.

- The International Sports and Exercise Nutrition Conference in Newcastle in December 2013 to present the lecture 'Female Athlete Triad Update', together with Margo Mountjoy of the IOC medical commission.

- The Danish Sport Medicine Meeting in Kolling and the Swedish Sport Federation International Sport Nutrition Conference in Stockholm in spring 2014 to give the lecture 'Mechanism and Consequences of Energy Deficiency'.

- The Danish Health Ministry working group which is developing national clinical guidelines for treatment of bulimia nervosa, as a representative of the Danish Society of Clinical Nutrition.

- The Olympiatoppen Nutritional Conference in Oslo in autumn 2014 to give a series of lectures.

ACKNOWLEDGEMENTS

Thank you, Lars, my patient and wonderful husband, and my sons Mark and Noah, for all your support and love. Du fattas mig, lilla älskade mamma.

Special thoughts to the legendary Professor Bengt Saltin for his enthusiastic encouragement and support of the LEA study and for sharing his great wisdom.

Åsa Tornberg and myself, together with Bengt Saltin, at the Scandinavian Congress of Medicine and Science in Sports in Malmö 2012, after we received honours for best poster and best oral presentation. Malin Eggertz Forsmark from the World Village of Women Sports was behind the camera.

SUMMARY

The Female Athlete Triad (Triad), which links low energy availability (EA), menstrual dysfunction (MD), and impaired bone health, is frequently reported, especially in leanness-demanding sports, making low EA a major nutritional concern for female athletes. Most female athletes have a body weight and body composition within the normal range, independent of eating behaviour and reproductive function. Body weight therefore seems to be preserved during long-term energy deficiency, potentially involving several metabolic mechanisms, such as a reduction in energy metabolism at rest (RMR). Menstrual dysfunction is common among female athletes, but often ignored and even regarded as a normal consequence of intense training, despite the fact that health consequences, including premature osteoporosis, are well documented. Restricted eating behaviour and MD have furthermore been associated with an increased risk of injuries, impaired performance, and potentially also an increase in cardiovascular risk factors. However, the prevalence of low EA among female athletes has scarcely been investigated.

The overall aims of this PhD thesis were to investigate the association between current EA, reproductive function, and energy metabolism in a group of Swedish and Danish female endurance athletes, and to identify dietary characteristics associated with current low or reduced EA and oligomenorrhea/functional hypothalamic amenorrhea (FHA) in subjects without the manifestation of eating disorders (ED) or disordered eating behaviour (DE). Another aim was to investigate the prevalence of current low and reduced EA and Triad related conditions in this group of female endurance athletes and to construct and validate a brief questionnaire (the LEAF-Q) that focused only on self-reported physiological symptoms linked to persistent energy deficiency, which could be routinely used in order to identify individuals at risk for the Triad.

The results (Paper I) indicated that athletes with current low and reduced EA, as well as those with oligomenorrhea/FHA, had lower RMR compared to those with either current optimal EA or eumenorrheic athletes. Furthermore, athletes with secondary FHA had increased work efficiency compared to eumenorrheic subjects, indicating a more profound metabolic adaptation in female athletes with clinical MD. All three Triad conditions were common in this group of athletes, despite a normal BMI range and body composition. Furthermore, issues and physiological symptoms related to current low and reduced EA and oligomenorrhea/FHA were not limited to impaired bone health, but also included hypoglycaemia, hypercholesterolemia, and hypotension. These findings emphasize the importance of the implementation of prevention, early identification, and treatment strategies, and that the findings of either one of these related subclinical and clinical features should necessitate careful assessments for the other conditions.

The results (Paper II) further revealed that diets lower in energy density, fat content, compact carbohydrate-rich foods and energy-containing drinks, together with higher fibre content, were associated with current low and reduced EA and oligomenorrhea/FHA, and may constitute targets for dietary intervention in order to prevent and treat these conditions. Even a slight increased drive to

lose or maintain a low body weight was associated with dietary characteristics likely to increase the risk of energy deficiency and oligomenorrhea/FHA.

The results (Paper III) showed that the LEAF-Q had acceptable sensitivity, specificity, and internal consistency, which indicates that it has the potential to be a useful self-reported screening tool to complement existing validated DE screening instruments for the identification of female athletes at risk for the Triad.

DANSK SAMMENFATNING

Den kvindelige idrætstriade (Triaden), der forbinder lav energitilgængelighed, menstruationsforstyrrelser og nedsat knogletæthed, er hyppigt forekommende, især inden for idrætsgrene hvor der er fokus på vægt og kropssammensætning. Lav energitilgængelighed er derfor et af de største ernæringsrelaterede problemområder blandt kvindelige atleter. De fleste kvindelige idrætsudøvere er normalvægtige og har en normal kropssammensætning uafhængigt af deres spiseadfærd, og om de har udebleven eller regelmæssig menstruation. Årsagen til at kropsvægten forbliver normal på trods af langvarig lav eller reduceret energitilgængelighed, er sandsynligvis metaboliske mekanismer, såsom et nedsat hvilestofskifte (RMR). Menstruationsforstyrrelser bliver ofte ignoreret inden for idrætten og endda betragtet som et naturligt resultat af hård træning, på trods af de alvorlige sundhedsmæssige konsekvenser det kan medføre såsom for tidlig knogleskørhed. Restriktiv spiseadfærd og menstruationsforstyrrelser er samtidig blevet forbundet med en øget risiko for skader og forringet præstationsevne samt kardiovaskulære risikofaktorer. På trods af de negative konsekvenser forbundet med lav energitilgængelighed er forekomsten blandt kvindelige atleter dårligt belyst.

De overordnede formål med denne ph.d.-afhandling var at undersøge sammenhængene mellem energitilgængelighed, reproduktion og energiomsætningen i en gruppe svenske og danske kvindelige udholdenhedsatleter samt at identificere kostmæssige faktorer associerede til lav energitilgængelighed og menstruationsforstyrrelser blandt udøvere uden forstyrret spiseadfærd. Et andet mål var at undersøge forekomsten af lav eller reduceret energitilgængelighed og Triade-relaterede symptomer i denne gruppe af udholdenhedsidrætsudøvere samt at konstruere og validere et kortfattet spørgeskema (LEAF-Q), der kun fokuserer på selvrapporterede fysiologiske symptomer forbundet med energimangel, og som rutinemæssigt kan bruges til at identificere individer i risiko for Triaden.

Resultaterne viste, at atleter med lav eller reduceret energitilgængelighed og udøvere med menstruationsforstyrrelser havde lavere RMR end atleter med optimal energitilgængelighed og udøvere med regelmæssig menstruation (Artikel I). Vi fandt desuden en øget arbejdseffektivitet blandt atleter med sekundær amenorré, hvilket indikerer en mere omfattende metabolisk adaptation hos idrætsudøvere med en klinisk menstruationsforstyrrelse. Resultaterne viste også, at lav og reduceret energitilgængelighed (oftest uden en spiseforstyrrelse), menstruationsforstyrrelser og nedsat knogletæthed var almindeligt i denne gruppe af kvindelige atleter, på trods af et BMI inden for normalområdet og normal kropssammensætning, samt at de sundhedsmæssige problemer og fysiologiske symptomer forbundet med lav og reduceret energitilgængelighed og menstruationsforstyrrelser ikke var begrænset til nedsat knogletæthed, men også omfattede lavt blodsukker, lavt blodtryk samt forhøjet kolesterol. Den høje forekomst af Triade-relaterede problemstillinger samt associerede kliniske fund understreger betydningen af implementering af strategier for forebyggelse, tidlig identifikation og behandling af atleter, som udviser de nævnte fysiologiske symptomer samt at fundet af hvilken som helst af disse associerede subkliniske eller kliniske fund bør medføre en udvidet udredning for tilstedeværelse af andre relaterede Triade-symptomer.

Resultaterne vedrørende kostfaktorer (Artikel II) viste, at en kost med lavere energitæthed, et mere restriktivt indtag af fedt, kompakte kulhydratrige fødevarer og energiholdig væske samt et højt kostfiberindtag var associeret til lav og reduceret energitilgængelighed og menstruationsforstyrrelser. Disse kostfaktorer bør der derfor fokuseres på ved ernæringsmæssig forebyggelse og behandling. Vores resultater indikerede også, at selv et lille øget fokus på vægttab eller det at bibeholde en lav kropsvægt var associeret til et kostmønster, der sandsynligvis øger risikoen for lav og reduceret energitilgængelighed og menstruationsforstyrrelser.

Resultaterne vedrørende udvikling og testning af LEAF-Q (Artikel III) viste en acceptabel sensitivitet og specificitet, hvilket indikerer, at LEAF-Q har potentiale til at være et brugbart screeningsværktøj til identifikation af kvindelige atleter med risiko for Triaden og kan supplere allerede eksisterende screeningsinstrumenter vedrørende forstyrret spiseadfærd for derved at sikre tidlig opdagelse og behandling.

ABSTRACT

Objectives: The female athlete triad (Triad), links low energy availability (EA) with oligomenorrhea/functional hypothalamic amenorrhea (FHA) and impaired bone health. The aims of this study were to examine associations between EA, oligomenorrhea/FHA and energy metabolism and to investigate the prevalence of Triad-associated conditions in Swedish and Danish endurance athletes. Furthermore, the aim was to describe dietary characteristics of athletes with low/reduced EA and/or oligomenorrhea/FHA but without disordered eating (DE). Finally, the aim was to develop and validate a screening tool designed to identify female athletes at risk for the Triad.

Methods: Athletes (n = 84) 18–39 yrs. of age, training ≥5 times/week were recruited from national teams, competitive clubs and a professional dancing company. They filled out the Low EA in Females Questionnaire (LEAF-Q), comprising questions regarding injuries, illness, dizziness, gastrointestinal and reproductive functions. Reliability and internal consistency were evaluated in a subsample of female dancers and endurance athletes (n = 37). Discriminant as well as concurrent validity were evaluated in endurance athletes from weight bearing endurance sports (n = 45), by testing self-reported data against clinical examinations including gynaecological examination; assessment of bone health (DXA); indirect respiratory calorimetry for assessment of RMR and work efficiency; diet and exercise measured 7-days to assess EA; eating disorder (ED)-examination; biochemical markers. Subjects with MD other than oligomenorrhoea/FHA (n = 5) were excluded when assessing energy metabolism and Triad conditions as was athletes with ED/DE (n = 11) and low dietary-record validity (n = 4) when assessing dietary characteristics.

Results: Subjects with low/reduced current EA (n = 25; < 188 kJ/kg FFM/day), had lower resting metabolic rate (RMR) compared to those with optimal EA (n = 15; ≥188 kJ/kg FFM/day) (P=0.003), as did subjects with oligomenorrhea/ FHA (n = 24) compared to eumenorrheic subjects (n = 16) (P=0.040). Subjects with secondary FHA (n = 14) also had increased work efficiency compared to eumenorrheic subjects (P=0.017). 63% had low/reduced current EA, 25% ED, 60% oligomenorrhea/FHA, 45% impaired bone health, and 23% had all three Triad conditions. 53% had low RMR, 25% hypercholesterolemia and 38% hypoglycaemia. There were no differences in energy intake (P=0.475) or current EA (P=0.977) between oligomenorrheic/FHA and eumenorrheic subjects. However, subjects with oligomenorrhea/FHA shared the same dietary characteristics as subjects with low/reduced current EA with a lower energy density (P=0.012 and P=0.020) and fat intake (P=0.047 and P=0.027). Oligomenorrheic/FHA subjects had, furthermore, a higher fibre intake (P<0.001). The 25-item LEAF-Q produced an acceptable sensitivity (78%) and specificity (90%) for correctly classifying current EA and/or reproductive function and/or bone health.

Conclusions: Athletes with low/reduced current EA and/or oligomenorrhea/FHA had lower RMR and those with secondary FHA also had increased work efficiency, indicating a more profound adaptation in female athletes with severe clinical MD. Triad-associated conditions were common in

11

this group of athletes, despite a normal BMI-range. Diets lower in energy density and fat content together with higher fibre content, were associated with low/reduced EA and oligomenorrhea/FHA and may constitute targets for dietary intervention in order to prevent/treat these conditions. The LEAF-Q is brief and easy to administer, and may be used as a complement to existing validated DE questionnaires, when screening female athletes at risk for the Triad, in order to enable early detection and intervention.

ABBREVIATIONS

BD score	Body dissatisfaction score (EDI-3)
BMD	Bone mineral density (g/m^2)
BMI	Body Mass Index [weight (kg) divided by height squared (m^2)]
DE	Disordered eating behaviour
DT score	Drive for thinness score (EDI-3)
DXA	Dual-energy x-ray absorptiometry
EA	Energy availability
ED	Eating disorders
EDE-16	Eating disorder examination, a semi-structured diagnostic interview
EDI-3	Eating Disorder Inventory-3
EDNOS	Eating disorders not otherwise specified
FFM	Fat-free mass
FHA	Functional hypothalamic amenorrhea
FSH	Follicle-stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
IGF-1	Insulin-like growth factor-1
LH	Luteinizing hormone
MET	Metabolic equivalents
MD	Menstrual dysfunction
NEAT	Non-exercise activity thermogenesis
PCOS	Polycystic ovary syndrome
RMR	Resting metabolic rate
mRMR	Measured resting metabolic rate
pRMR	Predicted resting metabolic rate
RMR_{ratio}	The ratio between measured and predicted RMR
T_3	Triiodothyronine
Triad	The Female Athlete Triad
TSH	Thyroid stimulating hormone
VO_{2peak}	Peak oxygen uptake (L/min or mL/kg/min)
WE%	Work efficiency percentage

DEFINITIONS

BMD *Z*-score | The number of standard deviations above or below the mean for the patient's age, sex, and ethnicity

BMD *T*-score | The number of standard deviations above or below the mean for a healthy 30-year-old adult of the same sex and ethnicity

Clinical Triad conditions | Low energy availability with or without eating disorders, oligomenorrhea/functional hypothalamic amenorrhea, and osteoporosis

Disordered eating | Eating Disorder Inventory (EDI-3) subscale drive for thinness score of $\geq$ 14 and/or a body dissatisfaction score $\geq$ 19

Eating disorders | Anorexia nervosa, bulimia nervosa, and eating disorders not otherwise specified (EDNOS) meeting the DSM-IV criteria and diagnosed by the Eating Disorder Examination (EDE-16) Interview

EDI-3 | Eating Disorder Inventory-3, a self-reported questionnaire that assesses disordered eating behaviour

Energy availability | Ingested energy remaining for all other metabolic processes after the energy cost of exercise has been subtracted

Low EA | Energy availability < 125 kJ (< 30 Kcal)/kg FFM/day

Reduced EA | Energy availability 125-187 kJ (30-44 kcal)/kg FFM/day

Optimal EA | Energy availability $\geq$ 188 kJ ($\geq$ 45 kcal)/kg FFM/day

Eumenorrhea | Menstrual cycles of 28 days $\pm$ 7 days

Functional hypothalamic amenorrhea | Either primary amenorrhea, defined as menarche after 15 years of age, or secondary amenorrhea, defined as absence of three or more consecutive menstrual cycles

Hypotension | Systolic blood pressure < 90 mmHg and/or diastolic blood pressure < 60 mmHg

Low BMD | *Z*-score of - 1 to - 2 in at least one of the measured sites

Low RMR	Ratio between the measured (mRMR) and predicted resting metabolic rate (pRMR) < 0.90
mRMR	Resting metabolic rate measured by indirect respiratory calorimetry using a ventilated open hood system
Normal BMD	Z-score $> - 1$ in all measured sites
Oligomenorrhea	Menstrual cycles > 35 days
Orthostatic hypotension	A fall in systolic blood pressure > 20 mmHg and/or a fall in diastolic blood pressure > 10 mmHg when moving from supine to standing position
Osteoporosis	Z-score $< - 2$ in at least one of the measured sites
Polycystic ovary syndrome	Enlarged ovaries with a volume greater than 10 mL and/or at least one ovary demonstrating 12 or more follicles in one plane, irregular menstrual cycle, and elevated androgen levels or otherwise stigmatized androgen
pRMR	Predicted resting metabolic rate using the Cunningham equation
Subclinical Triad conditions	Reduced energy availability with or without disordered eating behaviour, short luteal phase defect or anovulation, and low bone mineral density
The Female Athlete Triad	Continuum of energy availability with or without disordered eating behaviour, reproductive function, and bone health in female athletes
VO_{2peak}	Maximal aerobic capacity measured as the peak oxygen uptake during an incremental exercise test
Work efficiency / 100W * 100	[Energy expended (kJ/min) 100W - Energy expended (kJ/min) 0W]

INTRODUCTION

Athletes need to maintain sufficient energy and nutrient intake in order to avoid fatigue and injuries, as well as illness (Burke 2001, Rodriguez et al. 2009). Female athletes focusing on leanness, however, have been reported to have an increased risk of developing restricted eating behaviour (Manore et al. 2007); furthermore, disordered eating behaviour (DE) or eating disorders (ED) are frequently reported among female athletes (Gibbs et al. 2013a). Low energy availability (EA) with or without ED is related to oligomenorrhea/functional hypothalamic amenorrhea (FHA) and impaired bone health, a syndrome called the Female Athlete Triad (Triad) (Nattiv et al. 2007, De Souza et al. 2014). Menstrual dysfunction (MD) is reported to be common among female athletes, but is often ignored and regarded as a normal consequence of intense training, despite the fact that negative health consequences, including the risk of premature osteoporosis, are well documented (Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014). The hormonal synthesis and increased luteal phase thermogenesis are energy consuming processes (Harber 2004), and the energy metabolism at rest (RMR) changes up to 10 percent during the menstrual cycle, with peaks during the late luteal phase or the early follicular phase (Henry et al. 2003). Oligomenorrhea/FHA, therefore, may lead to lower energy needs. Most female athletes with long-term energy deficiency are reported to have a steady body weight and body composition within the normal range, independent of their reproductive function (Redman and Loucks 2005). Therefore, several metabolic mechanisms might be involved, such as a reduction in RMR and in non-exercise activity thermogenesis (NEAT) (Redman et al. 2009), as well as in increased work efficiency (Goldsmith et al. 2010).

Energy availability is defined as the ingested energy remaining for all other metabolic processes after the energy cost of training has been subtracted (Loucks and Thuma 2003). Experimental studies in sedentary eumenorrheic women have shown that five days of EA less than 125 kJ/kg FFM/day reduces blood glucose levels, suppresses the pulsatility of gonadotropin-releasing hormone (GnRH) and hypothalamic-pituitary-axis hormones, like luteinizing hormone (LH) and triiodothyronine (T_3), elevates cortisol (Loucks and Thuma 2003), and reduces biomarkers of bone formation (Ihle and Loucks 2004) (Figure 1). Several possible reasons for low EA in female athletes have been suggested, such as difficulties in eating enough during periods of high intensity training or an intentional restriction of food to obtain a low body weight (Nattiv et al. 2007). This behavioural pattern seems frequent; indeed 24 percent of female elite endurance athletes have been reported as having DSM-IV diagnosed ED (Sundgot-Borgen and Torstveit 2004). Furthermore, restricted eating behaviour and MD have been associated with an increased risk of injury, impaired performance, and may also potentially increase cardiovascular risk factors, gastrointestinal problems, and metabolic alterations (De Souza et al 2014, Mountjoy et al 2014). The prevalence of low EA with or without ED/DE among female athletes has, however, been little investigated (Gibbs et al. 2013a).
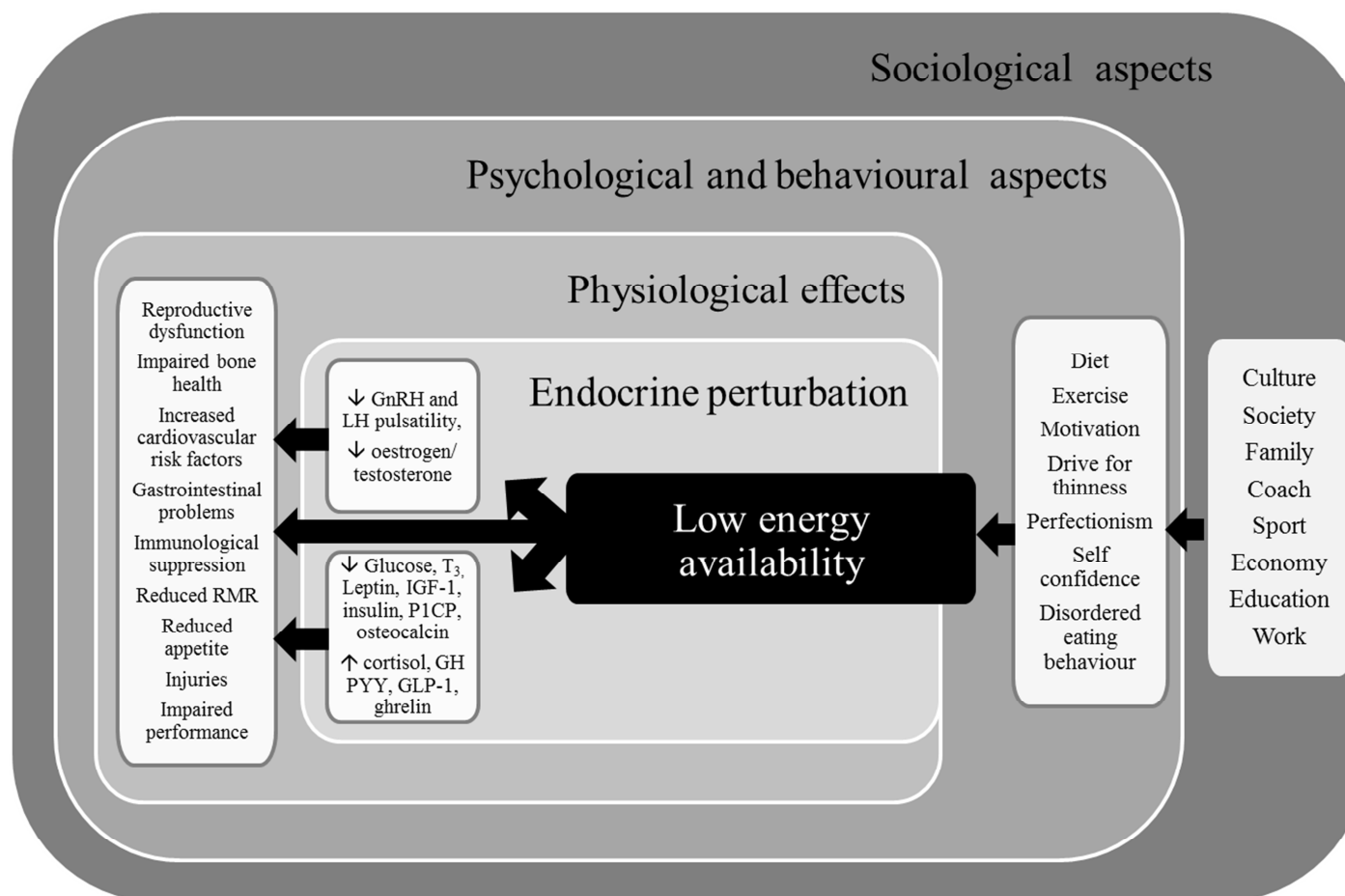
Figure 1 Potential cause and effect diagram of low energy availability in athletes

The figure illustrates examples of potential sociological, psychological, and behavioural causes of low energy availability (EA) and its physiological effects in adult athletes (Sundgot-Borgen 1994, Ihle and Loucks 2004, Bonci et al. 2008, Sundgot-Borgen and Torstveit, 2010, Gordon 2010, Warren 2011, Larson-Meyer et al. 2012, Barrack et al 2013). Abbreviation: GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; T3: triiodothyronine; IGF-1: Insulin-like growth factor 1; P1CP: carboxy-terminal propeptide of type I procollageni n serum; GH: growth hormone; PYY: peptide YY; GLP-1: glucagon-like peptide-1.

BACKGROUND

The Female Athlete Triad and Related Conditions

Female athletes, especially athletes in sports that focus on leanness, are reported to have an increased risk of developing restricted eating behaviour and low EA (Manore et al. 2007, Barrack et al. 2013). Persistent low EA, with or without an ED, is related to oligomenorrhea/FHA and impaired bone health, which are all symptoms of the Female Athlete Triad (Triad) (Nattiv et al. 2007, De Souza et al. 2014). The term 'Female Athlete Triad' was introduced in 1992 (Yeager et al. 1993), and the first Triad Position Stand from the American College of Sports Medicine (ACSM) was published in 1997 and revised ten years later (Nattiv et al. 2007). The Triad is described as developing along a continuum of severity from optimal EA, eumenorrhea, and normal bone mineral density (BMD) through subclinical conditions, such as reduced EA with or without DE, subclinical MD (short luteal phase defect or anovulation), and low BMD (Z-score of -1 to -1.9) until it ends with severe, clinically overt conditions associated with low EA (< 125 kJ/kg FFM/day) with or without an ED, oligomenorrhea/FHA, and osteoporosis (Z-score ≤ -2) (Nattiv et al. 2007). Most research on the Triad has been performed on young adult female athletes, and there are great variations in the reported prevalence of subclinical and clinical Triad conditions, depending on the sport investigated, the competitive level, and the methods and clinical definitions used. Between 16%–60% of the investigated athletes have been reported to exhibit one Triad condition, while 3%–27% have been reported to have two Triad conditions. The reported prevalence of athletes who exhibit all three Triad conditions is as low as 0%–16% (Gibbs et al. 2013a). Energy metabolism (Harber 2004, Warren 2011) and the cardiovascular system (Warren et al. 2011, Rickenlund et al. 2005) have been reported to affect athletes with MD and restricted eating behaviour. Athletes with MD have also been reported to have an increased risk of musculoskeletal injuries (Rauh et al. 2010, Thein-Nissenbaum et al. 2012). Since adequate nutrition is essential, especially for the development and accumulation of bone minerals during adolescence (Barrack et al. 2013), prevention and early intervention of the Triad is recommended (Nattiv et al. 2007, De Souza et al. 2014).

In 2014, a Consensus paper on the diagnosis and treatment of the Triad was published by the Female Athlete Triad Coalition (De Souza et al. 2014). In both the ACSM 2007 Position Stand and the recent Triad Coalitions Consensus Paper, other medical issues related to the components of the Triad were mentioned (Nattiv et al. 2007, De Souza et al. 2014). These include cardiovascular issues (impaired endothelium-dependent vasodilatation and lipid dysfunction), impaired perfusion of working muscles, impaired skeletal muscle oxidative metabolism, problems with the gastrointestinal system, and metabolic and endocrine perturbations (Figure 1). Eating disorders and oligomenorrhea/FHA are regarded as serious clinical outcomes. Impaired bone health is a particularly serious outcome, since it is likely irreversible (Nattiv et al. 2007, De Souza et al. 2014). In 2014, to address that low EA affects both men and women, and that there are more aspects of physiological function, health, and performance affected than just reproductive function and bone health, the International Olympic Committee (IOC) published their new Consensus Statement (Mountjoy et al. 2014) to replace its previous statement concerning the Triad

(Drinkwater et al. 2006). The IOC introduced a new concept: Relative Energy Deficiency in Sport (RED-S) and a risk-assessment and return-to-play model developed and utilized since 2012 by The Norwegian Olympic and Paralympic Committee and Confederation of Sports (Skaaderud et al. 2012). Because RED-S was introduced after the LEA study was completed, the original Triad terminology is used in this PhD thesis.

In summary, there are strong scientific evidence concerning the causality between low EA and the endocrine perturbation related to subclinical and clinical MD and impaired bone health in women. There is, furthermore, a growing body of evidence that low EA and the subsequent endocrine alterations have other health-impairing consequences in addition to oligomenorrhea/FHA and impaired bone health, and that low EA also exists in male athletes with similar physiological consequences. There is, however, no study that has simultaneously investigated the association between subclinical and clinical Triad conditions, including EA in female athletes, as well as their impact on performance and multiple related medical issues such as dyslipidaemia, endocrine and metabolic alterations.

Energy Availability in Sports

It has been suggested that EA is a physiologically useful concept with regard to estimating the energetic state in female athletes, as it reflects the remaining ingested energy for all metabolic processes after the energy cost of exercise has been subtracted (Loucks et al. 1998, Loucks and Thuma 2003, Loucks 2014), in contrast to energy balance, which is the relationship between energy intake and the total energy expenditure. Total energy expenditure can be divided into RMR, which includes the energy costs of basic physiologic functions, such as cellular maintenance, thermoregulation, growth, reproduction, and immunity. Furthermore, total energy expenditure includes NEAT, energy expenditure during exercise, and diet induced thermogenesis (DIT). Resting metabolic rate is mainly determined by fat-free mass (FFM), but is also affected by energy balance and comprises 55%–65% of the total energy expenditure in normal sedentary people (Speakmen and Selman 2003). Exercise increases total energy expenditure *per se* (Westerterp et al. 1992). Highly trained endurance athletes who are in energy balance have been reported to have elevated RMR post-exercise that can be maintained at least for thirty-nine hours after the last training session (Sjödin et al. 1996), while athletes with MD have been reported to have lower RMR compared to eumenorrheic subjects (Myerson et al. 1991, Lebenstedt et al. 1999). Therefore, measuring energy balance in female athletes with low energy intake and MD might result in a more neutral energy balance than expected, due to metabolic adaptations (Loucks et al. 2011).

Clinical studies on eumenorrheic sedentary women have reported that EA less than 125 kJ/kg FFM/day over more than five days causes a reduction in glucose availability, LH-pulsatility, T_3, insulin, leptin, and IGF-1, as well as increased cortisol, growth hormone (GH) (Loucks and Heath 1994, Loucks et al. 1998, Loucks and Thuma 2003) and bone resorption markers (Ihle and Loucks 2004) (Figure 1). Therefore, low T_3 and leptin, as well as elevated cortisol levels, have been suggested as biomarkers to be used when screening female athletes for the Triad (Nattiv et al. 2004, De Souza et al. 2014, Mountjoy et al. 2014) (Figure 1

and 2). Hypoglycaemia, clinically low T_3, and elevated cortisol and total cholesterol levels are more often seen in patients with anorexia nervosa (Warren 2011), female athletes, and recreationally active women with MD compared to eumenorrheic subjects (Harber et al. 1998, De Souza et al. 2003, Rickenlund et al. 2005, De Souza et al. 2008). Furthermore, since an elevated cortisol to insulin ratio is evidence of accelerated proteolysis, and GH to insulin ratio with elevated urine ketones are evidence of an accelerated lipolysis (Loucks 2014), these might be potential biomarkers for persistent low energy and glucose availability in athletes. The new Consensus Statement by the Triad Coalition suggests that a BMI of less than 17.5 kg/m^2 in athletes over 18 years of age can indicate low EA (De Souza et al. 2014). Most female athletes, however, have been reported to have a body weight within the normal range, independent of their reproductive function (Redman and Loucks 2005), and athletes with ED have been found to be underweight, at normal weight, or even overweight (Torstveit and Sundgot-Borgen 2012). Therefore, it is rare for female athletes in most sports to be underweight and this should be considered an indicator of severe, persistent energy deficiency.

In eumenorrheic weight-stable sedentary women, the average EA has been reported to be ~188 ± 25 kJ/kg FFM/day (Loucks et al. 1998, Loucks and Thuma 2003), and physically active women are recommended to have an EA of at least 188 kJ/kg FFM/day to ensure adequate energy for all physiological functions (De Souza et al. 2014). Subclinical EA has been suggested to be an EA less than 188/kg FFM/day (Gibbs et al. 2013a), and it is recommended that athletes aiming to lose body weight or body fat follow a diet and training regime that provides EA of 125–188 kJ/kg FFM/day (Loucks et al. 2011). No study concerning the impact of EA, however, has been performed on elite athletes, and no study has investigated whether the same thresholds for EA are applicable in a more long-term perspective. When the LEA study was initiated, only two studies had reported EA in free-living recreationally active women and female athletes (De Souza et al. 1998, Hoch et al. 2009). Since 2011, ten studies have been published (Hoch et al. 2011, Schaal et al. 2011, Reed et al. 2011, Reed et al. 2012, Gibbs et al. 2013b, Koehler et al. 2013, Woodruff and Meloche 2013, Guebels et al. 2014, Van Heest et al. 2014, Lagowska et al. 2014) (Table 1), reflecting the growing interest in this topic. Five of these studies have reported the prevalence of low EA: 77% in professional female ballet dancers (Hoch et al. 2011), 51% and 58% in female and male athletes from mixed sports (Kohler et al. 2013), 20% in female volley ball players (Woodruff and Meloche 2013), 12%–33% in female elite soccer players, depending on the time during the season (Reed et al. 2012), and 6% in high school female athletes from mixed sports (Hoch et al. 2009). In six of these studies, EA related to reproductive function have been reported (De Souza et al. 1998, Reed et al. 2011, Guebels et al. 2014, Logowska et al. 2014, Van Heest et al. 2014), and the weighted mean EA from these studies is 109 kJ/kg FFM/day in athletes and recreationally active women with MD, compared to the 127 kJ/kg FFM/day in eumenorrheic subjects (Table 1). There are, however, great variations in the studies and overlap between the groups in the reported EA: 50–142 kJ/kg FFM/day in subjects with MD, and 97–176 kJ/kg FFM/day in eumenorrheic subjects. In this thesis, optimal EA is operationally defined as EA ≥ 188 kJ/kg FFM/day, reduced EA is operationally defined as 125-187 kJ/kg FFM/day, and low EA is operationally defined as < 125 kJ/kg FFM/day.

In summary, clinical studies on eumenorrheic, sedentary women have reported that low EA defined as less than 125 kJ/kg FFM/day over more than five days cause endocrine alterations in eumenorrheic women. Therefore, it is recommended that physically active women have an EA of at least

188 kJ/kg FFM/day to ensure adequate energy for all physiological functions. Few studies have investigated the prevalence of low or reduced EA in female athletes, and no study has investigated the impact of EA on elite athletes or if the same thresholds for low EA are applicable in a more long-term perspective.

Aetiology of Low Energy Availability in Female Athletes

Female athletes are reported to have similar or even lower daily energy intake compared to sedentary women (Wilmore et al. 1992, Dueck et al. 1996). However, underreporting or under-eating are well documented behaviours during prospective dietary recording (Burke 2001, Jonnalagada et al. 2010) and can therefore explain some of the discrepancies between reported energy intakes and energy needs in female athletes, and hence a potentially false low EA. The high prevalence of ED/DE and physiological symptoms of persistent low EA, such as oligomenorrhea/FHA and reduced RMR, however, indicates that many female athletes are inadvertently failing to balance energy expenditure with adequate energy intake (Gibbs et al. 2013a, Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014). The suggested aetiology of low EA in female athletes with or without the presence of ED/DE includes an intentional restriction of food intake, especially of energy dense foods, or an increase in energy expenditure in order to obtain and maintain a low body weight. Low EA could also emerge unintentionally and be primarily expenditure-driven, due to the difficulty of increasing food intake sufficiently to meet energy requirements during periods with large training volumes (Stubbs et al. 2004, Nattiv et al. 2007, De Souza et al. 2014), especially on a diet characterized by a low energy density.

Nutritional Recommendations and Dietary Intake in Female Endurance Athletes

Some endurance training programs have a predicted exercise energy expenditure of 2.9–4.2 MJ per training session (Burke 2001), but energy requirements for female endurance athletes may vary considerably, with documented energy needs reaching levels of around 20 MJ/day over several days, corresponding to a PAL of 3.6 (Sjödin et al. 1996). The weighted mean energy intake in female eumenorrheic endurance athletes and endurance trained women pooled from a number of studies, however, is only 9.2 MJ/day (164 kJ/kg body weight/day) and 8.4 MJ/day (152 kJ/kg body weight/day) in endurance athletes with MD (Table 2) and, therefore, is similar to the 8.8–9.4 MJ/day recommended to Nordic, sedentary women aged 18–60 years (Nordic Council of Ministers 2013). Underreporting or under-eating is a potential bias in dietary surveys and studies on the accuracy of food diaries in the general population, which are reported to underreport 20% of true energy intake (Schoeller 1990, Black 2000). Burke have reported that the following errors might occur in self-reported food diaries in athletic populations: a) alteration in the dietary intake during the period of recording so that it fails to reflect the usual intake; b) inaccurate recording of the dietary intake to improve the perception of what they are eating; and c) errors of quantification or description in recording the food intake (Burke 2001). The high prevalence of physiological symptoms of energy deficiency found in female athletes, such as subclinical and clinical MD and lowered RMR (Gibbs et al. 2013a, Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014) indicates, however, that the discrepancy between energy intake and estimated energy needs reported in these dietary surveys can only be partly explained by underreporting.
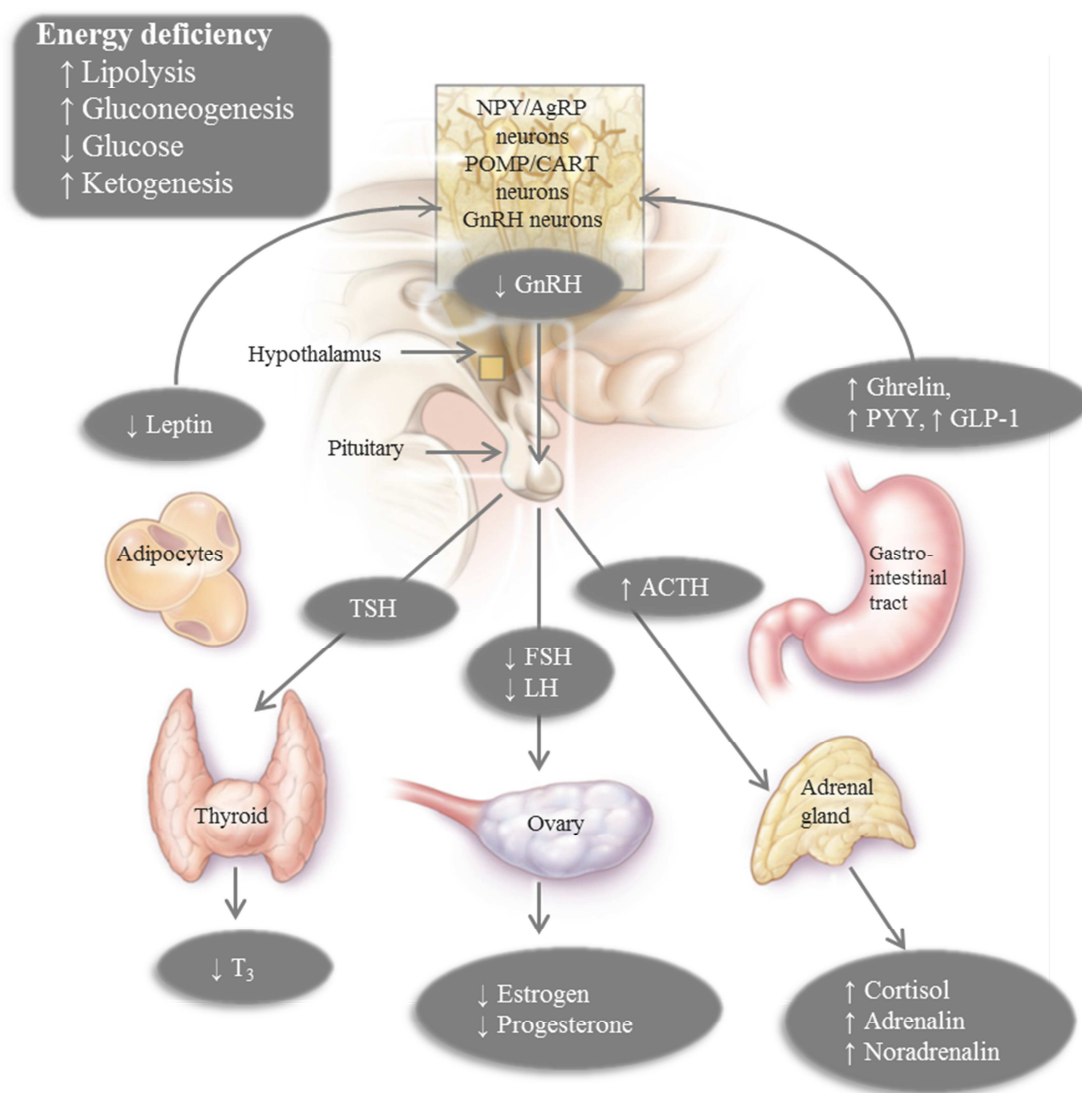
Figure 2 Potential Endocrine Alterations due to Energy Deficiency

The figure is modified from Loucks and Thuma 2003, Bonci et al. 2008, Gordon 2010, Warren 2011 and Larson-Meyer et al. 2012. The arcuate nucleus in the hypothalamus contains neurones that coordinates the signals and control food intake (orexigenic neuropeptide Y [NPY]/agouti-related peptide (AgRP) and anorexigenic peptide proopiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART)). Leptin, ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) are some of the hormones involved in the regulation of appetite. Leptin, mainly produced in fat cells, suppresses appetite. PYY and GLP-1 also have anorexigenic actions while ghrelin is orexigenic and stimulates appetite. Energy deficiency reduces leptin levels, which enhances the release of NPY/AgRP and increases appetite. However, elevated PYY and GLP-1generates an overall anorexogenic effect and might, therefore, increase the risk of energy deficiency. High intensity exercise has been shown to acutely suppress appetite and the effect of ghrelin, GLP-1, and PYY in eumenorrheic female athletes. Insufficient availability of metabolic fuels, especially glucose, will be detected by cells in the hindbrain, where NPY neurons inhibit gonadotropin-releasing hormone (GnRH) pulsatility. The suppression of GnRH reduces the LH-pulsatility and the FSH production, which leads to a reduced production of oestrogen and progesterone and disruption of the feedback mechanism. Ghrelin, leptin, and PYY might be indirectly involved in the suppression of GnRH pulsatility, and thereby in the development of MD. Abbreviation: ACTH: adrenocorticotropic hormone; FSH: follicle stimulating hormone; TSH: thyroid stimulating hormone; T_3: triiodothyronine.

Table 1 Studies that have measured energy availability in athletes and recreational active women

Reference	Subjects	Methods			DE	Reproductive function	BMD	Remarks	EA (kJ/ kg FFM/day)
		Energy intake	Exercise energy expenditure	Exercise definition					
De Souza et al. 1998	Recreational runners; Subclinical MD (n=29), EUM (n=24)	3x7 days prospective dietary records	Simultaneously estimated by activity logs and HR.	Running and other weight-bearing PA	-	Menstrual history and repeated sex hormones	-	EEE NA for SA or EA for FFM	Subclinical MD: 98 EUM: 97
Hoch et al. 2009	Mixed female athletes (n=80), Sedentary controls (n=80)	3 days prospective weighed dietary record	Simultaneously estimated by activity logs and calculated using METs.	Sport participation	EAT-26	Menstrual history and sex hormones	Yes	EEE NA for SA	Athletes: 6% low EA, 30% reduced EA; Controls: 4% low, 35% reduced EA.
Hoch et al. 2011	Professional female ballet dancers (n=22)	3 days prospective dietary record	Simultaneously estimated by accelerometers, individually calibrated at 2 self-selected intensities of exercise.	All PA	EDE-Q	Menstrual history and sex hormones	Yes	Low/negative EA defined as a negative value. EEE NA for SA. EA NA for FFM. Endothelial function (FMD) determined.	77% had low EA
Schaal et al. 2011	Runners and triathletes; MD (n=6), EUM (n=6)	7 days prospective weighed dietary record	Simultaneously estimated by activity logs, HR, and intensity using the 10-point RPE scale. EEE was estimated on the basis of RPE and HR during training to matched O_2 consumption and RER during laboratory testing.	Self-defined training verified by RPE	EDE-Q	Menstrual history and diagnosis verified by physician	-	EEE NA for SA. Catecolamines and BP measured. POMS questionnaire.	MD: 75 EUM: 121
Reed et al. 2011	Recreationally active women: MD (n=12), EUM (n=13)	2 x 3 days prospective dietary records	HR and activity logs during a 7-day period at baseline. METs for exercise sessions without HR monitoring.	All purposeful exercise >10 min with a HR >90 beats/min but not SA	EDI, TFEQ, psychologist	Menstrual history and sex hormones	-	EEE NA for SA. RMR and satiety (PYY) measured. Food group variety and energy density estimated.	MD: 121** EUM: 176

Table 1 continued									
Reference	Subjects	Methods							EA (kJ/kg FFM/day)
		Energy intake	Exercise energy expenditure	Exercise definition	DE	Reproductive function	BMD	Remarks	
Reed et al. 2012	Female soccer players (n=19)	3x3 days prospective dietary records	Simultaneously estimated using HR during team training sessions. HR and activity logs during individual training. METs for exercise sessions without HR monitoring.	Team training sessions and purposeful exercise sessions	EDI	Menstrual history	-	T_3 measured	Pre-season: 180 Mid-season: 146 Post-season: 188 12%–33% low EA, depending on the time during the season
Gibbs et al. 2013b	Exercising women; Dietary restraint (n=30), non-dietary restraint (n=56)	3 days prospective weighed dietary record	Simultaneously estimated using 7 days activity logs and HR. Individual VO_{2peak} values were used to compute EEE. METs for exercise sessions without HR monitoring.	All purposeful exercise, but not SA	TFE Q	Menstrual history and daily sex hormones (one cycle)	-	RMR measured	Dietary restraint: 150* Non-dietary restraint: 176
Koehler et al. 2013	Athletes from mixed sports; Men (n=160), Women (n=185)	7 days prospective dietary record with 193 foods listed with standard portion sizes.	Simultaneously estimated using activity records, including a list of exercise-related activities with different intensity levels and SA and METs.	Exercise related activities	-	-	-	EA corrected with age-specific factors for EE. Leptin, IGF-1, T_3 and insulin measured.	Male endurance athletes (n=22): 113, 58% low EA; female endurance athletes (n=18): 151, 51% low EA.
Woodruff, Meloche 2013	Volleyball players (n=10)	2x7 days prospective dietary records	Simultaneously assessed from accelerometers and METs, based on dates and times of practices, warm-ups, and games.	Volleyball practice, warm-ups, and games	-	Menstrual history	-	EEE NA for SA.	178

		Methods						Remarks	EA (kJ/kg FFM/day)
Reference	Subjects	Energy intake	Exercise energy expenditure	Exercise definition	DE	Reproductive function	BMD		
Guebels et al. 2014	Endurance trained women; MD (n=8), EUM (n=9)	2x7 days prospective weighed dietary records	Simultaneously assessed from activity logs, accelerometers, and METs.	*1*: All planned exercise *2*: All planned exercise + transport-related activities *3*: Exercise ≥ 4 METs *4*: Exercise > 4 METs	EDI	Menstrual history and repeated sex hormones	-	EEE NA for SA, RMR measured	MD: 142 EUM: 158
Lagowska et al. 2014	Mixed athletes with MD (n=45)	7 days prospective dietary record using a photographic album of dishes.	HR monitors for 3 days. For each subject, the relationship between HR and VO_2 was established for lying in supine position, sitting quietly, standing quietly, and continuous graded exercise on a cycle ergometer.	Exercise activities	-	Menstrual history, sex hormones, and gynecological ultrasound examination	-	EEE NA for SA, RMR measured	MD: 118
Van Heest et al. 2014	Female swimmers; MD (n=5), EUM (n=5)	7x3 days prospective weighed dietary records	7 days training logs and swimming specific EE table.	Swimming training	-	Prospective menstrual cycle record during 12 weeks. Sex hormones at 0 and 2 weeks.	-	EEE NA for SA. Performance assessed after 12-week training program. RMR, T_3, IGF-1 measured.	MD: 50* EUM: 125

Abbreviation: EEE: exercise energy expenditure; EI: energy intake; EA: energy availability; MD: menstrual dysfunction; EUM: eumenorrhea; PA: physical activity; HR: heart rate; DE: disordered eating behaviour; NA.: not adjusted; SA: sedentary activities; N.R.: not reported; RPE: ratings of perceived exertion; MET: Metabolic Equivalent of Task; FMD: flow mediated dilation; DXA: dual-energy x-ray absorptiometry; EAT-26: eating attitudes test-26; EDE-Q: eating disorder examination questionnaire; TFEQ: Three-Factor Eating Questionnaire; BP: blood pressure; POMS: profile of mood states; RMR: resting metabolic rate; T_3: Triiodothyronine; IGF-1: Insulin growth factor-1. * $P < 0.05$ and ** $P < 0.01$ compared to eumenorrheic or non-dietary restraint subjects.

Some studies have reported a lower energy intake in female endurance athletes with MD compared to eumenorrheic athletes (Nelson et al. 1986, Thong et al. 2000, Kaiserauer et al. 1989, Tomten and Høstmark 2006), or an increased training volume and thereby a potentially lower EA (Drinkwater et al. 1984, Harber et al. 1998, Cobb et al. 2003) (Table 2). However, many studies have found similar energy intakes and training volumes in female endurance athletes with MD, as in eumenorrheic athletes (Marcus et al. 1985, Deuster et al. 1986, Myerson et al. 1991, Snead et al. 1992, Wilmore et al. 1992, Laughlin and Yen 1996, Perry et al. 1996, Lebenstedt et al. 1999, Pettersson et al. 1999, Gremion et al. 2001, Schaal et al. 2011).

Adequate carbohydrate intake is important for the effective restoration of liver glycogen to maintain normal blood glucose levels and thereby facilitate LH pulsatility (Figure 2) (Loucks et al. 1998, Loucks and Thuma 2003, Loucks 2014). A sufficient carbohydrate intake is also important in order to replenish muscle glycogen storage after training, since reduced carbohydrate availability for the working muscles limits performance during prolonged sub-maximal exercise, as well as during repeated bouts of high intensity exercise (Costill et al. 1973, Hawley and Burke 2010, Burke et al. 2011). Therefore, the recommended intake of carbohydrates is 5–7 g/day for athletes with a moderate exercise program of 1–1½ h/day in order to endure daily glycogen recovery (Burke et al. 2011). In female endurance trained athletes reported to run between 40 to 119 km/week or to train 6.4 to 10.4 h/week, the weighted mean intake of carbohydrates are 5.5 g/kg/day in athletes with MD and 5.4 g/kg/day in eumenorrheic athletes (Table 2).
The recommended protein intake for female endurance athletes in energy balance is 1.2–1.7 g/kg /day (Philips and Van Loon 2011). The weighted mean intake reported in athletes with MD is 1.3 g/kg/day, and in eumenorrheic athletes, it is 1.4 g/kg/day. Periods of low energy and/or carbohydrate intake may increase amino acid oxidation and hence protein requirements and 1.8–2.0 g/kg/day of protein is recommended during periods of energy deficiency in order to minimise the loss of FFM as well as to optimise glycogen storage (Burke et al. 2011, Philips and Van Loon 2011).

A lower intake of fat is frequently reported in female endurance athletes with MD compared to eumenorrhoeic athletes, independent of any differences in total energy intake (Deuster et al. 1986, Nelson et al. 1986, Kaiserauer et al. 1989, Laughlin and Yen 1996, Harber et al. 1998, Thong et al. 2000, Cobb et al. 2003, Tomten and Høstmark 2006) (Table 2). In a study by Horvath et al. (2000), *ad libitum* energy intake from a low-fat diet (17 E%) during a month only provided 60% of the required energy for female runners. The higher the contribution percentage was of energy intake fat, the lower the energy deficiency. The weighted mean fat intake reported in female endurance athletes with MD is 22 E% compared to 29 E% in eumenorrheic athletes (Table 2), indicating that diets with a lower fat content might increase the risk of low/reduced EA on a long-term basis.

In summary, most studies investigating EA, dietary intake, and training regimes in female endurance athletes have reported similar results in MD and eumenorrheic subjects. It is, therefore, possible that the threshold of low EA established in a laboratory setting that predicts the decline in LH pulsatility in eumenorrheic, sedentary women (Loucks and Thuma 2003) may be higher or may vary for maintaining oligomenorrhea/FHA in free-living athletes (Gibbs et al. 2013b, Ciadella-Kam et al. 2014). Furthermore, it is possible that specific dietary characteristics reported in female athletes with MD, such as lower energy density, a low fat content, and a high fibre intake might increase the risk of long-term

energy deficiency at a degree large enough to maintain the suppress of the hypothalamic-pituitary axis and, thereby, lead to oligomenorrhea/FHA. Therefore, more studies assessing EA and dietary characteristics related to reproductive function in a larger population of free-living athletes are needed.

The Disordered Eating Spectrum

Since weight influences performance in many sports, low EA could derive from an intentional restriction of food intake in order to obtain a low body weight (Nattiv et al. 2007). Most DE typically begins as a voluntary restriction of food intake, where the restricted eating behaviour progresses to chronic dieting and weight fluctuation, with increasingly pathological eating and weight-control behaviours with/or without excessive training (Sundgot-Borgen and Torstveit 2010). The DE continuum ends with overt clinical ED, where athletes struggle with abnormal eating behaviours, distorted body image, weight fluctuation, and extreme dieting, with the use of pathological compensatory strategies, such as fasting, dehydration, purging (i.e. vomiting), laxatives, and weight loss drugs (Sundgot-Borgen et al. 2013).

Predisposition to develop an ED is dependent on sociocultural, demographic, environmental, biological, psychological, and behavioural factors (Sundgot-Borgen et al. 2013) (Figure 1). In addition to dieting, personality factors such as perfectionism, pressure to lose weight, frequent weight cycling, early start of sport-specific training, overtraining, injuries, and coaching behaviour seem to be important risk factors in athletes (Sundgot-Borgen 1994, Sundgot-Borgen and Torstveit 2010). Athletes considered to be particularly vulnerable for the DE spectrum are those participating in sports that emphasize leanness: aesthetic sports (e.g., diving, figure skating, and gymnastics), endurance sports (e.g., distance running, triathlon, and swimming), and sports that require weight categories for competition (e.g., lightweight rowing and wrestling) (Byrne and McLean 2002, Sundgot-Borgen and Torstveit 2004, Torstveit and Sundgot-Borgen 2005a, Nichols et al. 2007). When using the DSM-IV criteria, 20% of Norwegian female elite athletes were reported as having overt ED, as compared to 9% of age-matched controls, while the prevalence among female endurance athletes has been reported to be 24% (Sundgot-Borgen and Torstveit 2004).

The most frequently reported ED diagnosis among female elite athletes is an eating disorder not otherwise specified (EDNOS, DSM-IV). Relatively few meet the criteria for bulimia nervosa or anorexia nervosa, especially the latter (Sundgot-Borgen and Torstveit 2004, Martinsen and Sundgot-Borgen 2013).

There are several standardized, self-reported questionnaires designed and validated to screen for DE in the general population, such as the Eating Attitudes Test (EAT) (Garner et al. 1982), the Three-Factor Eating Questionnaire (TFEQ) (Stunkard and Messick 1985), Eating Disorder Inventory (EDI) (Garner 2004), Eating Disorder Examination-Questionnaire (EDE-Q) (Fairburn et al. 2008), and the SCOFF Questionnaire (Hill et al. 2009). These screening tools, however, are not evaluated in athletic populations; hence the resultant information may not be accurate for athletes (Fairburn and Beglin 1994).

Table 2 Dietary intake in female endurance athletes divided by reproductive function

Reference	Methods	Athletes	Reproductive function	Training	BMI (kg/m²)	Body fat (%)	EI (MJ/day)	EI (kJ/kg/day)	CHO (g/kg/day)	Protein (g/kg/day)	Fat (g/kg/day)	Fat (E%)	Fibre (g/day)
Drinkwater et al. 1984	3 days food record. Self-reported DE excluded. Menstrual history, sex hormones (once a week x 4)	Runners	MD (n=14)	67 km/ week**	19.7	15.7	6.8	125	4.1	1.2	1.0	32.6	-
			EUM(n=14)	40 km/week	21.1	16.9	8.1	142	4.4	1.1	1.4	37.6	
Marcus et al. 1985	3 days food record. DE N.A. Menstrual history, oestrogen	Runners	MD (n=11)	93 km/week	18.6	10.0	5.3	107	-	1.0	-	-	-
			EUM (n=6)	93 km/week	21.5	11.1	7.2	133		1.3			
Deuster et al. 1986	3 days food record. DE N.A. Menstrual history, oestrogen (oligomenorrhea excluded)	Runners	MD (n=12)	119 km/week	18.3	11.2	9.0	181	6.4	1.5	1.4*	29.0*	9.0
			EUM (n=33)	113 km/week	19.6	12.2	10.4	199	6.2	1.6	1.9	36.3	7.0
Nelson et al. 1986	3 days food record. AN assessed and excluded. Menstrual history, oestrogen, gynaecological examination, prim. FHA excluded	Runners	MD (n=11)	56 km/week	21.2	21.6	7.2*	126**	3.4*	0.7	0.6**	33.4	-
			EUM (n=17)	64 km/week	20.2	19.7	9.4	169	4.4	1.0	0.8	33.5	
Kaiserauer et al. 1989	3 days food record. DE N.A. Menstrual history, oral temp (6-12 weeks). Prim. FHA excluded	Runners	MD (n=8)	63 km/week	18.8	11.8	6.6*	134*	4.5*	1.0*	1.0*	27.8	-
			EUM (n=9)	50 km/week	19.5	10.7	10.4	193	3.3	1.6	1.8	35.9	
Myerson et al. 1991	7 days food record. EAT-26, self-reported AN, BN excluded. Menstrual history, sex hormones.	Runners	MD (n=7)	84 km/week	19.4	14.6	7.2	141	4.6	1.0	1.0	27.1	6.4
			EUM (n=10)	83 km/week	19.5	15.0	8.1	158	5.3	1.3	1.3	31.1	7.3
Snead et al. 1992	7 days food record. EDI. Menstrual history, sex hormones, gynaecological examination.	Runners	MD (n=12)	46 km/week	20.3	20.1	8.6	149	4.9	1.2	1.2	30.2	-
			EUM (n=19)	49 km/week	21.8	22.1	8.3	140	4.2	1.3	1.2	32.7	

Table 2
continued

Reference	Methods	Athletes	Reproduc- tive function	Training	BMI (kg/m²)	Body fat (%)	EI (MJ/ day)	EI (kJ/kg/ day)	CHO (g/kg/ day)	Protein (g/kg/ day)	Fat (g/kg/ day)	Fat (E%)	Fibre (g/ day)
Wilmore et al. 1992	3 days food & activi-ty records. DE N.A. Menstrual history, sex hormones: once a week x 4.	Runners	MD (n=8)		18.4	10.8	7.4	145					
			EUM (n=5)	N.A	18.7	10.3	7.1	136	-	-	-	-	-
Laughlin, Yen 1996	7 days food & activi-ty records, EDI. Self-reported ED exclud-ed. Menstrual history, sex hormones, prim. FHA excluded	Runners, triath-letes	MD (n=8)	4.5 MJ/day	19.4	16.0	8.8	160	6.9**	1.2	0.6*	13.2*	25**
			EUM (n=8)	3.8 MJ/day	19.6	15.9	7.3	125	4.4	1.0	0.9	24.4	17
Perry et al. 1996	3 days food record, EAT-26. Menstrual history, oestrogen	Runners, triath-letes	MD (n=6)	59 km/week	20.6	19.1	7.8	143	4.2	1.3	0.4	10.9	-
			EUM (n=10)	63 km/week	21.0	18.6	8.5	156	5.9	1.3	0.7	17.0	
Harber et al. 1998	4 x days food rec-ords. DE N.A. Men-strual history, sex hormones; EUM: 3 times/week during 1 cycle, MD: once a week x 4.	Runners, cyclists, triath-letes	MD (n=8)	10.4 h/week*	18.6*	13.9	8.9	176	6.7	1.4	1.0	21.5*	-
			EUM (n=11)	7.2 h/week	20.5	16.8	8.7	156	5.5	1.3	1.1	25.6	
Pettersson et al. 1999	7 days food record. DE N.A. Menstrual history, sex hor-mones.	Runners	MD (n=10)	10.0 h/week	19.8	18.4	7.7	140	5.5	1.3	0.7	20.1	-
			EUM (n=10)	9.0 h/week	21.2	23.6	8.7	151	6.4	1.2	1.0	25.9	
Lebenstedt et al. 1999	7 days food record, Three-Factor Eating Questionnaire. Self-reported MD exclud-ed. MD assessed by morning saliva pro-gesterone (1 cycle).	Runners, triath-letes	Subclinical MD (n=12)	7.4 h/week	21.0	25.9	10.0	166	4.5	1.2	1.4	33.7	-
			EUM (n=21)	6.4 h/week	22.1	25.0	9.8	151	4.8	1.2	1.6	33.5	

Table 2
continued

Reference	Methods	Athletes	Reproductive function	Training	BMI (kg/m²)	Body fat (%)	EI (MJ/day)	EI (kJ/kg/day)	CHO (g/kg/day)	Protein (g/kg/day)	Fat (g/kg/day)	Fat (E%)	Fibre (g/day)
Thong et al. 2001	7 days food & activity records. Self-reported ED excluded. Menstrual history, sex hormones measured twice during 1 cycle. Oligomenorrhea, prim. FHA excluded	Cyclists, runners	MD (n=5)	106 km/week	18.9	14.6	7.0*	133**	5.5	1.0	0.7	17.0*	-
			EUM (n=8)	102 km/week	19.3	15.2	9.5	180	6.8	1.5	1.2	25.0	
Gremion et al. 2003	2x5 days food record. Self-reported DE. Menstrual history, sex hormones: 1/week during 3 cycles.	Runners	MD (n=11)	86 km/week	18.8	12.3	9.6	188	7.1	1.7	1.4	28.1	-
			EUM (n=10)	93 km/week	19.3	15.0	8.9	169	5.0	1.4	1.6	36.4	
Cobb et al. 2003	Food frequency questionnaire, EDI. Menstrual history.	Runners	MD (n=58)	63 km/week*	21.1	22.7	9.3	160		1.5	0.6*	15.3*	32.0
			EUM (n=33)	53 km/week	21.5	23.9	9.4	160	-	1.5	0.8	18.7	28.1
Tomten, Høstmark 2006	3 days weighed food records. Self-reported DE. Menstrual history, sex hormones.	Runners	MD (n=10)	7.5 h/week	20.7	19.8	9.7**	164**	5.7	1.5	1.0**	24.0(*)	-
			EUM (n=10)	6.7 h/week	20.0	18.1	12.3	217	7.0	1.7	1.7	30.0	
Schaal et al. 2011	7 days food records. EDE-Q. Menstrual history, oligomenorrhea/FHA verified by physician. Prim. FHA excluded.	Runners, triathletes	MD (n=6)	62 km/week	19.0	14.0	8.9	160	5.4	1.2	1.1	28.0	-
			EUM (n=6)	56 km/week	20.4	17.0	9.2	156	4.7	1.4	1.1	28.0	
Weighted mean		MD (n=217)			20.0	17.9	8.4	152	5.5[1]	1.3[3]	0.9[5]	21.9[5]	26.0[7]
		EUM (n=240)			20.6	18.2	9.2	164	5.4[2]	1.4[4]	1.3[6]	29.0[6]	16.3[8]

Only studies comparing dietary intake between menstrual dysfunction (MD) and eumenorrheic (EUM) endurance athletes are included. Abbreviation: AN: anorexia nervosa; BMI: body mass index; BN: bulimia nervosa; CHO: carbohydrates; EDE-Q: eating disorder questionnaire; EDI: eating disorder inventory; EI: energy intake; EEE: exercise energy expenditure; EUM: eumenorrhea; FFM: fat-free mass; FHA: functional hypothalamic amenorrhea; N.A: not assessed. *$P<0.05$, (*) $P=0.05$, **$P<0.01$ compared to EUM subjects. In the calculation of weighted mean, the following number of subjects were included: carbohydrates [1](n=134), [2](n=190), protein [3](n=209), [4](n=235), fat [5] (n=198), [6](n=229), and dietary fibres [7](n=84), [8](n=85).

Several DE screening tools targeting female athletes, such as the Athletic Milieu Direct Questionnaire (AMDQ) (Nagel et al. 2000), the Female Athlete Screening Tool (FAST) (McNulty et al. 2001), the College Health Related Information Survey (CHRIS-73) (Steiner et al. 2003), the Athlete Questionnaire (Hinton and Kubas 2005), and the Brief Eating Disorders in Athletes Questionnaire (BEDA-Q) (Martinsen et al. 2014), have also been developed. However, in order to diagnose clinical, overt ED, self-report questionnaires should be complemented with in-depth personal interviews that use a semi-structured format, such as the Eating Disorder Examination (EDE) Interview, which is considered to be the gold standard method (Fairburn and Beglin 1994, Sundgot-Borgen 2004, Fairburn et al. 2008, Martinsen and Sundgot-Borgen 2013). Furthermore, Black et al. (2003) developed the Physiological Screening Test (PST), a combined self-reported questionnaire regarding physiological symptoms (dizziness, gastrointestinal problems, and menstrual cycles per year) with measurements of skin folds, waist and hip ratio, standing diastolic blood pressure, and enlarged parotid glands.

Torstveit et al. (2008) evaluated the predictive power of three different models with regard to the detection of ED in female athletes. Model one was comprised of self-reported ED using the EDI-2 questionnaire; model two included a self-reported ED, low BMI (< 18.5 kg/m^2), EDI-2 drive for thinness score (DT-score) $\geq$ 15, EDI-2 body dissatisfactions score (BD-score) $\geq$ 14, and the use of pathological weight control methods (diet pills, hunger-repressive pills, laxatives, diuretics, or vomiting); and model three was made up of a self-reported MD, as well as stress fractures, in addition to all the variables of model two. Torsteveit et al. (2008) found that model one raised the probability of a clinical ED diagnosis among athletes in non-leanness sports, while model three, which comprising all the Triad components predicted ED in the whole group of athletes as well as in athletes in leanness-demanding sports.

It has been suggested that an elevated EDI drive for thinness score (DT-score), using a lower RMR$_{ratio}$ as confirmation of energy deficiencies in the group with an elevated DT-score, is an indicator of energy deficiency in recreationally active women (Gibbs et al. 2011). Several studies have found that athletes with subclinical MD and FHA are more likely to have an elevated DT-score or increased dietary restraint than eumenorrheic athletes (Lebenstedt et al. 1999, Warren et al. 1999, Cobb et al. 2003, Gibson et al. 2004, Nichols et al. 2006, Reed et al. 2011, Gibbs et al. 2011). Therefore, an elevated DT-score might be a useful screening tool to identify female athletes at risk for ED/DE and MD. Even though the prevalence of low EA is assumed to be high in female athletes and assumed to exist without the presence of ED/DE (Nattiv et al. 2007, Gibbs et al. 2013a, De Souza et al. 2014, Mountjoy et al. 2014), there are no screening tools currently developed to detect self-reported physiological symptoms of the Triad without ED/DE.

In summary, athletes participating in sports that emphasize leanness are considered to be particularly vulnerable for ED/DE; for example, the reported prevalence among female endurance athletes is 24%. There are screening tools developed to identify DE in female athletes. However, since low EA is reported also to exist without ED/DE, screening tools designed to detect self-reported physiological symptoms of the Triad without ED/DE are, therefore, needed.

Low Energy Availability without Disordered Eating Behaviour

Low/reduced EA could emerge unintentionally, because of the difficulty of increasing food intake sufficiently to meet energy requirements during periods with a high training volume and/or high intensity exercise (Stubbs et al. 2004, Nattiv et al. 2007). Certainly lifestyle and the effort required to obtain and prepare food can also make it difficult to increase the energy intake during times of with a high training volume, including the inhibitory effect of fatigue and fewer opportunities to participate in food-related activities on days in which a substantial number of hours are devoted to exercise. It has been reported that ad libitum energy intake in female athletes does not compensate for increased exercise energy expenditure, even after 27–48 days (Horvath et al. 2000, Whybrow et al. 2008), especially among female athletes eating bulky fibre-rich foods and low-fat diets (Horvath et al. 2000).

Appetite Regulation

The regulation of body weight is partially controlled by signals processed in the hypothalamus that reflect long-term energy balance and modulate day-to-day energy intake as well as energy expenditure (Huda et al. 2006). The arcuate nucleus in the hypothalamus contains two subsets of neurones that coordinate the signals and control food intake; one group contains neurones that express the orexigenic peptides, neuropeptide Y (NPY), and agouti-related peptide (AgRP), and the other expresses anorexigenic peptides derived from pro-opiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART) (Bing et al. 1996, Schwartz et al. 2000). When one group of neurons is activated, the other is inhibited, and thereby either an increase or decrease in energy intake is promoted (Schwartz et al. 2000, Druce et al. 2004). Leptin, ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) are some of the hormones involved in the regulation of appetite (Figure 2). Therefore, a combination of endocrine, behavioural, and psychological aspects is involved in controlling energy intake (Huda et al. 2006, Valassi et al. 2008, Warren 2011, Hirschberg 2012).

Leptin is a small anorexigenic polypeptide that suppresses appetite and is mainly produced in fat cells (Valassi et al. 2008, Fuqua and Rogol 2013), and the blood levels are proportional to fat stores (Keire et al. 2000). Low EA reduces the levels of leptin (Loucks and Thuma 2003, Donato et al. 2011), which enhances the release of NPY and AgRP, and increases the motivation to eat (Donato et al. 2011). Lowered levels of leptin have been reported in patients with ED (Warren 2011, Singhal et al. 2014) and in recreationally active women with MD (De Souza et al. 2004, De Souza et al. 2007, Schneid et al. 2009). Leptin receptors are expressed in many different organs and tissues, including the anorexigenic POMC-producing neurons as well as the orexigenic NPY and AgRP-producing neurons, which are thought to mediate most of the effects of leptin on food intake, energy expenditure, and reproductive function (Warren 2011, Donato et al. 2011).

While PYY and GLP-1 are primarily secreted by endocrine L cells of the intestine in response to intraluminal nutrients (Ballantyne 2006) that have anorexigenic actions (Huda et al. 2006), ghrelin secreted from the stomach is orexigenic and stimulates appetite and food intake. The levels of PYY and GLP-1 increase after food intake and promote satiety (Gradt et al. 1992, Keire et al. 2000, Huda et al. 2006). Elevated levels of PYY have been reported in athletes with MD (Russell et al. 2009) and in recreationally active women with MD (De Souza and Williams 2004, De Souza et al. 2007, Schneid et al. 2009), and, along with elevated levels

of GLP-1, have also been reported in in patients with anorexia nervosa (Warren 2011, Singhal et al. 2014). Increased PYY and GLP-1 generates an overall anorexogenic effect, and therefore might increase the risk for low EA due to a reduced appetite (Warren 2011).

High intensity exercise has been shown to have an acute suppressive effect on appetite (Larson-Meyer et al. 2012), rather than the converse. Hubert et al. (1998) showed that short-term food deprivation increased hunger in exercising women, but the same energy deficit produced by exercise energy expenditure did not. King et al. (1997) demonstrated that a substantial increase in energy expenditure due to intense exercise does not automatically lead to increased hunger or energy intake within 48 hours in healthy young men, and therefore a nutritional challenge for athletes could be that there is no strong drive to automatically match energy intake to exercise energy expenditure. The acute reduction of appetite after exercise is probably driven by the same complex changes in the appetite-regulating hormones as energy deficiency, since ghrelin, PYY, and GLP-1 have been reported to be increased in eumenorrheic female athletes after having run for one hour at 70% of their VO_{2max} (Larson-Meyer et al. 2012).

Energy Density

Energy density is the amount of energy (kJ) expressed per weight of food and is mainly influenced by the macronutrient composition and water content of the diet. The energy density of a food or a meal is more influenced by the content of fat than carbohydrates and protein, due to its higher energy density (38 kJ/g fat versus 17 kJ/g for carbohydrates and protein) than water content, since water lowers the energy density of foods, due to its contribution to weight, but not to energy (Rolls 2009). Additionally, adding bulky fibre to a diet will also reduce energy density (Slavin 2009). Exercising women with MD have been reported to eat diets characterized by a lower energy density than the diets of eumenorrheic women (Reed et al. 2011), and elite female soccer players with low EA have been reported to eat lower energy dense dinners than soccer players with EA greater than 125 kJ/kg FFM/day (Reed et al. 2014). The energy density of foods and meals affects satiety, which refers to the effects of food intake after eating has ended, as well as to satiation, which only refers to the process that leads to the termination of a meal (Rolls 2009). Rolls et al. have demonstrated the association between energy density and satiety in several studies, showing a 12%–20% lower energy intake in a test meal after eating a low energy density pre-load meal, such as an apple (15%) (Flood-Obbagy and Rolls 2009), soup (20%) (Flood and Rolls 2007) or a large salad (12%) (Rolls et al. 2004), as compared to consuming no pre-load meal.

Some dietary fibres, such as pectin (found in fruits and potatoes) and grains (found in oats, barley, rye, and wheat), form a gel-like substance in the stomach and delay the rate of food-emptying into the duodenum, which results in increased satiation and a prolonged feeling of satiety. Hence, these dietary fibres limit the intake of energy in a meal as well as delay the initiation of hunger, and thereby postpone the next meal (Cox 2006). Other types of fibres, such as cellulose and hemicellulose (found in vegetables and cereal bran), are not digested in the small intestine and bind with water in the large intestine, which may have a laxative effect (Lupton et al. 2006) and cause gastrointestinal dis-

comfort, a common problem among endurance athletes (Fallon 2006). Gastrointestinal problems due to a high fibre intake might potentially further decrease the motivation to eat, and therefore increase the risk for low/reduced EA by several mechanisms. Although Lloyd et al. (1987), Warren et al. (1994), and Laughlin and Yen (1996) identified a high fibre intake as a potential nutritional concern in female athletes with MD more than two decades ago, most studies investigating food intake in female endurance athletes have not assessed the intake of dietary fibres (Table 1). However, studies of sedentary premenopausal women have reported a negative association between fibre intake and oestrogen levels (Laughlin et al. 1998, Aubertin-Leheudre et al. 2008, Gaskins et al. 2009). The presumed mechanism involves oestrogens binding to fibre in the colon, which reduces the reabsorption of circulating oestrogen (Aubertin-Leheudre et al. 2008) and hence increases the excretion of oestrogens in the faeces. Oestrogen excreted from bile needs to be hydrolysed before it can be reabsorbed. An additional presumed oestrogen-lowering effect of fibre is linked to reduced β-glucuronidase activity in the colon, which leads to the reduced reabsorption of oestrogen (Aubertin-Leheudre et al. 2008).

In summary, low EA could occur intentionally in athletes who intend to lose weight or maintain a lower body weight; that is, female athletes have been reported to avoid compensating for increased exercise energy expenditure, especially those female athletes eating a diet with a low energy density. Low EA could also emerge unintentionally in athletes, since energy deficiency, as well as high intensity exercise, seems to generate similar endocrine alterations that generate an overall anorexogenic effect. An excessive intake of dietary fibres could, by several mechanisms, potentially increase the risk for low EA and oligomenorrhea/FHA. Very few studies, however, have reported dietary characteristics associated with EA and reproductive function in female athletes.

Potential Impact on Performance

Endurance training increases the fatigue resistance of contracting skeletal muscles during prolonged submaximal exercise by enhancing the maintenance of metabolic control (Burke et al. 2011, Hawley and Burke 2010) and circulatory effects, and by increasing the storage capacity for glycogen and lipids in the skeletal muscle (Costill et al. 1973). These adaptations include a shift towards more fat being oxidized during submaximal exercise (Hawley and Burke 2010). Trained, eumenorrheic, weight-stable women seem to have similar changes in substrate utilisation during moderate and high intensity exercise as trained men (Rominj et al. 2000). Carbohydrate availability is regulated directly in relation to exercise intensity; furthermore, low muscle glycogen concentrations (both diet and exercise induced) are associated with fatigue during prolonged, submaximal endurance training (< 85% peak oxygen uptake (VO_{2peak}) lasting more than two hours (Burke et al. 2011). Blood glucose levels have been shown to influence athletic performance (Costill et al. 1973, Bangsbo et al. 1992), and low levels of blood glucose have been reported in patients with ED (Warren 2011) and in female athletes with MD (Laughlin and Yen 1996). Therefore, persistent low energy and carbohydrate availability also seem to promote a shift in the working skeletal muscle's selection of metabolic fuels from glucose to fatty acid oxidation; furthermore, as EA declines, β-oxidation of fatty acids in the liver produces acetyl-CoA faster than the tricarboxylic acid cycle can accept, and the excess is converted to ketone bodies (primarily β-hydroxybutyrate and acetoacetate), released into the blood, and made available to be oxidized by

the brain (Figure 2). Another potential glucoregulatory mechanism is an increased hepatic gluconeogenesis, especially from amino acids, which have been obtained from the breakdown of skeletal muscle, accelerated by an increased cortisol/insulin ratio. Insulin and GH, along with the catecholamines, regulate the cycling of fatty acids in adipose tissue, and therefore the GH/insulin ratio is an index of lipolytic drive, which is accelerated by low EA (Loucks 2014). T_3, which stimulates mitochondrial ATP production and effects neural function, skeletal muscle protein turnover, fibre type expression, and Ca^{2+} uptake (Wrutniak-Cabello et al. 2001), is also lowered by chronic low glucose availability (Loucks 2014). Through these mechanisms, low T_3 levels reduce the ability for skeletal muscle to produce mechanical work (Loucks 2014).

There are few studies to date that have investigated the effect of MD on performance. Harber et al. (1996) reported that athletes with MD had a lower T_3 level and slower creatine phosphate recovery rates than eumenorrheic athletes, implying that the recovery rate of skeletal muscle might be altered in athletes with MD and influence their ability to perform repeated bouts of exercise. Recently, Van Heest et al. (2014) reported a 10% decline in the performance of young elite swimmers with chronic ovarian suppression and metabolic and hormonal perturbation, which was secondary to energy deficiency, during a 12-week competitive season; in contrast, there was an 8% improvement in the performance of the eumenorrheic group.

In summary, low energy and glucose availability and the appurtenant endocrine alterations are likely to influence substrate utilisation and performance of female athletes. Only two studies have investigated the association between oligomenorrhea/FHA and performance, and both studies indicate that oligomenorrhea/FHA have negative effects.

Assessing Energy Availability

Loucks et al. have recommended that EA should be calculated by subtracting exercise energy expenditure from the energy intake adjusted for FFM (Loucks et al. 1998, Loucks and Thuma 2003, Loucks et al. 2011). Furthermore, that exercise energy expenditure should only represent the amount of additional energy that has been expended as a result of exercise activity. Thus, the energy cost of daily living during the period of exercise should be subtracted from the estimates of exercise energy expenditure (Loucks et al. 1998). There are, however, several methodological difficulties involved in estimating EA, such as timing of the assessment in order to collect representative data of the athletes' habitual EA.

Some studies that have estimated EA have used a three- or seven-day food diary to estimate energy intake (Table 1) (Hoch et al. 2009, Hoch et al. 2011, Schaal et al. 2011, Gibbs et al. 2013b, Koehler et al. 2013, Lagowska et al. 2014), and some studies have performed repeated estimates of energy intake (De Souza et al. 1998, Reed et al. 2011, Reed et al. 2012, Woodruff and Meloche 2013, Guebels et al. 2014, Van Heest et al. 2014). Estimated energy intake and exercise energy expenditure are examined simultaneously in almost all of the studies, though the method used to estimate exercise energy expenditure varies. For example, a few studies have used activity logs and heart rate monitors, and have estimated exercise energy expenditure during training on the basis of heart rate in relation to O_2 consumption and the respiratory exchange ratio during laboratory testing

(Schaal et al. 2011, Gibbs et al. 2013b, Logawska et al. 2014). Accelerometers monitoring bodily movements have been used in other studies in order to assess exercise energy expenditure (Hoch et al. 2011, Woodruff and Meloche 2013, Guebels et al. 2014), although accelerometers have been reported to underestimate exercise energy expenditure at more rigorous exercise levels (Plasqui and Westerterp 2007, Nichols et al. 2010). A frequently used method, although less precise, is the use of prospective activity logs to calculate exercise energy expenditure from the corresponding Metabolic Equivalent of Task (MET) (Ainsworth et al. 2000) or other tables of exercise energy expenditure (De Souza et al. 1998, Hoch et al. 2009, Koehler et al. 2013, Van Heest et al. 2014), while some studies have used a combination of activity logs and heart rate monitors and/or accelerometers (Reed et al. 2011, Reed et al. 2012, Guebels et al. 2014). The recommended adjustment of exercise energy expenditure for the energy expenditure of sedentary activity, which would have occurred instead of exercise, (Loucks et al. 1998) has only been used in a few of the studies (Reed et al. 2012, Gibbs et al. 2013b, Koehler et al. 2013).

Another methodological issue when assessing EA is how to define exercise. In the existing literature, different terminology and definitions have been used, such as all sport or exercise sessions/activities (Hoch et al. 2009, Schaal et al. 2011, Reed et al. 2011, Koehler et al. 2013, Van Heest et al. 2014, Lagowska et al. 2014), all physical activity (Hoch et al. 2011), all weight-bearing physical activity (De Souza et al. 1998), all physical activity except daily living activities (e.g., cleaning the house or walking the dog) (Reed et al. 2012, Gibbs et al. 2013b), or as all physical activity > 4 MET (Guebels et al. 2014), corresponding to moderate activities, such as playing golf or vigorous housekeeping (Ainsworth et al. 2000). Logically, an athlete working full time in a physically demanding trade, (e.g., as a physiotherapist), is likely to have a higher level of NEAT, and thereby an increased total energy expenditure compared to an athlete who is a full time student or a professional athlete. Therefore, a physiological purposeful definition for exercise energy expenditure when estimating EA is needed.

Four studies have assessed EA in female athletes (Schaal et al. 2011, Woodruff and Meloche 2013, Van Heest et al. 2014, Lagowska et al. 2014). It should be noted that two of these studies have a sample size of five and six subjects in each group (Schaal et al. 2011, Van Heest et al. 2014). In the study by Lagowska (2014), forty-five MD subjects, but no eumenorrheic subjects, were investigated; in contrast, Woodruff and Meloche (2013) examined ten eumenorrheic, but no MD subjects. There are several methodological diversities in the assessment of EA between these studies and none have adjusted exercise energy expenditure for sedentary activities as recommended by Loucks and Thuma (2003).

In summary, no study assessing EA has used gold standard methods to diagnose subjects with ED. Only two studies with small sample sizes have assessed EA in female athletes as related to their reproductive function, and none of these have used recommended methods when calculating EA. More studies assessing EA using the recommended methods are therefore needed.

Reproductive Function

The menstrual cycle is regulated via cross-talk over the hypothalamic pituitary ovarian axis (positive and negative feedback). The concentration of sex steroids changes during the menstrual cycle (Figure 3), and the feedback mechanism is regulated by the interplay between GnRH in the hypothalamus and changes in the circulating pituitary gonadotropins, in the LH and follicle stimulating hormone (FSH), and in the gonadal hormones (oestrogen and progesterone) (Harber 2004) (Figure 2). Over the period of one cycle, oestrogens increase in eumenorrheic women, peaking between day 12 and day 14 (end of the follicular phase), and again, at a lower level, between day 19 and day 24 (mid luteal phase) (Figure 3). Concomitantly, the pulsatility of LH increases from 65 to 80 per minute during the follicular phase to 185 to 200 per minute in the luteal phase, and then decreases again (Harber 2004).
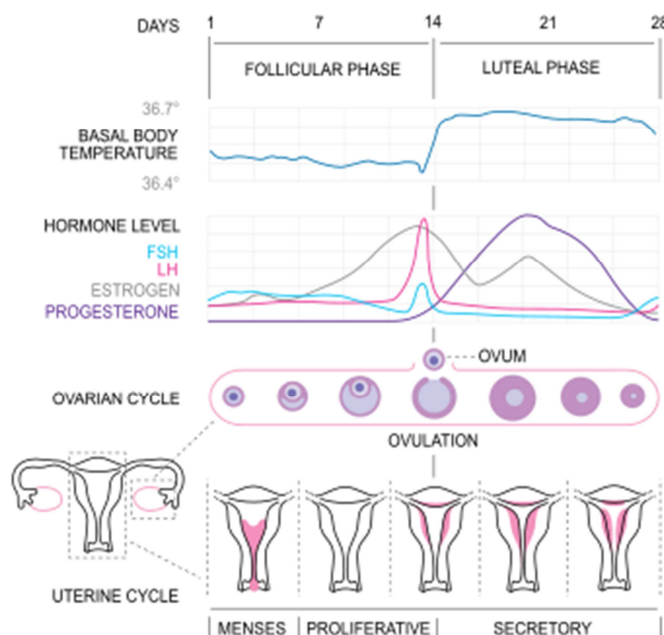
Figure 3 The menstrual cycle

The figure illustrates the menstrual cycle divided in the follicular and luteal phases, the time for ovulation, and the uterine cycle divided into the menstruation, proliferation, and secretary phases. Furthermore, it illustrates the fluctuations of sex hormones and body temperature during the menstrual cycle.

Menstrual Dysfunction

The continuum of reproductive function in female athletes linked to EA ranges from eumenorrhea, to subclinical MD issues such as luteal phase defects and anovulation, to clinical MD issues such as oligomenorrhea (menstrual cycle longer than 35 days) and FHA (primary amenorrhea: menarche

after 15 years of age, and secondary amenorrhea: absence of bleeding for at least three consecutive menstrual cycles) (Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014).

The estimated prevalence of MD ranges from 2%–46% among athletes, depending on the methodology used for assessment, the sport, and the diagnostic definition used, as compared to 2%–5% in non-exercising women; furthermore, the reported prevalence of MD has been shown to be similar among competitive and recreationally active women (Gibbs et al. 2013a). In sedentary women, polycystic ovarian syndrome (PCOS) is reported to be the most frequent reason for oligomenorrhea/amenorrhea (Solomon et al. 2002), and is also reported to be frequent among female elite athletes with MD (Hagmar et al. 2009). Although MD is common among female athletes, it is often ignored and regarded as a natural result of intense training (Beals and Meyer 2007), despite the fact that health consequences are well documented and include lower bone mineral density with an increased risk of premature osteoporosis (Constantini and Warren 1994, Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014) and an increased risk of developing cardiovascular diseases, in terms of PCOS (Solomon et al. 2002).

Aetiology of Menstrual Dysfunction in Female Athletes

Exercise or other physiologically distressing conditions per se, such as sleep deprivation, have been demonstrated not to disrupt reproductive function as long as the energy intake is not restricted (Loucks et al. 1998, Friedl et al. 2000), while persistent limited availability of metabolic fuels has frequently been reported to be the primary cause of the development of oligomenorrhea/FHA (Loucks et al. 1998, Cumming and Cumming 2001, Loucks and Thuma 2003, Wade and Jones 2004). Hormonal synthesis, follicular development, endometrial proliferation, and luteal phase thermogenesis are energy consuming processes (Harber 2004), and experimental studies on healthy women have shown that EA of < 125 kJ/kg FFM/day for five days is enough to disrupt the pulsatility of LH and to reduce oestrogen and progesterone levels in healthy sedentary women (Loucks and Thuma 2003). The LH pulsatility is reported to be more sensitive to low EA in young women with a gynaecological age (time since menarche) < 14 years of age, as compared to women with a gynaecological age > 14 years of age (Loucks 2006a).

Energy Availability and Menstrual Dysfunction

Insufficient availability of metabolic fuels, especially glucose, will be detected by cells in the hindbrain, where neurons producing NPY and catecholamine (noradrenalin and dopamine) project to the forebrain where they inhibit GnRH pulsatility mediated by the corticotrophin-releasing hormone (CRH) (Wade and Jones 2004) and gamma-amino butyric acid (GABA) (Gordon 2010, Warren 2001). The suppression of GnRH reduces the LH-pulsatility and the FSH production in the pituitary glands, which leads to a reduced production of oestrogen and progesterone in the ovaries, and to a disruption of the feedback mechanism (Figure 2). Liver glycogen (about 80–120g) is the principal store of glucose available to the brain, but it is only a one-day supply, and half is depleted during the overnight fast (Loucks 2014). Loucks and Thuma (2003) reported in their study on sedentary eumenorrheic women that, as EA during five days of testing were reduced to 84 kJ/kg FFM/day, the effects on plasma glucose were minimized by multiple neuroendocrine glucoregulatory mechanisms. However, when EA was further reduced to 42 kJ/kg FFM/day, the carbohydrate intake was reduced to ~155 g/day,

of which skeletal muscle oxidized ~85 g/day during exercise. The remaining carbohydrate availability was only ~70 g/day, and within five days, the liver glycogen stores became depleted, and the body's mechanisms were unable to maintain normal plasma glucose levels.

Ghrelin and leptin have also been suggested to be indirectly involved in the suppression of GnRH pulsatility, and thereby in the development of MD (Couzinet et al. 1999, Schneid et al. 2009, De Souza 2010, Donato et al. 2011, Fuqua and Rogol 2013). In rodent models, high PYY levels have been reported to decrease GnRH-mediated LH secretion (Fernandez-Fernandez et al. 2005, Pinilla et al. 2007), and higher PYY has been reported in exercising women with MD than in eumenorrheic exercising women (Schneid et al. 2009).

Assessing Menstrual Dysfunction in Athletes

Menstrual dysfunction is common among female endurance athletes, indeed a prevalence of 63% has been reported (Pollock et al. 2010). As previously described, PCOS is also reported in female elite athletes (Hagmar et al. 2009). The aetiology of PCOS is not related to low EA, and therefore a clinical diagnosis of oligomenorrhea/FHA requires a pertinent evaluation of sex hormones and ultrasound examination by a skilled gynaecologist in order to establish if the origin of the MD is due to a suppression of the hypothalamic pituitary ovarian axis (De Souza et al. 2014). Many studies, however, have used only self-reported menstrual history (Kaiserauer et al. 1989, Deuster et al. 1996, Pettersson et al. 1999, Cobb et al. 2003, Torstveit and Sundgot-Borgen 2005b, Nichols et al. 2007, Pollock et al. 2010, Rauh et al. 2010, Barrack et al. 2011, Woodruff and Meloche 2013) or a combination of menstrual history and a single measurement of sex hormones (Marcus et al.1985, Myerson et al. 1991, Wilmore et al. 1992, Laughlin and Yen 1996, Perry et al. 1996, Harber et al. 1998, Hoch et al. 2003, Warren et al. 2003, Gibson et al. 2004, Rickenlund et al. 2005, Tomten and Høstmark 2006, Hoch et al. 2009, Hoch et al. 2011, Ackerman et al. 2011) when diagnosing MD in female athletes and recreationally active women. A few studies have included a gynaecological ultrasound examination in the diagnostic assessment (Nelson et al. 1986, Snead et al. 1992, Hagmar et al. 2009, Schaal et al. 2011, Lagowska et al. 2014), while many studies have included, instead, repeated measurements of sex hormones in blood, urine, or saliva during one or more menstrual cycles (Drinkwater et al. 1984, De Souza et al. 1998, Lebenstedt et al. 1999, Thong et al. 2000, Gremion et al. 2001, Schneid et al. 2009, Reed et al. 2011, Guebels et al. 2014).

In summary, the causal link between low energy and glucose availability and the endocrine perturbation related to oligomenorrhea/FHA, especially in young women, is well documented. Nonetheless, oligomenorrhea/FHA is common among female endurance athletes it is often ignored, despite the fact that the increased risk of premature osteoporosis is documented. Since there are different types of MD, clinical diagnosis requires a pertinent evaluation of sex hormones and ultrasound examination by a skilled gynaecologist in order to establish the origin of the MD.

Impact on Energy Metabolism

Most female athletes are lean, but have a body weight and body composition within the normal range, independent of their reproductive function (Redman and Loucks 2005). A stable body weight, however, might be an unreliable indicator of sufficient energy intake in female athletes. When energy intake is inadequate for maintenance of all basal physiological processes, the allocation is prioritized

to those processes that are essential for immediate survival (Wade and Jones 2004). Body tissue might therefore be preserved during long-term low EA by metabolic adaptations, such as a reduction in RMR, by reduced NEAT, or by increased work efficiency as reported in overweight subjects (Redman et al. 2009, Goldsmith et al. 2010).

Some studies show that RMR changes up to 10% during the menstrual cycle (Solomon et al. 1982, Meijer et al. 1992, Henry et al. 2003), with an increase in the last part of the luteal phase (7–10 days before menstruation), followed by a sudden drop in the early follicular phase (the first days of bleeding), with a gradual return to normal levels within 7–10 days (Solomon et al. 1982), as seen in Figure 3. The change in RMR is suggested to be mediated by the changes in progesterone (Solomon et al. 1982); additionally, Dalvit (1981) reported that the increased energy expenditure in the luteal phase is compensated for by an increase in food intake during the 10 days following, in the follicular phase. Biedleman et al. (1995) reported a higher RMR in female eumenorrheic athletes than in eumenorrheic sedentary women; otherwise, studies have found similar RMR in eumenorrheic women independent of the level of physical activity (Mulligan and Butterfield 1990, Myerson et al. 1991, O'Donnell et al. 2009). Patients with anorexia nervosa have MD, since it is one of the DSM-IV diagnostic criteria (American Psychiatric Association 2013); furthermore, many studies have reported a higher RMR in eumenorrheic athletes, (Myerson et al. 1991, Lebenstedt et al. 1999, Schneid et al. 2009, Van Heest et al. 2014) as well as in eumenorrheic recreationally active women, (De Souza et al. 2008, O'Donnell et al. 2009, Reed et al. 2011) than in MD subjects, (Table 3). Only Guebels et al. (2014) have reported a higher RMR in recreationally active women with MD than in eumenorrheic subjects. The reported mean RMR pooled from these studies indicates that RMR is ~130 kJ/kg FFM/day in (sedentary eumenorrheic women as well as athletes and recreationally active women) compared to ~118 kJ/kg FFM in athletes and recreational active women with MD, while RMR is reported to be ~110 kJ/kg FFM/day in patients with anorexia nervosa (Platte et al. 2000). Most RMR measurements in eumenorrheic women have been performed in the follicular phase, suggesting a higher mean RMR during the whole menstrual cycle in eumenorrheic women, and that the energy preserving effect, as a result of MD, could therefore be as high as 0.5–1.1 MJ/day with a FFM of 45 kg.

The ratio (RMR_{ratio}) between the measured RMR (mRMR) and the predicted RMR (pRMR) has also been reported to be low in patients with anorexia nervosa (0.60 to 0.80) (Platte et al. 2000, Marra et al. 2002). An $RMR_{ratio} < 0.90$ has been used in some studies with recreationally active women as an indicator of low EA instead of assessing EA (De Souza et al. 2007, De Souza et al. 2008, Schneid et al. 2009, Gibbs et al 2011). Since FFM is the main determinant of RMR (Speakman and Selman 2003), the Cunningham equation has been found to be the best predictive equation for RMR in female athletes (Thompson and Manore 1996). In the studies investigating RMR_{ratio} in recreationally active women (De Souza et al. 2007, De Souza et al. 2008, Schneid et al. 2009, Gibbs et al. 2011) and female athletes (Lagowska et al. 2014, Van Heest et al. 2014), the equation of Harris Benedict, which only includes age, height, and weight, has been used, which might underestimate pRMR, and thereby overestimate RMR_{ratio} in lean athletes.

Several of the studies assessing EA in recreationally active women and female athletes (Table 2) have also measured RMR (Reed et al. 2011, Gibbs et al. 2013b, Guebels et al. 2014, Van Heest et al. 2014, Lagowska et al. 2014), but none have reported the RMR results in relation to current EA, independent of reproductive function,

Table 3 Resting metabolic rate in women divided by reproductive function

Reference	Population	Reproductive function	BMI (kg/m²)	RMR (kJ/kg FFM/day)	Day in cycle
Mulligan & Butterfield 1990	Sedentary women	EUM (n=5)	N.R	124	
	Endurance athletes	EUM (n=7)	N.R	122	7-10/21-25
Myerson et al. 1991	Sedentary women	EUM (n=10)	22.6	136	
		MD (n=7)	19.4	107§*	
	Distance runners	EUM (n=10)	19.5	130	5-10
Willmore et al. 1992	Distance runners	MD (n=8)	18.4	125	
		EUM (n=5)	18.7	121	N.R
Biedleman et al. 1995	Sedentary women	EUM (n=10)	21.6	124	
	Distance runners	EUM (n=10)	21.5	132§	3-10
Thompson & Manore 1996	Endurance athletes	EUM (n=13)	20.0	139	0-5
Smith et al. 1997	Sedentary women	EUM (n=12)	21.6	134	1-7
Lebenstedt et al. 1999	Endurance athletes	MD (n=12)	22.2	116*	
		EUM (n=21)	21.0	124	0-8
De Souza et al. 2008	Recreationally active women	MD (n=15)	20.6	123**	
		EUM (n=29)	21.5	131	0-6
O´Donnell et al. 2009	Sedentary women	EUM (n=9)	22.2	142	
	Recreationally active women	MD (n=8)	21.0	117§*	
		EUM (n=14)	21.2	133	2-6
Schneid et al. 2009	Sedentary women	EUM (n=8)	22.3	139	
	Recreationally active women	MD (n=20)	20.8	119**	2-6
		EUM (n=20)	21.6	131	
Reed et al. 2011	Recreationally active women	MD (n=12)	20.5	110**	21-27
		EUM (n=13)	21.2	125	
Guebels et al. 2014	Recreationally active women	MD (n=8)	22.0	131*	1-7
		EUM (n=9)	23.2	122	
Lagowska et al. 2014	Mixed athletes	MD (n=45)	20.6	121	-
Van Heest et al. 2014	Adolescent swimmers	MD (n=5)	25.0	95*	N.R
		EUM (n=5)	21.0	118	
Weighted averages	Sedentary women	EUM (n=54)	22.0	134	
	Athletes	MD (n=77)	20.8	118	
		EUM (n=71)	20.5	128	
	Recreationally active women	MD (n=63)	20.9	120	
		EUM (n=85)	21.6	129	

Abbreviation: RMR: resting metabolic rate; BMI: body mass index; EUM: eumenorrhea; MD: Menstrual dysfunction; N.R.: not reported. § $P<0.05$ and §§ $P<0.01$ compared to sedentary women. *$P<0.05$ and **$P<0.01$ compared to eumenorrheic athletes/recreationally active women.

and therefore, it is not known whether a lowered RMR_{ratio} is only a useful biomarker for long-term persistent energy deficiency with MD, or if it also is a useful indicator of more short-term low/reduced EA, that could be used to find athletes at risk for other Triad conditions.

Maintenance of a body weight 10% above or below the customary weight for lean or obese individuals has been linked to greater changes in the total energy expenditure than can be explained by altered body weight or body composition, and are suggested to be due mainly to altered skeletal muscle work efficiency at low levels of power generation (Goldsmith et al. 2010). Increased work efficiency, seen as a reduction in energy spent in order to produce a given amount of external work, has been reported during low intensity exercise in dieting sedentary subjects (Redman et al. 2009, Goldsmith et al. 2010), but also in hyperactive anorexia nervosa patients (Platte et al. 2000). However, no difference in work efficiency was reported in a small study that compared RMR and work efficiency in eumenorrheic female runners in severe negative energy balance (n = 7) and eumenorrheic controls in energy balance (n = 5) (Mulligan and Butterfield 1990). Whether differences in work efficiency can be detected in a larger study population when comparing eumenorrheic athletes to athletes with oligomenorrhea/FHA, has not been investigated.

In summary, when EA is inadequate for maintenance of all basal physiological processes in female athletes, the allocation seems to be prioritized to processes that are essential for immediate survival, and many studies have reported a lower RMR in women with MD than in eumenorrheic women. No study, however, has investigated the association between RMR and low or reduced EA in female athletes. Furthermore, it would be relevant to investigate if the metabolic adaptation in female athletes also includes a lowered NEAT (reduced sedentary activities and/or increased work efficiency during sedentary activities).

Impact on Bone Health

Impaired bone health is a severe complication of low EA and oligomenorrhea/FHA (Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014), in which the endocrine alterations due to low EA contribute to bone loss (Warren 2011, Scofield and Hecht 2012). The International Society for Clinical Densitometry recommends using an age-matched *Z*-score instead of a *T*-score to estimate the level of BMD in premenopausal women and children (Nattiv et al. 2007). Weight-bearing exercise normally has beneficial effects on bone mineral accumulation, especially in adolescence (Barrack et al. 2010a), and it is common for athletes to have a minimum 5%–15% higher BMD and peak bone mass than non-athletes (Nattiv et al. 2007, Barrack et al. 2010a). The ACSM Position Stand and the IOC defines the continuum of bone health as normal BMD (*Z*-score ≥ -1), low BMD, or BMD below the expected range for age (*Z*-score < -1 to -1.99) and osteoporosis with secondary clinical risk factors for fracture (*Z*-scores ≤ -2) (Nattiv et al. 2007). The reported prevalence of impaired bone health in female athletes ranges from 0%–40% in terms of low BMD, and 0%–15% in terms of osteoporosis (Gibbs et al. 2013a). Bone mineral density reflects a lifelong history of EA, reproductive function, and mechanical loading, and declines with the number of menstrual cycles missed since menarche (Lloyd et al. 1987). The aetiology of impaired bone health in female ath-

letes is therefore complex, involving the cumulative history of exercise, energy, and nutrient availability, genetics, and reproductive and endocrine function (Lebrun 2006, Nattiv et al. 2007).

Bone is an organ, one that reshapes itself, has constant turnover, and adapts to changes in the biomechanical forces from physical activities. Under normal conditions, there is a continuous cyclic remodelling of bone, where osteoclasts remove old and micro-damaged bone by acidification and proteolytic digestion, and osteoblasts begins the replacement process of forming new and mechanically stronger bone by forming osteoid (an extracellular organic matrix of un-calcified collagen and other proteins), which is the first step in the osteogenesis. Osteoblasts that develop from mesenchyme stem cells in the bone marrow are responsible for the subsequent matrix mineralization, leading to the formation of bone. Osteoblasts produce osteocalcin, which is the most abundant non-collagenous protein of bone matrix. The osteoblasts possess receptors for parathyroid hormone and oestrogen, but also for other bone stimulating factors, such as growth factors (Manolagas and Jilika 1995, Kini and Nandeesh 2012).

Physical activity acts mainly through the osteocytes. The hormonal regulation of bone and bone mineral metabolism results from the interactions between parathyroid hormone, calcitonin, and vitamin D in order to regulate the absorption and release of calcium and phosphorous in the bone, the kidneys, and the gastrointestinal tract. The greatest bone formation occurs during the growth spurt that accompanies adolescence, and the majority of adult levels of bone mass are already achieved by 18 years of age, followed by only small amounts added until BMD reaches its peak (peak bone mass) by the end of the third decade (Kini and Nandeesh 2012). A study on adolescent and young, sedentary women reported that bone density in the trabecular vertebral bone in the spine already reaches its peak near the end of the second decade (Gilsanz et al. 1988). The achievement of optimal BMD and the regulation of bone maintenance therefore depend upon a combination of mechanical, hormonal, and dietary factors (Lebrun 2006), and an adequate hormonal status (oestrogen and progesterone), and sufficient nutrition (energy intake, calcium, protein, and other bone-building materials), especially during adolescence, are essential (Barrack et al. 2010a,b).

Restricted eating behaviour and endurance running in five or more seasons have been reported to be predictors of low BMD in adolescent endurance runners (Warren and Perlroth 2001), while adolescent athletes with MD have more frequent incidences of impaired bone microarchitecture than non-athletes (Ackerman et al. 2011). Factors affecting bone formation are reported to be sensitive to restricted eating behaviour, and that suppression may occur when EA is 125 kJ/kg FFM/day, and perhaps at even higher levels of EA (Ihle and Loucks 2004). Disordered eating behaviour significantly affects BMD in female endurance athletes, even in the absence of MD (Cobb et al. 2003, Barrack et al. 2008). The effect of EA on BMD is thought to be mediated by endocrine factors, including an oestrogen-dependent pathway increasing bone resorption (Barrack et al. 2010b), as well as an oestrogen-independent pathway decreasing bone formation (Warren and Perlroth 2001) and sustained malnutrition with low calcium and vitamin D intake (Nattiv et al. 2007).

The principal role of oestrogen in bone turnover is to suppress osteoclast activity (Ihle and Loucks 2004). Oestrogen receptors have been found on osteoblast-like cells, and hypoestrogenism seems to increase bone resorption (Fabbri et al. 1991). In a study on women with MD and hyperprolactinemia, bone mass in the peripheral cortical bone was only slightly decreased from age-matched controls, while the metabolically active spinal trabecular bone was decreased by 20% to 30% (Cann et al. 1984). The oestrogen-independent pathway involves the suppression of insulin, T_3, IGF-1, and leptin, as well as an increase of ghrelin and PYY, factors affecting bone formation (Warren and Perlroth 2001). Oligomenorrhea/FHA (De Souza et al. 2004) and anorexia nervosa are strongly associated with increased cortisol levels, which negatively affect bone metabolism and BMD, both directly via the inhibition of osteoblast proliferation and differentiation, as well as via the stimulation of osteoclast proliferation, and indirectly via its antagonistic effects on bone growth (Howgate et al. 2013). These endocrine alterations are thought to contribute to a negative effect on bone turnover in athletes (Ihle and Loucks 2004, Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014) and in patients with active anorexia nervosa (Howgate et al. 2013), leading to increased bone resorption in relation to bone formation.

A 3-year longitudinal study of forty adolescent endurance runners found that 13 of 15 runners with low bone mass at baseline also exhibited low bone mass at the 3-year follow-up assessment, despite a ~5kg increase in body weight and an increase in the number of menses in the past year (Barrack et al. 2011). Oestrogen replacement alone, which is a frequent treatment for female athletes with MD, may prevent further bone loss (Hergenroeder et al. 1997, Cobb et al. 2003), but clinical trials in young women with MD and low EA have reported that lost bone is not fully replaced by oestrogen replacement therapy (Hergenroeder et al. 1997, Warren et al. 2003), by the return of menstrual cycles (Drinkwater et al. 1986, Keen and Drinkwater 1997, Warren et al. 2002, Warren et al. 2003) or weight gain (Soyla et al. 2002). Energy and nutrient restrictions and low oestrogen levels, together and independently, therefore pose a significant risk for low BMD and osteoporosis in physically active women.

The bones are anabolically stimulated by the strain developed at the particular site of impact; furthermore, the ground reaction force and/or the force induced by muscle contractions will put a load on the skeleton and exert a positive effect on bone remodelling and modelling (Kerr et al. 2006). Varied and large mechanical loading patterns on the bone, such as compression, tension, bending, shear, and rotation, have the largest potential effect on bone formation (Wulff et al. 2012). Several studies on female elite athletes from weight-bearing endurance sports have reported a negative association between training hours/week and the whole body, as well as the lumbar spine BMD in subjects with energy deficiency and/or MD (Robinson et al. 1995, Cobb et al. 2003, Hind et al. 2006, Pollock et al. 2010, Barrack et al. 2010b). Endurance athletes with low EA and MD may therefore be at an even greater risk for low BMD (Barrack et al. 2010a, Tenforde and Fredericson 2011).

In summary, bone health reflects a lifelong history of EA and reproductive function and mechanical loading. The aetiology of impaired bone health in female endurance athletes is therefore complex, involving training hours/week, low energy and nutrient availability, MD, and endocrine perturbation.

AIM

The overall aim of this project was to study the prevalence and potential dietary risk factors and consequences of current low EA and menstrual dysfunction in a group of female elite endurance athletes. The specific research questions were:

➢ Do subjects with current low and reduced EA display adaptations in their energy metabolism in the form of lower RMR and higher work efficiency compared to subjects with optimal EA?

➢ Do subjects with oligomenorrhea/FHA display adaptations in their energy metabolism in the form of lower RMR and higher work efficiency compared to eumenorrheic subjects?

The results concerning the energy metabolism are presented in Paper I, 'Energy Availability and the Female Athlete Triad in Elite Endurance Athletes'.

➢ Do subjects with current low and reduced EA report a lower energy intake and/or have higher exercise energy expenditure compared to subjects with current optimal EA?

➢ Do subjects with oligomenorrhea/FHA report lower energy intake and/or have higher exercise energy expenditure, and thereby a current lower EA, compared to eumenorrheic subjects?

➢ What dietary characteristics are associated with current low and reduced EA in female endurance athletes without ED/DE?

➢ What dietary characteristics are associated with oligomenorrhea/FHA in female endurance athletes without ED/DE?

The results concerning dietary characteristics associated with current EA and reproductive function are presented in Paper II, 'Lower Energy Density and High Dietary Fibre Intake are Dietary Concerns in Female Endurance Athletes'.

➢ What is the prevalence of the Triad and its related conditions in a group of Swedish and Danish elite female endurance athletes?

The results concerning the prevalence of the Triad conditions are presented in Paper I: 'Energy Availability and the Female Athlete triad in Elite Endurance Athletes'.

➢ What physiological symptoms related to the Triad are relevant to include in a brief self-reported screening instrument in order to identify female athletes at risk for the Triad?

The development procedure and the results from the validation of the Low Energy Availability in the Female Athlete Questionnaire (LEAF-Q) are presented in Paper III, 'The LEAF-Questionnaire: A Screening Tool for the Identification of Female Athletes At Risk for the Female Athlete Triad'.

METHODS

Permission to undertake the study was provided by the Swedish and Danish Confederation of Sports, Team Denmark, the Data Inspectorate, and the Regional Ethical committees, both in Sweden and Denmark. The recruitment procedure, all methods, and statistical analysis used are described in detail in Papers I, II, and III.

Recruitment

The study population was recruited through the Danish and Swedish sports federations in endurance sports, competitive endurance sports clubs, and a professional dancing company. Interested athletes received the study protocol and the first version of the LEAF-Q. The athletes who returned the questionnaire and acknowledged that they were interested in further participation were contacted by the research group; a total of 120 athletes volunteered (Figure 4). Subjects included were women, 18–39 years of age, who trained a minimum of five times per week. A sub-sample of endurance athletes and dancers (n = 37) participated only in the initial reliability testing of the LEAF-Q. Before inclusion in the clinical examination procedure, all subjects were informed of all study procedures and signed an informed consent form. Exclusion criteria included: pregnancy, chronic illness, smoking, use of any forms of contraceptives other than oral, inability or unwillingness to discontinue oral contraceptives for at least six weeks prior to the investigation, and any injuries preventing the athlete from training ≥ 2 weeks. In the clinical examination procedure, including the clinical verification of self-reported symptoms from the LEAF-Q (Paper III), only athletes from weight-bearing endurance sports participated. Forty-five athletes completed the study protocol; twenty-five were not able to participate, eleven were excluded, and two dropped out. When investigating the association between current EA, reproductive function, and energy metabolism, as well as the prevalence of Triad and associated conditions, subjects with a MD other than oligomenorrhea/FHA were excluded, and forty subjects were included in the analyses (Paper I). In the section investigating dietary characteristics associated with current EA and reproductive function, eleven subjects with ED/DE and four subjects identified as having low validity of their reported energy intake (Black 2000), besides the five subjects with MD other than oligomenorrhea/ FHA, were excluded; and a total of twenty-five subjects were included in the analyses of dietary characteristics (Paper II).
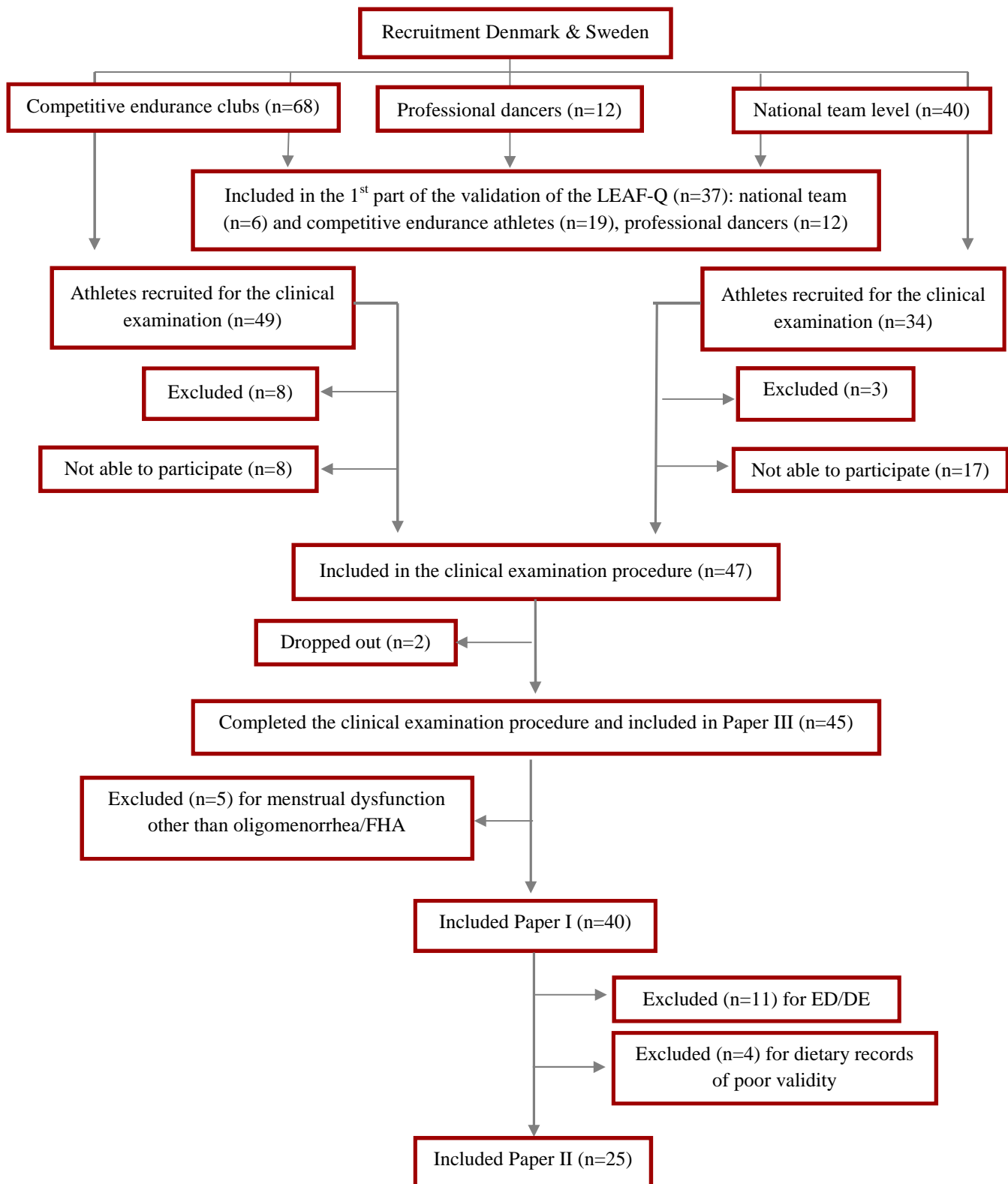
```
                        ┌─────────────────────────────────┐
                        │  Recruitment Denmark & Sweden   │
                        └─────────────────────────────────┘
        ┌───────────────────────────┼───────────────────────────┐
┌─────────────────────────┐  ┌───────────────────────┐  ┌─────────────────────────┐
│ Competitive endurance   │  │ Professional dancers  │  │ National team level     │
│ clubs (n=68)            │  │ (n=12)                │  │ (n=40)                  │
└─────────────────────────┘  └───────────────────────┘  └─────────────────────────┘
```

Included in the 1st part of the validation of the LEAF-Q (n=37): national team (n=6) and competitive endurance athletes (n=19), professional dancers (n=12)

Athletes recruited for the clinical examination (n=49)

Athletes recruited for the clinical examination (n=34)

Excluded (n=8)

Excluded (n=3)

Not able to participate (n=8)

Not able to participate (n=17)

Included in the clinical examination procedure (n=47)

Dropped out (n=2)

Completed the clinical examination procedure and included in Paper III (n=45)

Excluded (n=5) for menstrual dysfunction other than oligomenorrhea/FHA

Included Paper I (n=40)

Excluded (n=11) for ED/DE

Excluded (n=4) for dietary records of poor validity

Included Paper II (n=25)

Figure 4 A flow chart of the recruitment and inclusion of subjects

The figure illustrates the recruitment and inclusion procedure of subjects. Abbreviations: FHA: functional hypothalamic amenorrhea; ED/DE: Eating disorders/disordered eating behaviour.

The Clinical Examination Procedure

The clinical examination procedure was performed on two consecutive days (Figure 5), followed by a seven-day registration period in the athletes' normal environment.
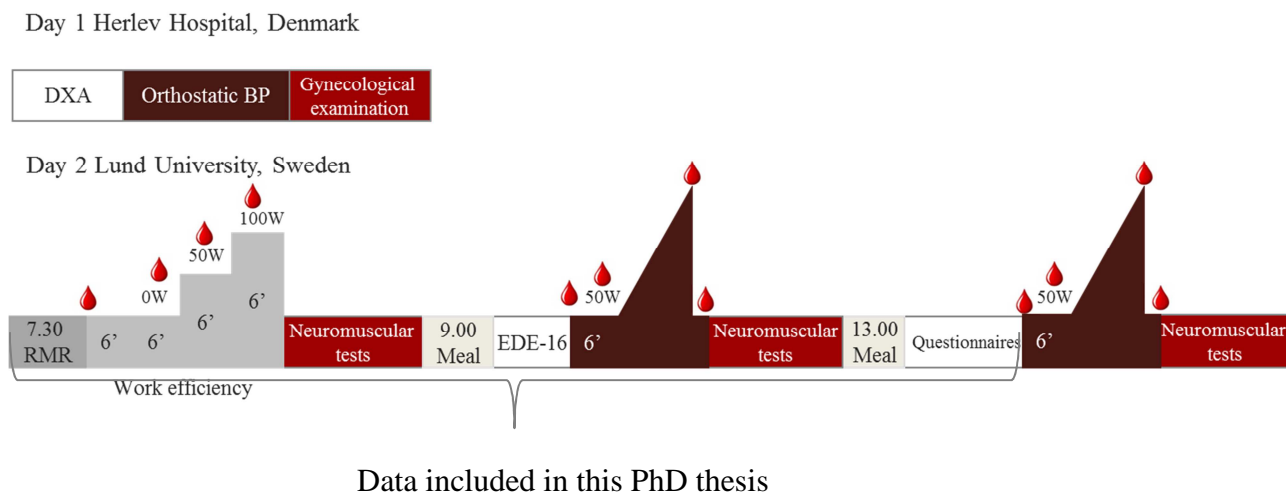

Day 1 Herlev Hospital, Denmark

Day 2 Lund University, Sweden

Data included in this PhD thesis

Figure 5 Clinical examination protocols

Abbreviations: DXA: dual-energy x-ray absorptiometry; BP: blood pressure; RMR: resting metabolic rate; EDE-16: Eating Disorder Examination edition nr 16.

Assessing Energy Availability and Dietary Intake

Food intake and training were simultaneously recorded by the subjects for seven consecutive days to calculate current EA, and FFM was determined by dual-energy x-ray absorptiometry (DXA). The timing of the examination and registration period was planned individually for each subject in order to choose a period reflecting their habitual food habits and exercise regimes. Heart rate monitors and training logs were used to assess exercise energy expenditure. An individual equation provided the basis for the calculation of exercise energy expenditure using the heart rate measurement for each training session, and regression lines were calculated for the corresponding values of heart rate and energy expenditure during the incremental exercise test in the laboratory (Tomten and Høstmark 2006). In the calculation of EA, exercise energy expenditure represented only the energy (kJ) attributable to training, so the estimate of the energy expended for RMR and NEAT throughout the duration of the training was subtracted from the estimate of exercise energy expenditure. For more details, see Papers I and II.

Prospective dietary intake was calculated from weighed food records. Dietary energy density, defined as the amount of energy (kJ) per gram of consumed food (Stubbs et al. 2004), was calculated for all food, including dairy products. Food group variety was analysed by calculating the mean number of

servings of nine different food groups over the seven days. The nine food groups in this study were defined as 1) fruits and vegetables, 2) whole grain products, 3) compact carbohydrate-rich foods, 4) nuts and seeds, 5) meat, 6) dairy products, 7) sports products, 8) fats and oils and 9) energy-containing drinks. For more details, see Paper II.

Examination of Reproductive Function

The first day of data collection involved examinations of bone health, blood pressure, and reproductive function (Figure 5). The subjects were instructed not to exercise for more than thirty minutes at low or moderate intensity on the day before the first day of examination, and to arrive at the clinic in a fasted (from midnight) and rested state.

Menstruating athletes were examined by an experienced gynaecologist, who performed a transvaginal ultrasound examination in the early follicular phase on the third to fifth day of menstruation. The maximum number of ovarian follicles present in a single plane was counted, and total volume was assessed. Sex hormone status (oestrogen, progesterone, LH, FSH, sexual hormone binding globulin [SHBG], prolactin, dehydroepiandrosteron-sulfate [DHEA-S], androstenedione, and total testosterone) was measured, and anamnestic assessment (e.g., age of menarche, previous menstrual irregularities, use of hormonal contraceptives, and number of menstrual cycles during the last year), were recorded using the LEAF-Q. Subjects using oral hormonal contraceptives were requested to stop for a minimum of six weeks prior to the gynaecological examination. The half-life of oral hormonal contraceptives is approximately 24 hours; therefore six weeks was deemed an adequate washout period for all drugs to be eliminated from the system and to ensure that endogenous production of sex steroids was measured. Subjects not recovering their menstrual bleeding within the allotted six weeks were, after the gynaecological examination, contacted monthly by the research team during a follow-up period of a minimum of three months. Subjects were then classified with eumenorrhea (menstrual cycles of 28 days ± 7 days and sex hormones within the normal range); oligomenorrhea (menstrual cycles > 35 days where causes other than hypothalamic suppression had been ruled out); FHA (either primary or secondary where causes other than hypothalamic suppression had been ruled out) (Nattiv et al. 2007); other MD (anatomic defects, hyperprolactinemia, or other dysfunctional ovarian conditions); or polycystic ovary syndrome (PCOS), involving at least two of the following: 1) enlarged ovaries with a volume greater than 10 mL and/or ≥ one ovary demonstrating ≥ 12 follicles in one plane, 2) irregular or absence of bleeding, and 3) elevated androgen level, or otherwise androgen stigmatized.

Assessment of Energy Metabolism

The second day of data collection (Figure 5) included examinations of energy metabolism, aerobic capacity, and an assessment of ED/DE. RMR was assessed after an over-night fast using a ventilated open hood system. After voiding, subjects lay down for 15 minutes before measurements of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were taken over 35 minutes.

Work efficiency was assessed by a standardized test protocol in the fasted state, initiated by the subject seated on a bicycle ergometer for six minutes, followed by cycling for six minutes at 0 W, 50 W, and 100 W, respectively. An air-tight mask covering the mouth and nose was used to measure respiratory gas exchange.

In order to calculate daily total energy expenditure, heart rate monitors were used to assess energy expenditure during bicycle transportation, while accelerometers were used for the assessment of NEAT. Subjects were instructed to wear an accelerometer on the wrist during sleep, and on the hip after getting up in the morning until bedtime; they were instructed to only to take it off during the processes of showering, swimming, bicycle transportation, and training.

Assessment of the Female Athlete Triad and Related Conditions

Besides EA and reproductive function, the presence of ED/DE, impaired bone health, and related conditions was investigated. Eating behaviour was assessed using the Eating Disorder Inventory (EDI-3); and a questionnaire was used to assess behaviour and attitudes related to DE behaviour, as well as to assess overt ED (Garner 2004). Subjects were categorized as having DE behaviour when the EDI risk subscale score for drive for thinness (DT) was ≥ 14, and/or a body dissatisfaction risk score ≥ 19, according to the classification by Garner (2004) and without the presence of DSM-IV-diagnosed ED (Garner 2004). The Eating Disorder Examination (EDE-16) (Fairburn et al. 2008) was used to determine whether subjects met the criteria for ED, according to the DSM-IV criteria for anorexia nervosa, bulimia nervosa, and ED not otherwise specified (EDNOS).

Heart rate and blood pressure were measured using an electronic sphygmomanometer. Hypotension was defined as a systolic blood pressure < 90 mmHg and/or diastolic blood pressure < 60 mmHg. DXA was used to determine fat-free mass, fat mass, and bone mass, respectively. Bone mineral density was determined for the whole body, lumbar spine (L1-L4), and hip. Subjects were classified as having normal BMD (Z-scores > -1 in all measured sites), low BMD (Z-score of -1 to -2 in at least one site), and osteoporosis (Z-score < -2 in at least one site), together with a minimum of one secondary risk factor, such as low EA, ED, and oligomenorrhea/FHA (Nattiv et al. 2007). Leptin, total T_3, cortisol, insulin, IGF-1, GH, cholesterol, and glucose were measured in the fasted and rested state, and analysed according to standardised procedures.

Development and Testing of the Low Energy Availability in Females Questionnaire (LEAF-Q)

The development and testing procedure of the Low Energy Availability in Females questionnaire (LEAF-Q) are presented in Paper III. Physiological symptoms in the literature frequently associated with long-term low EA and/or other Triad conditions were included as variables in the LEAF-Q after being verified as being especially relevant by a collective of clinical experts in endocrinology, sports nutrition, medicine, and gastroenterology. Female professional dancers and endurance athletes from sports such as long-distance running and triathlons were chosen to be representatives for leanness-demanding sports, as they had an increased risk of the Triad. The study had two parts. In part one, the reliability of the LEAF-Q was assessed in a group of endurance athletes and dancers (n = 37). In part two, the self-reported symptoms reported on the LEAF-Q were verified in the group

of endurance athletes (n = 45) described earlier who completed the clinical assessment protocol. In this group of female endurance athletes, oligomenorrhea/FHA existed both with and without the presence of current low EA ($\leq$ 125 kJ/kg FFM), and therefore subjects were divided in half, with 50% being those with the current highest EA, and 50% being those with the current lowest EA, in order to examine discriminant validity. The statistical procedure for questionnaire validation described by Black et al. (2003) was used, where discriminant validity was assessed by testing the mean item score for each of the six variables for significant differences (two-sample *t*-tests) between the group with lower EA versus the group with higher EA, the group with MD versus the group with eumenorrhea, and the group with low BMD versus the group with normal BMD. To measure concurrent validity and the degree of association between the total LEAF-Q score, LEAF-Q variables, and Triad conditions, Pearson's correlation coefficient (*r*) was calculated. Furthermore, the contribution of LEAF-Q variables to the different Triad conditions was calculated using an ordinal logistic regression. To compare the means between subjects categorized at risk for the Triad versus those categorized with a low risk, the students' paired *t*-tests were used. Internal consistency between questionnaire variables was examined by Cronbach's alpha (Cortina 1993). The items found to significantly predict lower EA, MD, and/or low BMD were retained and summed up to provide an overall LEAF-Q score. In order to test the validity of the LEAF scale, sensitivity and specificity were calculated.

Statistics

The dataset was checked for missing data and non-normality using histograms before statistical tests were performed. Normally distributed data were described by mean ± SD, and non-normally distributed data were described by median and interquartile range (IQ 25 and IQ 75). In order to establish a clinically relevant difference in RMR of 418 ± 460 kJ (Lebenstedt et al. 1999) with a power of 90%, and at a significance level of 0.05, twenty subjects were needed in each group. When evaluating energy metabolism, the prevalence of subclinical and clinical Triad conditions, and related physiological conditions (Paper I), the subjects (n = 40) were divided by their clinical levels of low EA (< 125 kJ/kg FFM/day), their subclinical levels of reduced EA (125-188 kJ/kg FFM/day), and their optimal EA ($\geq$ 189 kJ/kg FFM/day). For more details, see Paper I. Since Triad and associated conditions occurred at all three levels of EA and since the group with current low EA was small after excluding subjects with ED/DE and those with poor validity food records, the groups with low and reduced EA were merged when assessing dietary characteristics. In order to explore potential nutritional predictors of oligomenorrhea/FHA, a multiple linear regression with backward selection was conducted after evaluating the co-linearity between the predictors by means of the variance inflation factors. A significance level of < 0.05 was used. In the validation procedure of the LEAF-Q, however, we followed our power estimates and divided the subjects into two equally large groups; half with the highest EA and the other half with the lowest EA. The statistical procedure used in the validation procedure of the LEAF-Q is briefly presented below and in detail in Paper III.

MAIN RESULTS

Energy Availability

Eight subjects had current low EA, while seventeen had reduced EA, and subsequently, 63% were characterised as having current low/reduced EA. Ten subjects were diagnosed with ED (one with anorexia nervosa, one with bulimia nervosa, and eight with EDNOS), and one subject was characterized as having DE. There was no difference in the current EA between subjects with or without ED/DE [154 ± 56 kJ/kg FFM/day versus 170 ± 56 kJ/kg FFM/day, ($P = 0.430$)]. There were no differences in the anthropometrical assessments between groups divided by EA or reproductive function, although subjects with current low/reduced EA reported a higher training volume compared to subjects with optimal EA (Table 4).

Reproductive Function

Twenty-four subjects (60%) were diagnosed with oligomenorrhea (n = 6) and FHA: primary FHA (n = 4) and secondary FHA (n = 14). For all subjects, including those discontinuing oral hormonal contraceptives, the levels of oestrogen, progesterone, and SHBG were within the normal range, confirming that a sufficient washout period was used to eliminate exogenous oestrogen and progesterone before examination. There were no differences in weight or body composition between subjects with oligomenorrhea/FHA and eumenorrheic subjects (Table 4). For more results and details concerning reproductive function, see Papers I and III.

Energy Metabolism

Resting metabolic rate expressed in relation to FFM, as well as the calculated RMR_{ratio}, was lower in subjects with current low/reduced EA and in subjects with oligomenorrhea/FHA compared to subjects with current optimal EA and eumenorrheic subjects (Table 5).

Subjects with oligomenorrhea/FHA and current optimal EA (n = 8) had higher RMR and RMR_{ratio} compared to subjects with oligomenorrhea/FHA and current low/reduced EA (n = 16; 128 ± 10 kJ/kg FFM/day versus 115 ± 8 kJ/kg FFM/day [$P = 0.004$]; and 0.92 ± 0.08 versus 0.84 ± 0.06 [$P = 0.013$]). Furthermore, there was a positive association between current EA and RMR (Figure 6). There were no differences in NEAT between the groups. There was a trend towards higher exercise energy expenditure in the group with current low/reduced EA, while no difference was found in work efficiency between the EA groups. There was, however, a trend towards a higher work efficiency in subjects with oligomenorrhea/FHA compared to eumenorrheic subjects; in addition, a sub analysis revealed that subjects with secondary FHA (n = 14) had a higher work efficiency compared

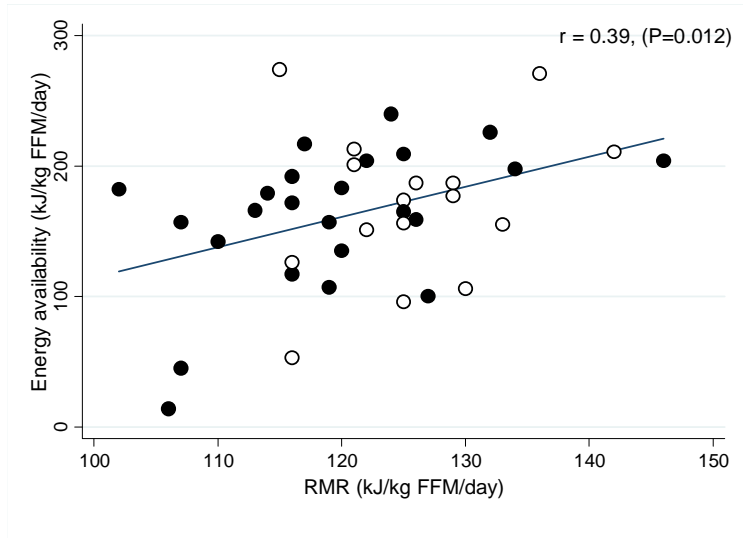to eumenorrheic subjects (Figure 7). For more results and details concerning energy metabolism, see Paper I.

Figure 6 The association between energy availability and resting metabolic rate

The figure illustrates the positive association between energy availability and resting metabolic rate (RMR) in relation to fat-free mass (FFM). Also shown is the regression line. Eumenorrhoeic athletes are represented by open circles and athletes with oligomenorrhoea/FHA are represented by filled circles.
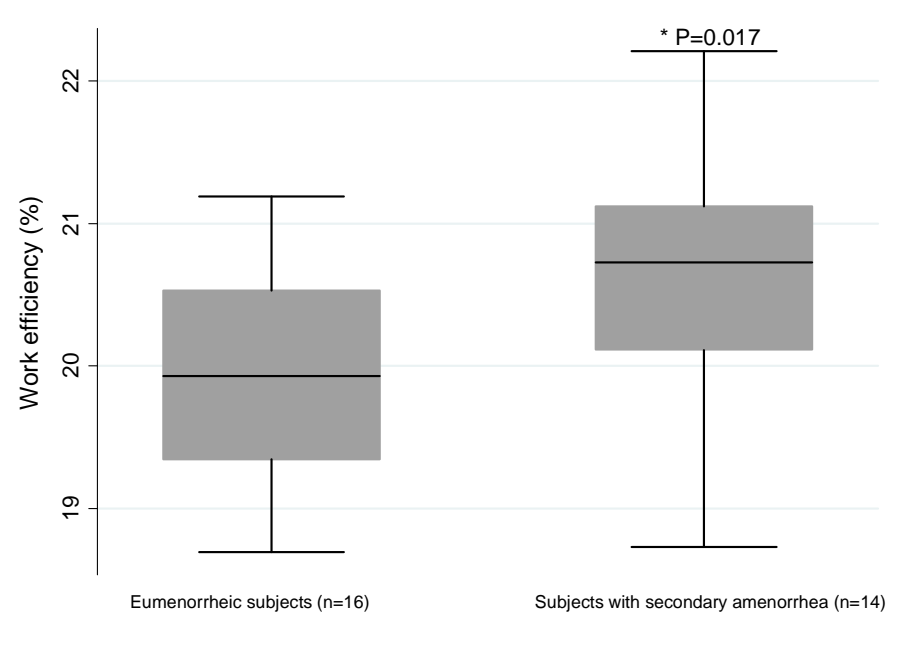

Figure 7 Work efficiency at 100W

The box plots show work efficiency (%) (median, min-max) in secondary functional hypothalamic amenorrhea subjects compared with eumenorrheic subjects at 100W work load.

Table 4 Description of subjects divided by current energy availability and reproductive function

Variable	All (n=40)	Optimal EA (n =15)	Low/reduced EA (n=25)	Eumenorrhea (n=16)	Oligomenorrhea/FHA (n=24)
Age (y)	26.3 ± 5.7	26.9 ± 6.0	26.0 ± 5.6	27.6 ± 5.6	25.5 ± 5.7
Height (cm)	168.9 ± 0.05	166.7 ± 0.04	170.2 ± 0.05	169.1 ± 0.05	168.8 ± 0.05
Weight (kg)	58.8 ± 7.1	57.1 ± 6.2	59.8 ± 7.5	60.2 ± 7.1	57.2 ± 6.6
BMI (kg/m^2)	20.6 ± 2.2	20.5 ± 1.7	20.6 ± 2.2	21.0 ± 1.9	20.1 ± 1.8
VO_{2peak} (l/min)	3.16 ± 0.4	3.15 ± 0.4	3.17 ± 0.4	3.21 ± 0.4	3.13 ± 0.4
VO_{2peak} (ml/kg/min)	55.4 [49.1-59.0]	55.7 [51.4-58.8]	55.0 [50.1-58.3]	55.0 [47.4-59.4]	55.4 [49.1-59.0]
Relative FM (%)¤	20.0 ± 3.6	20.0 ± 3.0	20.0 ± 3.9	20.9 ± 3.5	19.4 ± 3.4
FFM (kg)¤	46.1 [43.1-50.7]	44.1[41.1-49.5]	46.4 [44.2-50.8]	48.8 [44.4-50.8]	45.5 [42.8-50.1]
Exercise (h/week)	11.4 ± 4.5	9.6 ± 2.8	12.5 ± 5.0*	11.6 ± 4.2	11.3 ± 4.7

Abbreviation: EA: Energy availability; BMI: body mass index (kg/m^2); VO2$_{peak}$: peak oxygen uptake; FM: fat mass; FFM: fat-free mass. ¤Determined by DXA scan. Data are presented as mean ± SD for normal distributed data and as median and interquartile range [25-75] for skewed data. For a comparison of mean levels between the group with optimal EA versus low/reduced EA and the group with eumenorrhea versus oligomenorrhea/FHA, a two-way analysis of variance was used.*P = 0.049. No group interaction effect was found in any of the variables.

Table 5 Energy expenditure divided by energy availability and reproductive function

Variable	All (n=40)	Optimal EA (n=15)	Low/reduced EA (n=25)	Eumenorrhea (n=16)	Oligomenorrhea/ FHA (n=24)	P-value EA	Reproductive function
TEE (MJ/day)	12.42 ± 2.02	11.98 ± 1.23	12.69 ± 2.35	12.82 ± 2.12	12.07 ± 2.02	0.284	0.148
RMR (MJ/day)	5.74 ± 0.75	5.86 ± 0.62	5.66 ± 0.75	5.87 ± 0.82	5.52 ± 0.70	0.364	0.016
RMR (kJ/kg FFM/day)¤	122 ± 9.4	127 ± 9.3	119 ± 8.2	126 ± 7	119 ± 10	**0.003**	0.040
RMR$_{ratio}$	0.89 ± 0.08	0.93 ± 0.07	0.87 ± 0.07	0.93 ± 0.06	0.87 ± 0.08	**0.009**	0.008
NEAT (MJ/day)	1.93 ± 0.69	1.96 ± 0.56	1.90 ± 0.81	1.79 ± 0.61	2.03 ± 0.73	0.982	0.320
EEE (MJ/day)	3.68 ± 0.84	2.97 ± 0.84	4.10 ± 2.06	3.85 ± 1.54	3.49 ± 2.02	0.053	0.303
WE 100W (%)	20.3 ± 0.9	20.2 ± 1.0	20.3 ± 0.9	19.9 ± 0.7	20.5 ± 1.0	0.754	0.066

Abbreviation: EA: Energy availability; FFM: fat-free mass. Total energy expenditure (TEE): calculated as resting metabolic rate (RMR) + non-exercise physical activity (NEAT) + exercise energy expenditure (EEE) + diet induced thermogenesis (DIT; 10% of energy intake). RMR$_{ratio}$: the ratio between measured and predicted RMR using the equations of Cunningham, including FFM, ¤determined by DXA scan. Data are presented as mean ± SD for normal distributed data and as median and inter-quartile range [25-75] for skewed data. To investigate whether there were main effects of EA and reproductive function in relation to energy metabolism, a two-way analysis of variance was used. There were no group interaction effects on any of the measured variables.

Dietary Characteristics

Energy availability was positively associated with energy intake ($r = 0.53$; $P = 0.008$) and the intake of all macronutrients (carbohydrates [g/kg/day]: $r = 0.43$, $P = 0.034$; protein [g/kg/day]: $r = 0.41$, $P = 0.043$; and fat [g/kg/day]: $r = 0.57$, $P = 0.003$) and negatively associated with exercise energy expenditure ($r = -0.66$, $P = 0.002$). Furthermore, energy availability was positively associated with energy density, the intake from the food group fats and oils, as well as with energy-containing drinks (Figure 8). The diet of subjects with current low/reduced EA was characterised by lower energy density (5.7 ± 0.9 kJ/g versus 6.7 ± 0.7 kJ/g; $P = 0.020$), content of fat (1.4 ± 0.2 g/kg versus 1.9 ± 0.6 g/kg, $P = 0.027$) and energy-containing drinks [0.7 ± 0.8 servings/day versus 1.6 ± 1.2 servings/day, $P = 0.039$), compared to subjects with current optimal EA.

There were no differences in energy intake (11.19 ± 1.55 MJ/day versus 12.12 ± 2.31 MJ/day; $P = 0.475$) or current EA (170 ± 48 kJ/kg FFM/day versus 189 ± 56 kJ/kg FFM/day; $P = 0.977$) between subjects with oligomenorrhea/FHA and eumenorrheic subjects. However, subjects with oligomenorrhea/FHA shared the same dietary characteristics as subjects with current low/reduced EA; a lower energy density (5.7 ± 0.9 kJ/g versus 6.8 ± 0.7 kJ/g; $P = 0.012$), and a lower fat intake (81 ± 24 g/day versus 111 ± 34 g/day; $P = 0.047$). Dietary energy density was positively associated with the intake of fat ($r = 0.42$; $P = 0.0394$) and compact carbohydrate-rich foods ($r = 0.55$; $P = 0.005$), and was negatively associated with the intake of fibre ($r = -0.52$; $P = 0.008$), protein ($r = -0.42$; $P = 0.039$), and fruits and vegetables ($r = -0.64$; $P = 0.001$).
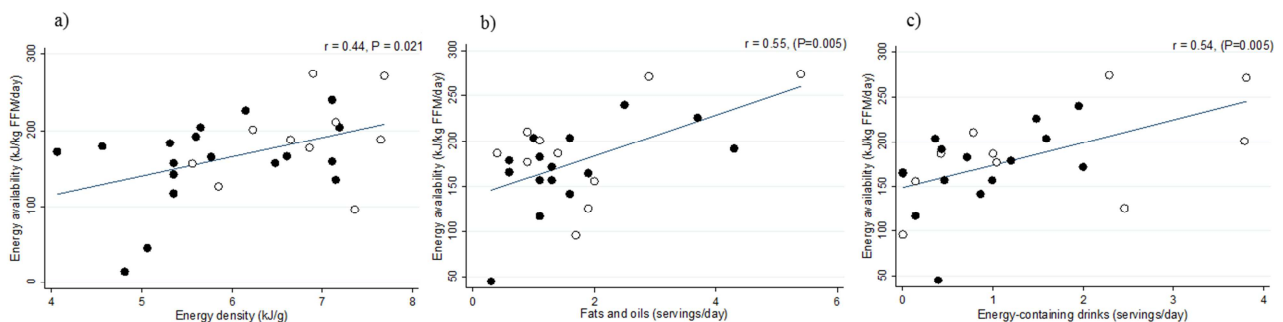

Figure 8 The association between energy availability and energy density as well as intake of different food groups

The figure illustrates the positive association between energy availability and a) energy density of the diet, b) the intake of fats and oils, and c) the intake of energy-containing drinks. Also shown are the regression lines. Eumenorrhoeic athletes are represented by open circles, and athletes with oligomenorrhoea/FHA are represented by filled circles.

Subjects with oligomenorrhea/FHA had a 23% higher total (g/day) and a 32% higher relative fibre intake (g/MJ) compared to the eumenorrhoeic group; fibre intake exceeded the Nordic nutritional recommendation of 25–35 g/day (Nordic Council of Ministers 2013) was more common among subjects with oligomenorrhea/FHA than among eumenorrhoeic subjects (12 out of 15 versus 3 out of ten; $P = 0.018$). Subjects with oligomenorrhea/FHA had a lower intake of compact carbohydrate-rich foods (3.5 ± 2.1 servings/day versus 5.7 ± 2.0 servings/day; $P = 0.019$) than eumenorrheic subjects.

Subjects with oligomenorrhea/FHA had a higher mean total EDI-3 score (25.5 ± 2.8 versus 23.0 ± 2.0; $P = 0.047$) and a higher DT-score (4.0 [2.0-6.0] versus 0.0 [0.0-1.0]; $P = 0.003$) than eumenorrhoeic subjects. There were positive associations between DT-score and the intake of dietary fibre ($r = 0.59$; $P = 0.003$), while there was a negative association with energy density ($r = -0.41$; $P = 0.045$), the intake of fat ($r = -0.41$; $P = 0.046$), and compact carbohydrate-rich foods ($r = -0.65$; $P = 0.001$).

A multiple regression analysis, which included all subjects, with a backward elimination containing EA, energy density, and relative intake of fat (E%) and fibre (g/MJ/day) as possible predictors of oligomenorrhea/FHA, showed a partial correlation coefficient (r^2) for a dietary fibre intake of 0.25 ($P < 0.001$), with an unstandardized regression coefficient (ß) of 0.37 (0.21–0.53; 95% confidence interval) and a mean variance inflation factor (VIF) of 1.41 (range 1.22–1.62). For more details concerning dietary intake and dietary characteristics, see Paper II.

The Female Athlete Triad and Related Conditions

Eighteen subjects (45%) had impaired bone health. Three subjects were diagnosed with osteoporosis in the lumbar spine, and fifteen had low BMD. Sixty-seven percent of the subjects with impaired bone health had oligomenorrhea/FHA, and 33% had ED/DE. There was a negative association between exercise and whole body, as well as lumbar spine BMD and lumbar spine Z-scores $r = -0.32$ ($P = 0.035$), $r = -0.33$ ($P = 0.037$), and $r = -0.36$ ($P = 0.023$), respectively.

The presence of subclinical MD was not assessed in this study, but when merging clinical and sub-clinical Triad conditions that were investigated (current reduced EA with or without DE and low BMD), 14 subjects (35%) had one condition, 13 (32%) had two, and nine (23%) displayed all three Triad conditions.

There were no differences in leptin, T_3, IGF-1, GH, cortisol, GH to insulin ratio, cortisol to insulin ratio, or glucose levels between the groups divided by EA. Subjects with oligomenorrhea/FHA, however, had lower T_3 (1.53 ± 0.05 nmol/L versus 1.69 ± 0.07 nmol/L; $P = 0.046$), lower fasting glucose (3.94 ± 0.47 mmol/L versus 4.39 ± 0.35 mmol/L; $P = 0.003$), and higher cortisol (504 ± 126 mmol/L versus 400 ± 140 mmol/L; $P = 0.021$), than eumenorrheic subjects. Ten subjects (25%) had elevated LDL cholesterol (≥ 3 mmol/L).

There was a negative association between current EA and total cholesterol (r = -0.36; P = 0.022), but there was no difference in total cholesterol between the groups divided by EA or reproductive function. Seven subjects had hypotension, and there was a positive association between current EA and supine systolic blood pressure (r = 0.32; P = 0.048), although there was no difference in blood pressure between the groups divided by EA or reproductive function.

All subjects with hypercholesterolemia, hypotension, and/or hypoglycaemia had at least one Triad condition, while all but two subjects with low RMR had at least one Triad condition. For more results and details concerning the prevalence and interrelationship between the Triad and related conditions, see Paper I.

The Low Energy Availability in Females Questionnaire (LEAF-Q)

During the first part of development and testing reliability (n = 37), internal consistency testing of the main variables resulted in an overall alpha of 0.86, suggesting a relatively high homogeneity of the LEAF-Q. The intra-class correlation coefficient was used in order to calculate the difference between the test and the test-retest score. Test-retest reliability was 0.79 after a two-week interval of retesting. In the second part of the development (n = 45), the variable scores for gastrointestinal symptoms, injuries, and MD showed significant differences between the tested Triad conditions—gastrointestinal symptom: current lower EA versus current higher EA (P = 0.023); injury: low BMD versus high BMD (P = 0.021); and menstrual function: MD versus eumenorrhea (P < 0.001). These three variables were significantly associated with a current lower EA and/or MD and/or low BMD; additionally, the mean variable score for the variables correlated with the total LEAF-Q score. The three variables had values of Cronbach's alpha $\geq$ 0.71 and were therefore retained. The variable score producing the highest sensitivity and specificity for the corresponding Triad end point was used as the cut-off for each item score ($\geq$ 2 for gastrointestinal symptoms, $\geq$ 2 for injuries, and $\geq$ 4 for MD). The total LEAF-Q score $\geq$ 8.0 produced a sensitivity of 78% and a specificity of 90% for correctly classifying current EA and/or reproductive function and/or bone health (Table 3). Subjects categorised by the questionnaire to be at risk for the Triad were leaner (19.2 $\pm$ 3.2% body fat versus 21.8 $\pm$ 3.4% body fat; P = 0.014) and had lower levels of leptin (0.21 $\pm$ 0.19 pg/mL/kg fat mass versus 0.29 $\pm$ 0.31 pg/mL/kg fat mass; P = 0.019), T_3 (1.54 $\pm$ 0.22 ng/mL versus 1.74 $\pm$ 0.29 ng/mL; P = 0.013), and glucose (4.0 $\pm$ 0.5 mmol/L versus 4.3 $\pm$ 0.3 mmol/L; P = 0.016) compared to subjects that were categorised as low risk; furthermore, there was a trend towards lower RMR_{ratio} for subjects at risk for the Triad (0.88 $\pm$ 0.08 versus 0.93 $\pm$ 0.05; P = 0.056). For more results and details concerning the development and testing of the LEAF-Q, see Paper III.

Table 5 Statistics from the LEAF-Q (%)

Verifying variables	Sensitivity	Specificity	False positive	False negative	Positive predicted value	Negative predictive value	Yield	Accuracy	Validity
Lower EA and/or MD and/or low BMD	78	90	20	15	97	44	71	80	68

Abbreviation and definition: The LEAF-Includes the 25 best discriminating items. EA: energy availability; MD: menstrual dysfunction such as oligomenorrhea, functional hypothalamic amenorrhea, poly cystic ovarian syndrome, or other MD; Low BMD: bone mineral density *z*-score < -1.

Sensitivity: Percentage of those who actually are positive who test positive; true positives/number of people who actually are positive x 100%. **Specificity**: Percentage of those who actually are negative who test negative; true negatives/number of people who actually are negative or healthy x 100%. **False positive**: Percentage that the test indicates as positive, but who are actually healthy; false positives/total number who are healthy x 100%. **False negative**: Percentage that the test indicates as healthy, but actually are positive; false negatives/total number who are positive x 100%. **Positive predictive value**: Percentage who test positive in comparison with the sum of those who test positive and are, and those who test positive, but aren't; true positives/(true positives + false positives) x 100%. **Negative predictive value**: Percentage who test negative in comparison with the sum of those who test negative and are, and those who test negative and are not; true negatives/(true negatives + false negatives) x 100%. **Yield**: Percentage of true positives in relation to total number tested; true positives/total x 100%. **Accuracy**: Percentage of true positives and true negatives in relation to total number tested; true positives + true negatives/total x 100%. **Validity**: Percentage who test positive by the test who actually are positive + percentage who test negative by the test who actually are negative -100; sensitivity + specificity -100%.

DISCUSSION

The LEA study is the first to examine the prevalence of current EA and Triad related conditions simultaneously with potential adaptations in energy metabolism in a group of elite female endurance athletes. Subjects with current low/reduced EA as well as those with oligomenorrhea/FHA showed signs of metabolic adaptation in the form of lower RMR. Subjects with secondary FHA also had increased work efficiency, indicating a more profound adaptation in female athletes with severe clinical MD. Long-term restricted eating behaviour and MD have been shown to be associated with an increased risk of muscle, skeletal, and joint injuries (Rauh et al. 2010, Thein-Nissenbaum et al. 2012), as well as with impaired performance (Harber et al. 1998, Van Heest et al. 2014). In the present study, 63% were found to have current low or reduced EA, while 28% were diagnosed with ED/DE. Sixty percent had oligomenorrhea/FHA, and 45% had impaired bone health. Twenty-three percent had all three Triad conditions when merging the assessed clinical and subclinical conditions. Triad and associated conditions, such as low RMR, hypoglycaemia, hypotension, and hypercholesterolemia, were common in this group of endurance athletes, despite a normal BMI and fat mass range. We did not find any differences in energy intake or current EA between eumenorrhoeic and oligomenorrhea/FHA athletes. We did, however, find that athletes with oligomenorrhea/FHA had similar dietary characteristics to athletes with current low/reduced EA without ED/DE, such as a lower energy density and a lower fat content. Furthermore, athletes with oligomenorrhea/FHA were more concerned with weight and had a diet with a higher percentage of dietary fibre and fewer compact carbohydrate-rich foods than eumenorrhoeic athletes. The testing of the LEAF-Q showed an acceptable sensitivity and specificity as well as internal consistency, indicating that the LEAF-Q has the potential to be a useful screening tool for the identification of female athletes at risk for the Triad and a relevant complement to existing validated DE screening instruments. The high prevalence of ED, oligomenorrhea/FHA, and impaired bone health found in this group of elite endurance athletes emphasizes the importance of prevention, early detection, and treatment of any of these associated conditions.

Energy Availability

In the present study, 43% (n = 17) had current EA between 125-188 kJ/kg FFM/day, levels that are recommended to athletes wanting to lose weight (Loucks et al. 2011), while 20% (n = 8) had current EA less than 125 kJ/kg FFM/day, which in clinical settings has been shown to suppress the hypothalamic-pituitary axis and bone formation markers in healthy, sedentary eumenorrhoeic women (Loucks and Thuma 2003, Ihle and Loucks 2004, Loucks et al. 2013). The prevalence of low EA found in the present study is similar to what has been reported in female volley ball players (Woodruff and Meloche 2013) and in female elite soccer players (Reed et al. 2012) (Table 1). When merging the groups with current low and reduced EA, the prevalence was higher in this group of endurance athletes (63%) compared to the 36% found in high school athletes from a variety of sports and the 39% in sedentary controls, as reported by Hoch and collaborators (2009). In the literature, EA less than 125 kJ/kg FFM/day is used as a threshold for an unsafe level in order to support the energy expenditure required for health, func-

tion, and daily living, once the cost of exercise activities is taken into account (De Souza et al. 2014, Loucks 2014). In the present study, however, we found that oligomenorrhea/FHA occurred at all three levels of current EA. Furthermore, subjects with current reduced EA had lower LH levels as well as lowered RMR compared to those with current optimal EA. Our results, therefore, support earlier indications that the long-term threshold of unsafe EA associated with oligomenorrhea/FHA might be higher (Gibbs et al. 2013b, Ciadella-Kam et al. 2014) than the threshold of less than125 kJ/kg FFM/day established by Loucks and Thuma (2003), which predicted the decline in LH pulsatility in eumenorrheic sedentary women.

There are several methodological issues that need to be addressed when assessing EA in female athletes, such as the ability to capture habitual EA, the ability to discriminate between ED/DE and under-reporting when performing nutritional surveys, and determining which definition and methods to use when calculating exercise energy expenditure (Table 1). Since the subjects in the LEA study were elite athletes, we defined exercise as: all training registered by the subjects in their training records, as in the studies by Schaal et al. (2011) and Lagowska et al. (2014). In the present study, seven subjects were identified as having a low validity energy intake, using the equation described by Black (2000). All of these subjects had, however, one or more physiological conditions associated with energy deficiency, such as low RMR (n = 4), ED/DE (n = 4), oligomenorrhea/FHA (n = 5), hypercholesterolemia (n = 2), and hypotension (n = 2). Therefore, we chose not to remove any of these subjects in the analysis of energy metabolism and Triad conditions. Energy availability in our eumenorrheic subjects (172 kJ/kg FFM/day) as well as in the oligomenorrheic/FHA subjects (161 kJ/FFM/day) was high compared with what has been reported in other studies: EA between 50–142 kJ/kg FFM/day in subjects with MD and 97-176 kJ/kg FFM/day in eumenorrheic subjects (Table 1). Dietary record validity has, however, only been assessed in four of these studies (Schaal et al. 2011, Woodruff and Meloche 2013, Guebels et al. 2014, Van Heest et al. 2014) using the standard Goldberg (1991) cut-off. Furthermore, none of these studies have used validated methods in order to diagnose subjects with ED. This could to some extent contribute to these low estimates of EA, since when we removed our subjects with ED/DE and those with poor validity records, the mean EA in our group of endurance athletes was even higher: 189 kJ/kg FFM/day in eumenorrheic subjects and 170 kJ/FFM/day in oligomenorrhea/FHA subjects. The studies most similar to the LEA study, in terms of study population and methodology, are the small studies performed by Schaal et al. (2011), which reported EA of 75 kJ/kg FFM/day in MD and 121 kJ/kg FFM/day in eumenorrheic endurance athletes, and Van Heest et al. (2014), which reported EA of 50 kJ/kg FFM/day in young elite swimmers with MD and 125 kJ/kg FFM/day in eumenorrheic subjects. Exercise energy expenditure in these studies was not adjusted for sedentary activities as recommended by Loucks and Thuma (2003), which would have increased EA. In our group of endurance athletes, the unadjusted EA in eumenorrheic subjects was 159 kJ/kg FFM/day and in oligomenorrheic/FHA subjects 150 kJ/kg FFM/day, and would have resulted in categorising eleven subjects with low EA (instead of eight), eighteen with reduced EA (instead of seventeen), and eleven with optimal EA (instead of fifteen). These results exemplify the methodological divergences and some of the difficulties in the assessment of EA. Regardless of the major differences in the reported EA; these combined results indicate that the reproductive function in some women is more robust or more sensitive than it is in others (Gibbs et al. 2013b, Ciadella-Kam et al. 2014).

Furthermore, our results indicate that the threshold for low EA established by Loucks and Thuma (2003) in a clinical setting may not similarly manifest in athletes under free-living conditions using self-reporting and field methods to determine EA (Ciadella-Kam et al. 2014, Mountjoy et al. 2014).

Eating Disorders and Disordered Eating behaviour

The prevalence of clinical ED in this population of Swedish and Danish female athletes (25%) was similar to the 24% found in Norwegian female elite endurance athletes (Sundgot-Borgen and Torstveit 2004), using the same method of careful diagnostic workout. The LEA study is the first study to investigate EA that has used a gold standard method to diagnose subjects with ED, and we found no difference in the energy intake or current EA in subjects with ED/DE than subjects without ED/DE. Normally, one would assume that athletes diagnosed with EDNOS would have persistent low or reduced EA, unless they reported healthier diets or practiced less restricted eating behaviour during the registration period because they were being monitored. Our results, however, are supported by similar findings reported in Norwegian elite athletes (Sundgot-Borgen 1993), exemplifying the complexity of the ED/DE spectrum and the Triad continuum. The fact that only one of the athletes met the criteria for anorexia nervosa might furthermore contribute to the explanation of the reported EA results, since athletes with EDNOS and bulimia nervosa might differ from day to day when it comes to energy intake, and since athletes with ED can be underweight, have normal weight, or even be overweight (Torstveit and Sundgot-Borgen 2012).

De Souza et al. (2007) used an elevated DT-score as a proxy indicator for energy deficiency in exercising women and found no difference in the current energy intake between exercising women with elevated versus normal DT-levels, while the prevalence of MD was higher in women with an elevated DT-score compared to those with a normal DT-score. Only seven of the twenty-five subjects with current low or reduced EA in the present study had ED/DE, and we did not find any differences in the DT-score between subjects divided by current EA. As mentioned earlier, several studies that investigated the Triad have found elevated DT-scores or increased dietary restraint in athletes with MD compared to eumenorrheic athletes (Lebenstedt et al. 1999, Warren et al. 1999, Cobb et al. 2003, Gibson et al. 2004, Nichols et al. 2006, Reed et al. 2011, Gibbs et al. 2011). Interestingly, we found an elevated DT-score even after excluding all subjects with ED/DE and those subjects underreporting their dietary intake. These observations indicate that athletes with oligomenorrhea/FHA are more concerned with weight. However, the DT-score is often not pathologically high; therefore, to only screen athletes for DE is probably not sufficiently sensitive as an indicator of athletes at risk for the Triad.

Reproductive Function

The 60% prevalence of oligomenorrhea/FHA that we found in our study is similar to the 64% among endurance athletes reported by Pollock and collaborators (2010) underpinning that oligomenorrhea/FHA is common among female endurance athletes. Hormonal contraceptives are commonly used as treatment for MD, but the effectiveness for improving BMD is inconclusive (Manore et al. 2007). To restore bleeding with hormonal contraceptives may therefore give the illusion of normality, while the underlying adverse endocrine factors partly remain. Fifteen of our subjects (38%) used hormonal contraceptives before inclusion in the study. Eight of them were diagnosed with oligomenorrhea/FHA after a minimum break of three months, but only two of them were previously unaware of their MD.

Polycystic ovarian syndrome is reported to be common among female elite athletes (Hagmar et al. 2009), and is not associated with hypothalamic inhibition because of energy deficiency. Among the forty-five subjects finishing the clinical protocol, four of the five subjects diagnosed with PCOS or a MD other than oligomenorrhea/FHA had current low/reduced EA, and all five had at least one Triad associated condition: two subject with hypercholesterolemia and low BMD, one subject with low RMR, one subject with hypoglycaemia and low BMD, and one subject with EDNOS and hypercholesterolemia. These findings emphasize the importance of differential diagnoses in order to secure proper intervention for MD in female athletes, and further indicate that assessment of coexisting Triad conditions could be relevant independent of the type of MD.

Energy Metabolism

Mean RMR among our subjects with current low/reduced EA was only 119 ± 8 kJ/kg FFM/day, similar to the RMR found in our subjects with oligomenorrhea/FHA and to the level reported earlier in athletes and in recreationally active women with MD (Table 3). These results support earlier findings that energy deficiency leads to metabolic adaptations, such as lower RMR, as an attempt to restore energy balance (Redman et al. 2009). The short-term effects of low or reduced EA are, therefore, more likely to affect outcomes such as RMR (Redman et al. 2009) and LH (Loucks and Thuma 2003), while outcomes such as oligomenorrhea/FHA and bone health are evidently not tightly coupled to the current EA provided in this study, but instead to the long-term effect of energy deficiency. As earlier described, the RMR_{ratio} has been reported to be low in patients with anorexia nervosa, and an RMR_{ratio} < 0.90 has been used in studies with recreationally active women as an indicator of low EA (De Souza et al. 2007, De Souza et al. 2008, Schneid et al. 2009, Gibbs et al. 2011). In the present study, subjects with current low/reduced EA, as well as subjects with oligomenorrhea/FHA, had similarly low RMR_{ratio}, supporting the use of a lowered RMR_{ratio} as a potential indicator of current low/reduced EA as well as long-term persistent energy deficiency and clinical Triad conditions. We also found that subjects with secondary FHA, beyond a lower RMR, also had increased work efficiency, suggesting more profound metabolic adaptations in female athletes with severe clinical MD. Improved work effi-

ciency has earlier been reported during low intensity exercise in weight-reduced sedentary subjects (Goldsmith et al. 2010), but not in endurance athletes, and not at higher work intensities.

Dietary Characteristics

We did not find any differences in energy intake or current EA between subjects with oligomenorrhea/FHA and eumenorrhea, which is in line with what has frequently been reported in the literature (Deuster et al. 1986, Myerson et al. 1991, Snead et al. 1992, Wilmore et al. 1992, Marcus et al. 1995, Laughlin and Yen 1996, Perry et al. 1996, Lebenstedt et al. 1999, Pettersson et al. 1999, Gremion et al. 2001, Schaal et al. 2011) (Table 2). We did, however, find that subjects with current low/reduced EA, as well as those with oligomenorrhea/FHA, had a lower energy dense diet, as previously reported in recreationally active women with MD (Reed et al. 2011), and a lower fat intake—a finding frequently report earlier in female endurance athletes (Deuster et al. 1986, Nelson et al. 1986, Kaiserauer et al. 1989, Laughlin and Yen 1996, Harber et al. 1998, Thong et al. 2000, Cobb et al. 2003, Tomten and Høstmark 2006). We also found a positive association between the energy density of the diet and the intake of fat and compact carbohydrate-rich foods, while energy density was negatively associated with fibre and protein content as well as a high intake of fruits and vegetables in these endurance athletes. Energy density is increased more by fat than carbohydrates or protein, due to fats higher energy content per weight (Rolls 2009), and lowered by the fibre content (Slavin 2009), mainly due to the binding of water, since water contributes to weight, but not to energy (Rolls 2009). Diets with low energy density have been shown to increase satiation and prolong satiety (Holt et al. 1995), and a diet with low energy density is therefore likely to increase the risk for low or reduced EA in female athletes, especially during periods with high training loads. We also found that EA was not only positively associated with the energy density of the diet and the intake of fat and oils, but also with the intake of energy-containing beverages. Surprisingly, 56% of the athletes in the present study drank less than 200 mL/day of energy-containing drinks.

As mentioned earlier, female athletes with MD have been reported to be more focused on weight and body image than eumenorrheic athletes. Myerson et al. (1991) reported an increased preoccupation with weight in female runners with MD compared to eumenorrheic subjects, even after excluding those with ED/DE, which supported our findings and indicated underlying behavioural factors that could increase the risk of low or reduced EA on the long term. We also found that a higher DT-score was associated with a diet with lower energy density, a higher intake of dietary fibres, and a more restricted intake of fat and compact carbohydrate-rich food. These results indicate that even a slightly increased drive to lose weight or to maintain a low body weight with a diet following the general dietary guidelines increases the risk for long-term energy deficiency and oligomenorrhea/FHA in athletes.

Even though we did not find any differences in the carbohydrate intake between eumenorrhoeic subjects and those with oligomenorrhea/FHA, the athletes with oligomenorrhea/FHA consumed less compact carbohydrate-rich foods, such as white rice, pasta, bread, and sweets —foods that are important constituents of a carbohydrate-rich diet that commonly are recommended to athletes in order

to enhance performance (Burke et al. 2011). In the present study, 72% exceeded the recommended daily intake of 3 g fibre/MJ in the Nordic countries (Nordic Council of Ministers 2013), and this excessive intake of dietary fibre was linked to oligomenorrhea/FHA. The higher fibre intake among oligomenorrhea/FHA subjects compared to eumenorrhoeic subjects, as we found, has been reported previously (Warren et al. 1994, Laughlin and Yen 1996, Reed et al. 2011). An excessive dietary fibre intake of more than 40 g/day, as seen among the female athletes with oligomenorrhea/FHA in the present study, might reduce energy absorption, prolong the feeling of satiety, increase satiation, and consequently increase the risk for low/reduced EA. Furthermore, an excessive fibre intake may lead to gastrointestinal disorders, such as bloating and diarrhoea (Luton and Trumbo 2006)—common problems among endurance athletes (Fallon 2006). Independent of the effect of fibre on energy intake, studies have reported a negative association between fibre intake and oestrogen levels in sedentary premenopausal women (Laughlin et al. 1998, Aubertine-Leheudre et al. 2008, Gaskins et al. 2009), and these results support our findings derived from the multiple regression analysis that a high fibre intake, independent of its effect on EA or energy density, could potentially increase the risk of oligomenorrhea/FHA in female athletes.

The Female Athlete Triad and Related Conditions

In the present study, 45% of the female athletes had impaired bone health. These alarming results are in line with the findings of Pollock et al. (2010), which showed impaired bone health in 41% of female elite endurance athletes. Weight-bearing exercise normally has beneficial effects on bone mineral accumulation, especially during adolescence, and it is common for athletes to have 5%–15% higher BMD and peak bone mass than non-athletes (Nattiv et al. 2007). We found a negative association between the volume of training and the whole body, as well as lumbar spine BMD, which was also reported in other studies with female elite athletes from weight-bearing endurance sports (Pollock et al. 2010, Barrack et al. 2010a). The endurance athletes in the present study were mostly runners and triathletes. Repetitive low impact training, such as middle and long-distance running and non-impact sports (e.g., swimming), do not have the same positive effect on BMD as high-impact training (e.g., gymnastics) or odd–impact training (e.g., soccer) (Tenforde and Fredericson 2011). Endurance athletes with low EA and oligomenorrhea/FHA may therefore be at an even greater risk for low BMD (Barrack et al. 2010a, Tenforde and Fredericson 2011) compared to eumenorrheic athletes from sports with mainly high or odd impact training. Bone mineral density reflects a lifelong history of mechanical load and hormonal milieu linked to EA and reproductive function. It declines with the number of menstrual cycles missed since menarche (Lloyd et al. 1987), and even though some of this relevant information was collected in the present study, the history of mechanical load and hormonal milieu's estimated effect on bone health was not specifically investigated. However, according to self-reported data, 44% of the eumenorrheic subjects had previously experienced oligomenorrhea/ FHA. Consequently, 77% of all women in this study at one point or another had experienced oligomenorrhea/FHA. The high prevalence of impaired bone health in this group of female endurance athletes clearly emphasizes the importance of prevention and early identification of athletes at risk for developing low BMD/osteoporosis.

The prevalence of athletes in this study who displayed at least one clinical condition of the Triad was high (50%). Most previous studies have merged subclinical and clinical Triad conditions, and a prevalence has been reported of 16%–60% of athletes having at least one condition (Gibbs et al. 2013a), as compared to 35% in the present study. Fifteen percent of the athletes in our study displayed two clinical Triad conditions, which is similar to the 18% earlier reported among Norwegian elite athletes by Torstveit and Sundgot-Borgen (2005). Only one athlete met all the clinical criteria for the Triad, which supports earlier findings of a low prevalence of all three extreme endpoints of the Triad (Torstveit and Sundgot-Borgen 2005a, Hoch et al. 2009). However, when merging clinical and subclinical Triad conditions, 23% of the athletes displayed all three components of the Triad. This is even higher than the 16% reported by Pollock et al. (2010), indicating that the coexistence of oligomenorrhea/FHA, current low or reduced EA with or without ED/DE, and impaired bone health are common among female elite endurance athletes. Energy availability, however, was not assessed in the study by Pollock, which probably explains the higher prevalence of Triad conditions in the present study. In the present study, 83% of those with impaired bone health, 79% of those with oligomenorrhea/ FHA, and all subjects with high cholesterol levels, hypotension, and hypoglycaemia and all but two subjects with low RMR had at least one additional Triad condition, indicating that these conditions are closely related.

An easier and less time-consuming method for estimating EA could be to use validated biomarkers. Leptin has been suggested as an important modulator of ovulatory function (Barrack et al. 2010b). Low levels of leptin, a marker for low body fat and restricted food intake (Warren 2011), have been reported among athletes with MD. Low leptin levels were also common in the present group of athletes (78%), despite the fact that the majority of the subjects had BMI and fat mass within the normal range. We found no difference in leptin levels between subjects with oligomenorrhea/FHA and eumenorrheic subjects, which supports earlier findings that leptin levels per se may not be associated with oligomenorrhea/FHA in exercising women (Corr et al. 2011). We did, however, find lower levels of leptin relative to fat mass in subjects categorised at risk for the Triad (total LEAF-Q score ≥ 8) than those with low risk (Paper III). These results indicate that in this group of female endurance athletes with generally low levels of leptin, an even lower level of leptin is associated with the manifestation of more than one Triad condition.

The subjects with oligomenorrhea/FHA had lower fasting glucose levels, lower T_3, and higher cortisol than the eumenorrheic subjects. Earlier research has suggested that GnRH neuron activity and LH pulsatility are regulated by brain glucose availability (Loucks et al. 1998, Loucks 2014). Both T_3 and glucose levels have further been shown to affect performance, in that glucose availability to the central nervous system and muscle cells is critical for performance during prolonged aerobic exercise (Bangsbo et al. 1992, Costill et al. 1993, Hawley and Burke 2010, Burke et al. 2011, Gejl et al. 2014), while low T_3 decreases the activity of the mitochondrial oxidative enzymes, leading to impaired oxidative capacity and post–exercise creatine phosphate recovery in the skeletal muscles (Harber et al. 1998, Wrutniak-Cabello et al. 2001, Loucks 2006b, Reuters et al. 2012). Only one subject with ED and secondary FHA, however, had clinically low levels of T_3, and another subject with secondary FHA had elevated cortisol levels, suggesting that

these biomarkers are indicators of long-term persistent energy deficiency and clinical Triad conditions, and probably not useful in identifying athletes with subclinical Triad conditions.

Oestrogen is known to affect lipid metabolism by decreasing LDL and increasing HDL cholesterol (Schnaper et al. 2000), and an unfavourable lipid profile, as we found in 25% of our subjects, has been reported in female athletes with FHA (Rickenlund et al. 2005). High total cholesterol is also a common finding in patients with anorexia nervosa (Meczekalski et al. 2013), and has been reported to decrease in these patients after weight gain (Ohwada et al. 2006). Most subjects with hypercholesterolemia in this study had a body weight within the normal range (n = 8), and 90% had current low or reduced EA and/or ED/DE, while 30% were still eumenorrheic, suggesting that alterations in cholesterol synthesis might be triggered by energy deficiency, despite normal weight and eumenorrhea.

Hypotension is a typical cardiovascular complication of anorexia nervosa (Meczekalski et al. 2013). In the present study, hypotension was associated with low/reduced EA as well as oligomenorrhea/FHA, although only one subject was diagnosed with anorexia nervosa, and most subjects with hypotension had a BMI within the normal range. Therefore, hypercholesterolemia and hypotension are, together with RMR_{ratio} and hypoglycaemia, potentially useful biomarkers when screening athletes at risk for the Triad.

Triad and associated conditions were common in this group of endurance athletes, despite a normal BMI and fat mass range. This is in line with earlier findings that restrained or delayed eating patterns are associated with higher fat mass percentage in female elite athletes (Deutz et al. 2000). These results are important, because athletes with low EA and ED/DE often are presumed to be underweight (De Souza et al. 2014).

The Low Energy Availability in Females Questionnaire (LEAF-Q)

The intension of the LEAF-Q was to construct a brief questionnaire focusing only on self-reported physiological symptoms linked to persistent energy deficiency, with or without ED/DE, which can be routinely used in order to identify individuals at risk of the Triad.

Efforts were made to improve content validity by thoroughly focusing on physiologic plausibility and examining the research literature to identify the most discriminating variables for subjects with long-term energy deficiency. Menstrual dysfunction was selected due to the strong scientific evidence concerning the causality between low EA and the endocrine perturbation related to subclinical and clinical MD (Loucks and Thuma 2003, Nattiv et al. 2007, De Souza et al. 2014, Mountjoy et al. 2014). The variable of gastrointestinal problems was selected since persistent energy deficiency causes mucosal atrophy characterized by diminished intestinal function as well as morphological changes (Shaw et al. 2012) that link lower EA to gastrointestinal problems, both commonly reported in female endurance athletes (Fallon 2006) and in patients with ED/DE (Rodriguez et al. 2009, De Souza et al. 2014, Mountjoy et al. 2014). In this group of female endurance athletes, the variable for gastrointestinal problems was verified by current lower

EA. The variable of injuries was selected because Rauh et al. (2010) and Thein-Nissenbaum et al. (2012) have reported an increased risk for muscular skeletal injuries in female athletes with restricted eating behaviour, MD, and low BMD. In this group of female endurance athletes, the LEAF injury score correlated with impaired bone health, but not with MD or current EA. It is well established that prolonged, exhaustive endurance exercise is capable of inducing skeletal muscle damage and temporary impairment of muscle function (Grobler et al. 2004), making the association between the LEAF injury score and impaired bone health physiological plausible. The initial test results appear promising. They indicate that the LEAF-Q is a potentially relevant complement to existing validated DE screening instruments to screen female athletes at risk for the Triad. However, expanded testing and development in similar and other sports as well as in recreationally active women is needed.

PRACTICAL IMPLICATIONS

The mismatch between current EA, ED/DE, oligomenorrhea/FHA, and impaired bone health in the present study confirms the complexity involved in the Triad continuum. Furthermore, the high prevalence of coexisting features, such as low RMR, hypotension, hypoglycaemia, and hypercholesterolemia confirms that other aspects of physiological functions and health, aside from MD and impaired bone health, are affected even if BMI and fat mass are within the normal range. Therefore, the findings of any one of these clinical features or Triad conditions in female athletes should be seen as an indication for further assessment of the conditions associated with energy deficiency, as emphasized by the IOC (Mountjoy et al. 2014).

The present study demonstrates that a diet with a lower energy density, a more restricted intake of fat, compact carbohydrate-rich foods, energy-containing drinks, and a high intake of dietary fibre are dietary risk factors associated with low/reduced EA and oligomenorrhea/FHA in female endurance athletes. Specific considerations relevant for use in nutritional prevention and treatment of low/reduced EA and oligomenorrhea/FHA are presented in Paper II. Our results further indicate that even a slightly increased drive to lose or maintain a low body weight is associated with dietary characteristics likely to increase the risk for low/reduced EA and oligomenorrhea/FHA. Handling the issue of leanness and body weight with care is therefore of paramount importance in sports environments. An athlete who wishes to lose weight or change body composition should be provided with professional counselling to ensure a short-term nutritional treatment plan with proper and effective guidelines for weight loss (Sundgot-Borgen et al. 2013) that will result in re-establishing optimal EA and weight stability.

Lower levels of T_3, even if they are within the normal range, and lower glucose, as we found in oligomenorrheic/FHA subjects, have been shown to affect neuromuscular function, decrease muscle strength (Harber et al. 1998, Wrutniak-Cabello et al. 2001, Loucks 2006b), and contribute to fatigue and delayed recovery (Costill et al. 1973, Bangsbo et al. 1992, Harber et al. 1998, Wrutniak-Cabello et al. 2001, Burke et al. 2011, Hawley and Burke 2010, Reuters et al. 2012, Loucks 2014, Gejl et al. 2014). As mentioned earlier, a slower recovery rate of skeletal muscles (Harber et al. 1996) and reduced performance have been reported in athletes with MD along with metabolic and hormonal alterations secondary to energy deficiency (Van Heest et al. 2014). Therefore, the prevention and treatment of long-term low or reduced EA and oligomenorrhea/FHA seem to be an important factor in the pursuit of optimal performance for female athletes.

LIMITATIONS AND STRENGTHS

This observational study does not document the effect of induction of low or reduced EA on metabolic adaptations or endocrine alterations, and cannot demonstrate a causative effect. Twenty-five of the 108 national team and competitive endurance athletes who initially agreed to participate in the LEA study changed their minds and stated that they were not able to participate after all. There is a risk of selection bias with a preponderance of subjects focusing on dieting and weight, as well as with those concerned about their health. On the other hand, it is possible that some athletes with problems such as ED/DE, especially in the group of national team athletes, were apprehensive about being detected and chose not to participate. The high number of variables assessed and statistically tested in the present study also increases the risk of type 1 errors and this part of the study should primarily be seen as explorative, with the need for additional studies of this kind for verifications.

However, our results suggest that the assessment of blood pressure and fasting blood glucose, as well as RMR_{ratio} and cholesterol, in addition to eating behaviour, reproductive function, and bone health, might improve clinical assessment in this population of women. The strength of this study is that subjects were standardized in the period prior to their examinations, and validated methods have been used to assess ED/DE, reproductive function, bone health, and energy metabolism. Anovulation and luteal phase defect have been reported to be high in exercising women (De Souza et al. 1998, De Souza et al. 2003, De Souza et al. 2010). Research by De Souza and collaborators has revealed that in order to accurately detect these subclinical MD, sex hormones must be measured serially throughout the menstrual cycle. The lack of such repeated measurements is a limitation in this study, and some of our eumenorrheic subjects could have had undetected, subclinical MD (De Souza et al. 2010). The assessment of EA at one point in time in a mixed population of women with ED, non-ED, and who under-reported, can provide results that do not represent the 'true' habitual EA. Although this study only provided a 'snapshot' of EA, it demonstrated that current EA was associated with physiological measures of RMR and LH. A limitation to the LEA study is the lack of well-matched non-athletic control subjects in order to assess whether there is an increased prevalence of these conditions in a population of elite female endurance athletes. The LEAF-Q has so far only been validated in this group of female endurance athletes and needs repeated testing in both similar and different athletic populations.

CONCLUSION

- ➤ Subjects with current low/reduced EA had lower RMR than subjects with optimal EA, but no difference in work efficiency was found.

- ➤ Subjects with oligomenorrhea/FHA had reduced RMR, and subjects with secondary FHA also had increased work efficiency when compared with eumenorrheic subjects.

- ➤ Subjects with current low/reduced EA had a lower energy intake and higher exercise energy expenditure than subjects with optimal EA.

- ➤ Subjects with oligomenorrhea/FHA had an energy intake and EA similar to the eumenorrheic subjects.

- ➤ The dietary characteristics associated with low/reduced EA in female endurance athletes without ED/DE were a lower energy density, lower fat content, and lower intake of energy-containing drinks.

- ➤ The dietary characteristics associated with oligomenorrhea/FHA in female endurance athletes without ED/DE were a lower energy density, lower fat content, and lower intake of compact-carbohydrate rich foods, as well as higher fibre content.

- ➤ The prevalence of the Triad as well as Triad related conditions was high in this group of Swedish and Danish elite female endurance athletes, despite BMI and body composition being within the normal range.

- ➤ The physiological self-reported symptoms found that related to the Triad and were included in the LEAF-Q were injuries, gastrointestinal problems, and menstrual dysfunction.

PERSPECTIVES

The prevention, early detection, and treatment of low/reduced EA and oligomenorrhea/FHA in female athletes are important to prevent health consequences and injuries, but also to optimise performance. However, the knowledge concerning the association between endocrine alterations in relation to energy deficiency and injuries, as well as performance and recovery is limited, and more studies in this field are needed.

There is a growing body of evidence that male athletes from leanness-demanding sports also suffer from the outcomes of energy deficiency (Dolan et al. 2011). Disordered eating behaviour also exists in male athletes (Filaire et al. 2007, Gotlz et al. 2013, Bratland-Sanda and Sundgot-Borgen 2013), and energy deficiency causes similar interruption of the GnRH and LH pulsatility, altered hormone levels (Ayers et al. 1985, MacConnie et al. 1986, Hackney et al. 1988, Hackney et al. 1990, Degoutee et al. 2006, Dolan et al. 2011, Hagmar et al. 2013), impaired bone health (Guillaume et al. 2012, Dolan et al. 2011, Olmedillas et al. 2011), and impaired reproductive function in male endurance athletes (De Souza et al. 1997, Cumming and Wheeler 1989). Hence, the IOC Consensus Statement (Mountjoy et al. 2014) that describes a broader concept affecting many aspects of physiological function, health and athletic performance in both female and male athletes, is welcome. Studies investigating the prevalence of the broader concept of RED-S in both male and female athletes are, therefore, needed. Furthermore, screening tools such as the LEAF-Q as well as DE-screening tools needs to be tested and evaluated in male athletes as well.

The IOC as well as the Triad Coalition Consensus Statement has presented practical clinical models for the management of affected athletes providing guidelines to risk assessment, treatment and return-to-play models (De Souza et al. 2014, Mountjoy et al. 2014) ready for the sport societies to implement in order to improve the medical management of both male and female athletes. However, further validation of tools and methods to accurately assess EA as well as biomarkers for RED-S in both male and female athletes are needed.

REFERENCE LIST

Abel MG., Hannon JC., Sell K., et al. Validation of the KenZ Lifecorder EX and ActiGraph GT1M accelerometers for walking and running in adults. Appl Physiol Nutr Metab 2008;33(6):1155-64.

Ackerman KE., Nazem T., Chapko D., et al. Bone Microarchitecture Is Impaired in Adolescent Amenorrheic Athletes Compared with Eumenorrheic Athletes and Nonathletic Controls. J Clin Endocrinol Metab 2011;96(10):3123-33.

Ainsworth BE., Haskell WL., Whitt MC., et al. Compendium of Physical Activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32(9):S498-S516.

American Psychiatric Association. Feeding and Eating Disorders. Diagnostic and Statistical Manual of Mental Disorders. Washington DC, 5th; 2013.

Aubertin-Leheudre M., Gorbach S., Woods M., et al. Fat/fiber intakes and sex hormones in healthy premenopausal women in USA. J Ster Biochem Molec Biol 2008;112(1-3):32-9.

Ayers JW., Komesu V., Romani T., et al. Anthropomorphic, hormone, and psychologic correlates of semen quality in endurancetrained male athletes. Fertil Steril 1985;43:917-21.

Ballantyne GH. Peptide YY(1-36) and Peptide YY(3-36): Part I. Distribution, Release and Actions. Obes Surgery 2006;16:651-8.

Bangsbo J., Graham TE., Kiens B., Saltin B. Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. J Physiol 1992;451:205-27.

Barrack MT, Rauh MJ, Nichols JF. Prevalence of and traits associated with low BMD among female adolescent runners. Med Sci Sports Exerc 2008;40:2015-21.

Barrack MT., Rauh MJ., Nichols JF. Cross-Sectional Evidence of Suppressed Bone Mineral Accrual Among Female Adolescent Runners. J Bone Min Res 2010a;25(8):1850-7.

Barrack MT., Van Loan MD., Rauh MJ., Nichols JF. Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover. Am J Clin Nutr 2010b;92(3):652-9.

Barrack MT., Van Loan MD., Rauh MJ., Nichols JF. Body Mass, Training, Menses, and Bone in Adolescent Runners: A 3-yr Follow-up. Med Sci Sports Exerc 2011;43(6):959-66.

Barrack MT., Ackerman KE., Gibbs JC. Update on the female athlete triad. Curr Rev Musculoskelet Med 2013;6:195-204.

Beals KA., Meyer NL. Female athlete triad update. Clin Sports Med 2007;26(1):69-89.

Benell KL., Brukner PD., Malcolm SA. Effect of altered reproductive function and lowered testosterone levels on bone density in male endurance athletes. Brit J Sports Med 1996;30:205-8.

Biedleman BE., Puhl JL., De Souza MJ. Energy balance in female distance runners. Am J Clin Nutr 1995;61:303-11.

Bing C., Wang Q., Pickavance L., Williams G. Regulatory Peptides in the Control of Food Intake and Metabolism. Biochem Soc Trans 1996;24(2):559-65.

Black AE. Critical evaluation of energy intake data using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. Intern J Obesity 2000;24:119-1130.

Black DR., Larkin LJ.., Coster DC., et al. Physiologic screening test for eating disorders/disordered eating among female collegiate athletes. J Athlet Train 2003;38(4):286-97.

Bonci CM., Bonci LJ., Granger LR., et al. National athletic trainers' association position statement: preventing, detecting, and managing disordered eating in athletes. J Athl Train 2008;43(1):80-108.

Bratland-Sanda S., Sundgot-Borgen J. Eating disorders in athletes: Overview of prevalence, risk factors and recommendations for prevention and treatment. Europ J Sport Sci 2013;13(5):499-508.

Burke L. Energy Needs of Athletes. Can J Appl Physiol-Rev 2001;26(Suppl):202-19.

Burke ML., Hawley JA., Wong SH., Jeukendrup A. Carbohydrates for training and competition. J Sport Sci 2011;29(S1):17-27.

Byrne S., McLean N. Elite athletes: Effects of the pressure to be thin. J Sci Med Sport 2002;5(2):80-94.

Cann CE., Martin MC., Genant HK., Jaffe RB. Decreased spinal mineral content in amenorrheic women. JAMA1984;251:626-9.

Ciadella-Kam L., Guebels CF., Maddalozzo GF., Manore M.M. Dietary Intervention Restored Menses in Female Athletes with Exercise-Associated Menstrual Dysfunction with Limited Impact on Bone and Muscle Health. Nutr 2014;6:3018-39.

Cobb KL., Bachrach LK., Greendale G., et al. Disordered eating, menstrual irregularity, and bone mineral density in female runners. Med Sci Sports Exerc 2003;35(5):711-9.

Constantini NW., Warren MP. Special Problems of the Female Athlete. Baillieres Clin Rheumatol 1994;8(1):199-219.

Corr M., De Souza MJ., Toombs RJ., Williams NI. Circulating leptin concentrations do not distinguish menstrual status in exercising women. Hum Reprod 2011;26(3):685-94.

Cortina JM. What is Coefficienct Alpha? An examination of Theory and application. J Appl Physiol 1993;78(1):98.104.

Costill D., Gollnick PD., Jansson ED., et al. Glycogen depletion pattern in human muscle fibers during distance running. Acta Physiol Scand 1973;89:374-83.

Couzinet B., Young J., Brailly S., et al. Functional hypothalamic amenorrhoea: a partial and reversible gonadotrophin deficiency of nutritional origin. Clin Endocrinol 1999;50(2):229-35.

Cox G. Special needs of the vegetarian athlete. In: Burke L, Deakin V, editors. Clinical Sports Nutrition. Third edition ed. McGraw Hill; 2006. p. 699-720.

Cumming DC., Wheeler GD., McColl EM. The effects of exercise on reproductive function in men. Sports Med 1989;7(1):17.

Cumming DC. Exercise-associated amenorrhea, low bone density, and estrogen replacement therapy. Arch Intern Med 1996;156:2193-5.

Cumming DC., Cumming CE. Estrogen replacement therapy and female athletes: Current issues. Sports Med 2001;31(15):1025-31.

Dalvit SP. The effect of the menstrual cycle on patterns of food intake. Am J Clin Nutr 1981;34:1811-5.

Degoutte F., Jouanel P., Begue RJ., et al. Food restriction, performance, biochemical, psychological, and endocrine changes in judo athletes. Intern J Sports Med 2006 Jan;27(1):9-18.

De Souza MJ., Miller BE. The effect of endurance training on reproductive function in male runners. A "volume threshold" hypothesis. Sports Med 1997;23(357):373.

De Souza MJ., Miller BE., Loucks AB., et al. High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. J Clin Endocrinol Metab 1998;83(12):4220-32.

De Souza MJ., Van Heest J., Demers LM., Lasley BL. Luteal phase deficiency in recreational runners: Evidence for a hypometabolic state. J Clin Endocrinol Metab 2003;88(1):337-46.

De Souza MJ., Williams NI. Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. Hum Reprod Update 2004;10(5):433-48.

De Souza MJ., Hontscharuk R., Olmsted M., et al. Drive for thinness score is a proxy indicator of energy deficiency in exercising women. Appetite 2007;48(3):359-67.

De Souza MJ., West SL., Jamal SA., et al. The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. Bone 2008;43(1):140-8.

De Souza MJ., Toombs RJ., Scheid JL., et al. High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. Hum Reprod 2010;25(2):491-503.

De Souza MJ., Nattiv A., Joy E., et al. 2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. Brit J Sports Med 2014;48(289):1-20.

Deuster PA., Kyle SB., Moser PB., et al. Nutritional Intakes and Status of Highly Trained Amenorrheic and Eumenorrheic Women Runners. Fertil Steril 1986;46(4):636-43.

Deutz RC., Benardot D., Martin DE., Cody MM. Relationship between energy deficits and body composition in elite female gymnasts and runners.Med Sci Sports Exerc 2000;32(3):659-68.

Dolan E., O'Connor H., O´Loughlin G., et al. Nutritional, lifestyle and weight-control practices of professional jockeys. J Sport Sci 2011;29:791-9.

Donato J., Cravo RM., Frazao R., Elias CF. Hypothalamic Sites of Leptin Action Linking Metabolism and Reproduction. Neuroendocrinol 2011;93(1):9-18.

Drinkwater B., Nilson K., Chesnut C., et al. Bone mineral content of amenorrheic and eumenorrheic atletes. New Engl J Med 1984;311(5):277-81.

Drinkwater BL., Nilson K., Ott S., Chesnut CH. Bone mineral density after resumption of menses in amenorrheic athletes. JAMA 1986;256:380-2.

Drinkwater BL., Loucks AB., Sherman RT., et al. Position stand on the female athlete triad: IOC Medical Commission Working Group Women in Sport. 2006:1-46.

Druce MR., Small CJ., Bloom SR. Minireview: Gut Peptides Regulating Satiety. Endocrinol 2004;145(6):2660-5.

Dueck CA., Matt KS., Manore MM., Skinner JS. Treatment of athletic amenorrhea with a diet and training intervention program. Intern J Sport Nutr 1996;6(1):24-40.

Fabbri G., Petraglia F., Segre A., M, et al. Reduced spinal bone density in young women with amenorrhoea. Eur J Obstet Gynecol Reprod Biol 1991;13;41(2):117-22.

Fairburn CG., Beglin SJ. Assessment of eating disorders: interview or self-report questionnaire? Int J Eat Disord 1994;16(4):363-70.

Fairburn CG., Cooper Z., O'Connor M. Eating Disorder Examination (Edition 16.0D). Cognitive Behavior Therapy and Eating Disorders.New York: Guilford Press; 2008.

Fallon K. Athletes with gastrointestinal disorders. In: Burke L, Deakin V, editors. Clinical Sports Nutrition. Third edition ed. McGraw Hill; 2006. p. 721-38.

Fernandez-Fernandez R., Aguilar E., Tena-Sempere M., Pinilla L. Effects of polypeptide YY(3–36) upon luteinizing hormone-releasing hormone and gonadotropin secretion in prepubertal rats: in vivo and in vitro studies. Endocrinol 2005;146:1403-10.

Filaire E., Rouviex M., Pannafieux C., Ferrard C. Eating attitudes, perfectionism and body-esteem of elite male judoists and cyclists. J Sports Sci Med 2007;6:50-7.

Flood JE., Rolls BJ. Soup preloads in a variety of forms reduce meal energy intake. Appetite 2007;49:626-34.

Flood-Obbagy JE., Rolls BJ. The effect of fruit in different forms on energy intake and satiety at a meal. Appetite 2009;52:416-22.

Friedl KE., Moore RJ., Reed W., et al. Endocrine markers of semistarvation in healthy lean men in a multistressor environment. J Appl Physiol 2000;88:1820-1830.

Fuqua JS., Rogol AD. Neuroendocrine alterations in the exercising human:Implications for energy homeostasis. Metab Clin Exper 2013;62:911-21.

Garner DM., Olmstead MP., Bohr Y., et al. The eating attitudes test: psychometric features and clinical correlates. Psychol Med 1982;12:871-8.

Garner DM. Eating Disorder Inventory-3. Professional Manual. Par Inc; 2004.

Gaskins AJ., Mumford SL., Zhang CL., et al. Effect of daily fiber intake on reproductive function: the BioCycle Study. Am J Clin Nutr 2009 Oct 1;90(4):1061-9.

Gejl KD., Hvid LG., Frandsen U., et al. Muscle glycogen content modifies SR Ca^{2+} release rate in elite endurance athletes. Med Sci Sports Exerc 2014;46:496-505.

Gibbs JC., Williams NI., Scheid JL., et al. The Association of a High Drive for Thinness With Energy Deficiency and Severe Menstrual Disturbances: Confirmation in a Large Population of Exercising Women. Intern J Sport Nutr Exerc Metab 2011;21(4):280-90.

Gibbs JC., Williams NI., De Souza MJ. Prevalence of individual and combined components of the female athlete triad. Med Sci Sports Exerc 2013a;45(5):985-96.

Gibbs JC., Williams NI., Mallinson RJ., et al. Effect of High Dietary Restraint on Energy Availability and Menstrual Status. Med Sci Sports Exerc 2013b;45(9):1790-7.

Gibson JH., Mitchell A., Harries MG., Reeve J. Nutritional and exercise-related determinants of bone density in elite female runners. Osteo Intern 2004;15(8):611-8.

Gilsanz V., Gibbens DT., Carlson M., et al. Peak trabecular vertebral density: a comparison of adolescent and adult females. Calcif Tissue Int 1988;43:260-2.

Goldberg GR., Black AE., Jebb SA., et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Europ J Clin Nutr 1991;45(12):569-81.

Goldsmith R., Joanisse DR., Gallagher D., et al. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. Am J Physiol Regul Integr Comp Physiol 2010:298;R79-R88.

Goltz FR., Stenzel LM., Schneider CD. Disordered eating behaviors and body image in male athletes. Revista Bras Psiquiatria 2013;35:237-42.

Gordon CM. Functional Hypothalamic Amenorrhea. New Engl J Medicine 2010 22;363(4):365-71.

Grandt D., Teyssen S., Schimiczek M., et al. Novel generation of hormone receptor specificity by amino terminal processing of peptide YY. Biochem Biophys Res Communic 1992;186(3):1299-306.

Gremion G., Rizzoli R., Slosman D., et al. Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. Med Sci Sports Exerc 2001;33(1):15-21.

Grobler LA., Collins M., Lambert MI., et al. Skeletal muscle pathology in endurance athletes with acquired training intolerance. Br J Sports Med 2004;38(6):697-703.

Guebels CF., Kam LC., Maddalozzo GF., Manore M.M. Active Women Before/After an Intervention Designed to Restore Menstrual Function: Resting Metabolic Rate and Comparison of Four Methods to Quantify Energy Expenditure and Energy Availability. Int J Sport Nutr Exerc Metab 2014;24:37-46.

Guillaume G., Chappard D., Audran M. Evaluation of the Bone Status in High-Level Cyclists. J Clin Densitom 2012;15(1):103-7.

Hackney AC., Sinning WE., Brout BC. Reproductive hormonal profiles of endurance-trained and untrained males. Med Sci Sports Exerc 1988;28(180):190.

Hackney AC., Sinning WE., Brout BC. Reduced serum testosterone and prolactin levels in distance runners. Int J Sports Med 1990;11(298):303.

Hagmar M., Berglund B., Brismar K., Hirschberg AL. Hyperandrogenism May Explain Reproductive Dysfunction in Olympic Athletes. Med Sci Sports Exerc 2009;41(6):1241-8.

Hagmar M., Berglund B., Brismar K., Hirschberg AL. Body composition and endocrine profile of male Olympic athletes striving for leanness. Clin J Sport Med 2013;23(3):197-201.

Harber VJ., Petersen SR., Chilibeck PD. Thyroid hormone concentrations and muscle metabolism in amenorrheic and eumenorrheic athletes. Can J Appl Physiol-Rev 1998;23(3):293-306.

Harber VJ. Energy balance and reproductive function in active women. Can J Appl Physiol-Revue 2004;29(1):48-58.

Hawley JA., Burke LM. Carbohydrate availability and training adaptation: effects on cell metabolism. Exerc Sport Sci Rev 2010;38(4):152-60.

Henry CJK., Lightowler HJ., Marchini J. Intra-individual variation in resting metabolic rate during the menstrual cycle. Br J Nutr 2003;89(6):811-7.

Hergenroeder AC., Smith EA., Shypilo R., et al. Bone mineral changes in young women with hypothalamic amenorrhea treated with oral contraceptives, medroxyprogesterone, or placebo over 12 months. Med Sci Sports Exerc 1997;43:959-66.

Hill LS., Reid F., Morgan JF., Lacey HJ. SCOFF, the development of an eating disorder screening questionnaire. Intern J Eat Dis 2009;43(4):344-51.

Hind K., Truscott JG., Evans JA. Low lumbar spine bone mineral density in both male and female endurance runners. Bone 2006;39(4):880-5.

Hinton PS., Kubas KL. Psychosocial correlates of disordered eating in female collegiate athletes: validation of the ATHLETE questionnaire. J Am Coll Health 2005;54(3):149-56.

Hirschberg Lindén A. Sex hormones, appetite and eating behaviour in women. Maturitas 2012;71:248-256

Hoch AZ., Dempsey RL., Carrera GF., et al. Is there an association between athletic amenorrhea and endothelial cell dysfunction? Med Sci Sports Exerc 2003;35(3):377-83.

Hoch AZ., Pajewski NM., Moraski L., et al. Prevalence of the Female Athlete Triad in High School Athletes and Sedentary Students. Clin J Sport Med 2009;19(5):421-8.

Hoch AZ., Papanek P., Szabo A., et al. Association Between the Female Athlete Triad and Endothelial Dysfunction in Dancers. Clin J Sport Med 2011;21(2):119-25.

Holt SH., Miller JC., Petocz P., Farmakalidis E. A satiety index of common foods. Eur J Clin Nutr 1995;49(9):675-90.

Horvath PJ., Eagen CK., Ryer-Calvin SD., Prendergast DR. The effects of varying dietary fat on the nutrient intake in male and female runners. J Am Coll Nutr 2000;19(1):42-51.

Howgate DJ., Graham SM., Leonidou A., et al. Bone metabolism in anorexia nervosa: molecular pathways and current treatment modalities. Osteop Intern 2013;24:407-21.

Hubert P., King NA., Blundell JE. Uncoupling the effects of energy expenditure and energy intake: Appetite response to short-term energy deficit induced by meal omission and physical activity. Appetite 1998 Aug;31(1):9-19.

Huda MS., Wilding JP., Pinkey JH. Gut peptides and the regulation of appetite. Obesity 2006;7:163-82.

Ihle R., Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. J Bone Min Res 2004;19(8):1231-40.

Jonnalagadda SS., Benardot D., Dill MN. Assessment of under-reporting of energy intake by elite female gymnast. Int J Sport Nutr Exerc Metab 2000;10(3):315-25.

Kaiserauer S., Snyder AC., Sleeper M., Zierath J. Nutritional, Physiological, and Menstrual Status of Distance Runners. Med Sci Sports Exerc 1989;21(2):120-5.

Keen AD., Drinkwater BL. Irreversible bone loss in former amenorrheic athletes. Osteop Intern 1997;7:311-215.

Keire DA., Mannon P., Kobayashi M., et al. Primary structures of PYY, [Pro34]PYY, and PYY-(3–36) confer different conformations and receptor selectivity. Am J Gastroinest Liver Physiol 2000;279:126-31.

Kerr D., Khan K., Bennell K. Bone, exercise and nutrition. In: Burke L, Deakin V, editors. Clinical Sports Nutrition. Third edition ed. McGraw Hill; 2006:237-62.

King NA., Lluch A., Stubbs RJ., Blundell JE. High dose exercise does not increase hunger or energy intake in free living males. Europ J Clin Nutr 1997;51(7):478-83.

Kini U., Nandeesh BN. Physiology of Bone Formation, Remodeling, and Metabolism. In: Fogelman I, Gnanasegaran G, Wall H, editors. Radionuclide and Hybrid Bone Imaging.Heidelberg: Springer-Verlag Berlin; 2012:29-57.

Koehler K., Achtzehn S., Braun H., et al. Comparison of self-reported energy availability and metabolic hormones to assess adequacy of dietary energy intake in young elite athletes. Appl Physiol Nutr Metab 2013;38:725-33.

Lagowska K., Kapczuk K., Fribe Z., Bajeska J. Effects of dietary intervention in young female athletes with menstrual disorders. J Intern Soc Sports Nutr 2014;11(21):1-8.

Larson-Meyer ED., Palm S., Bansal A., et al. Influence of running and walking on hormonal regulators of appetite in women. J Obes 2012;2012:doi:10.1155/2012/730409.

Laughlin GA., Yen SS. Nutritional and Endocrine-Metabolic Aberrations in Amenorrheic Athletes. J Clin Endocrinol Metab 1996;81(12):4301-9.

Laughlin GA., Dominguez CE., Yen SSC. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1998;83(1):25-32.

Lebenstedt M., Platte P., Pirke KM. Reduced resting metabolic rate in athletes with menstrual disorders. Med Sci Sports Exerc 1999;31(9):1250-6.

Lebrun C. Bone disease in female athletes. Women's Health Medicine 2006.

Lloyd T., Buchanan JR., Bitzer S., et al. Interrelationships of Diet, Athletic Activity, Menstrual Status, and Bone-Density in Collegiate Women. Am J Clin Nutr 1987;46(4):681-4.

Loucks AB., HeathEM. Dietary restriction reduces Luteinizing Hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. J Clin Endocrinol Metab 1994;78(4):910-915.

Loucks AB., Verdun M., Heath EM. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J Appl Physiol 1998;84(1):37-46.

Loucks AB., Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab 2003;88(1):297-311.

Loucks AB. The response of luteinizing hormone pulsatility to 5 days of low energy availability disappears by 14 years of gynecological age. J Clin Endocrinol Metab 2006a;91(8):3158-64.

Loucks AB. The endocrine system: Integrated influences on metabolism, growth and reproduction. In: Tipton C, editor. Thyriod hormone, or Triiodothyronine. In ACSM's Advanced exercise physiology. Baltimore: Lippincott Williams & Wilkins; 2006b;467-468.

Loucks AB., Kiens B., Wright HH. Energy availability in athletes. J Sports Sci 2011;29:S7-S15.

Loucks AB. Energy balance and energy availability. In: Maughan RJ, editor. Sports Nutrition. International Olympic Committee. 1 ed. John Wiley & Sons, Ltd; 2014:72-87.

Lupton JR., Trumbo PR.. Dietary Fiber. In: Shils ME, Shike M, Catharine Ross A, Caballero B, Cousins RJ, editors. Modern Nutrition in Health and Disease. Tenth edition ed. Lippincott Williams & Wilkins; 2006:83-91.

MacConnie SE., Barkan A., Lampman RM., et al. Decreased hypothalamic gonadotropin-releasing hormone secretion in male marathon runners. New Engl J Med 1986;315(7):411-7.

Manolagas SC., Jilika RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. New Engl J Med 1995;332:305-11.

Manore MM., Kam LC., Loucks AB. The female athlete triad: Components, nutrition issues, and health consequences. J Sports Sci 2007;25:S61-S71.

Marcus R., Cann C., Madvig P., et al. Menstrual Function and Bone Mass in Elite Women Distance Runners - Endocrine and Metabolic Features. Annals Intern Med 1985;102(2):158-63.

Marra M., Polito A., De Filippo E., et al. Are the general equations to predict BMR applicable to patients with anorexia nervosa? Eat Weight Disord 2002;7(1):53-9.

Martinsen M., Sundgot-Borgen J. Higher prevalence of eating disorders among adolescent elite athletes than controls. Med Sci Sports Exerc 2013;45(6):1188-1197.

Martinsen M., Holme I., Pensgaard AM., et al. The Development of the Brief Eating Disorder in Athletes Questionnaire. Med Sci Sports Exerc. 2014 Aug;46(8):1666-75.

McNulty KY., Adams CH., Anderson JM, Affenito SG. Development and validation of a screening tool to identify eating disorders in female athletes. J Am Diet Assoc 2001;101(8):886-92.

Meczekalski B., Podfigurna-Stopa A., Katulski K. Long-term consequences of anorexia nervosa. Maturitas 2013;75(3):215-20.

Meijer GA., Westerterp KR., Saris WH., Tenhoor F. Sleeping Metabolic-Rate in Relation to Body-Composition and the Menstrual-Cycle. Am J Clin Nutr 1992;55(3):637-40.

Mountjoy M., Sundgot-Borgen J., Burke L., et al. The IOC concensus statement: beyond the Female Athlete Triad - Relative Energy Deficiency in Sport (RED-S). Br J Sports Med 2014;48:491-7.

Mulligan K., Butterfield GE. Discrepancies Between Energy-Intake and Expenditure in Physically Active Women. Br J Nutr 1990;64(1):23-36.

Myerson M., Gutin B., Warren MP., et al. Resting Metabolic-Rate and Energy-Balance in Amenorrheic and Eumenorrheic Runners. Med Sci Sports Exerc 1991;23(1):15-22.

Nagel DL., Black DR., Leverenz LJ., Coster DC. Evaluation of a screening test for female college athletes with eating disorders and disordered eating. J Athlet Train 2000;35(4):431-40.

Nattiv A., Loucks AB. Manore M., et al. The female athlete triad. Med Sci Sports Exerc 2007;39(10):1867-82.

Nelson ME., Fisher EC., Catsos PD., et al. Diet and Bone Status in Amenorrheic Runners. Am J Clin Nutr 1986;43(6):910-6.

Nichols JF., Rauh MJ., Lawson MJ., et al.. Prevalence of the female athlete triad syndrome among high school athletes. Arch Pediatr Adolesc Med 2006;160(2):137-42.

Nichols JF., Rauh MJ., Barrack MT., et al. Disordered eating and menstrual irregularity in high school athletes in lean-build and nonlean-build sports. Intern J Sport Nutr Exerc Metab 2007;17(4):364-77.

Nichols JF., Aralis H., Merino SG., et al. Utility of the Actiheart Accelerometer for Estimating Exercise Energy Expenditure in Female Adolescent Runners. Int J Sport Nutr Exerc Metab 2010;20(6):487-95.

Nordic Council of Ministers. Nordic Nutrition Recommendations 2012, part 1 - summary, principles and use. 5th edition ed. Norden; 2013.

O'Donnell E., Harvey PJ., De Souza MJ. Relationships between vascular resistance and energy deficiency, nutritional status and oxidative stress in oestrogen deficient physically active women. Clin Endocrinol 2009;70(2):294-302.

O'Donnell E., De Souza MJ. Increased serum adiponectin concentrations in amenorrheic physically active women are associated with impaired bone health but not with estrogen exposure. Bone 2011;1;48(4):760-7.

Ohwada R., Hotta M., Oikawa S., Takano K. Etiology of hypercholesterolemia in patients with anorexia nervosa. Int J Eat Disord 2006;39(7):598-601.

Olmedillas H., Gonzalez-Aguero A., Moreno LA., et al. Bone Related Health Status in Adolescent Cyclists. Plos One 2011;6(9):e2481.

Perry AC., Crane LS., Applegate B., et al. Nutrient intake and psychological and physiological assessment in eumenorrheic and amenorrheic female athletes: A preliminary study. Intern J Sport Nutr 1996;6(1):3-13.

Pettersson U., Stålnacke BM., Ahlenius GM., et al. Low bone mass density at multiple skeletal sites, including the appendicular skeleton in amenorrheic runners. Calcif Tissue Intern 1999;64(2):117-25.

Philips SM., Van Loon LC. Dietary protein for athletes: From requirements to optimim adaptation. J Sports Sci 2011;29(S1):29-38.

Pinilla L., Fernandez-Fernandez R., Roa J., et al. Selective role of neuropeptide Y receptor subtype Y2 in the control of gonadotropin secretion in the rat. Am J Physiol Endocrinol Metabol 2007;293:1385-92.

Plasqui G., Westerterp KR. Physical activity assessment with accelerometers: An evaluation against doubly labeled water. Obesity 2007 Oct;15(10):2371-9.

Platte P., Lebenstedt M., Ruddel H., Pirke KM. Energy cost of physical activity in patients with anorexia nervosa. Europ Eat Dis Rev 2000;8(3):237-44.

Polito A., Fabbri A., Ferro-Luzzi A., et al. Basal metabolic rate in anorexia nervosa: Relation to body composition and leptin concentrations. Am J Clin Nutr 2000;71(6):1495-502.

Pollock N., Grogan C., Perry M., et al. Bone-Mineral Density and Other Features of the Female Athlete Triad in Elite Endurance Runners: A Longitudinal and Cross-Sectional Observational Study. Intern J Sport Nutr Exerc Metab 2010;20(5):418-26.

Rauh MJ., Nichols JF., Barrack MT. Relationships Among Injury and Disordered Eating, Menstrual Dysfunction, and Low Bone Mineral Density in High School Athletes: A Prospective Study. J Athlet Train 2010;45(3):243-52.

Redman LM., Loucks AB. Menstrual disorders in athletes. Sports Med 2005;35(9):747-55.

Redman LM., Heilbronn LK., Martin CK., et al. Metabolic and Behavioral Compensations in Response to Caloric Restriction: Implications for the Maintenance of Weight Loss. Plos One 2009:9;4(2).

Reed JL., Bowell JL., Hill BR., et al. Exercising women with menstrual disturbances consume low energy dense foods and beverages. Appl Physiol Nutr Metab 2011;36(3):382-94.

Reed JL., De Souza MJ., Williams NI. Changes in energy availability across the season in Division I female soccer players. J Sport Sci 2012;31(3):314-24.

Reed JL., De Souza MJ., Kindler JM., Williams NI. Nutritional practices associated with low energy availability in Division I female soccer players. J Sports Sci Medicine 2014, DOI:10.1080/02640414.2014.908321.

Reuters V., Almeida CP., Teixedia TP., et al. Effects of subclinical hypothyroidism treatment on psychiatric symptoms, muscular complaints, and quality of life. Arq Bras Endocrinol Metab 2012;56:128-36.

Rickenlund A., Eriksson MJ., Schenck-Gustafsson K., Hirschberg AL. Amenorrhea in female athletes is associated with endothelial dysfunction and unfavorable lipid profile. J Clin Endocrinol Metab 2005;90(3):1354-9.

Robinson TL., Snowharter C., Taaffe DR., et al. Gymnasts Exhibit Higher Bone Mass Than Runners Despite Similar Prevalence of Amenorrhea and Oligomenorrhea. J Bone Min Res 1995;10(1):26-35.

Rodriguez NR., DiMarco NM., Langley S. et al. Nutrition and Athletic Performance. Med Sci Sports Exerc 2009;41(3):709-31.

Rolls BJ., Roe LS., Meengs JS. Salad and Satiety: Energy Density and Portion Size of a First-Course Salad Affect Energy Intake at Lunch. J Am Diet Assoc 2004;104:1570-6.

Rolls BJ. The relationship between dietary energy density and energy intake. Physiol Behav 2009;97:609-15.

Rominj JA., Coyle EF., Sidossis LS., et al. Substrate metabolism during different exercise intensities in endurance-trained women. J Appl Physiol 2000;88:1707-14.

Russell M., Stark J., Nayak S., et al. Peptide YY in adolescent athletes with amenorrhea, eumenorrheic athletes and non-athletic controls. Bone 2009;45:104-9.

Schaal K., Van Loan MD., Casazza GA. Reduced Catecholamine Response to Exercise in Amenorrheic Athletes. Med Sci Sports Exerc 2011;43(1):34-43.

Schnaper HW., McGuire J., Runyan C., Hubchak SC. Sex steroids and the endothelium. Currt Med Chemistry 2000;7(5):519-31.

Schneid JL., Williams NI., West SL., et al. Elevated PYY is associated with energy deficiency and indices of subclinical disordered eating in exercising women with hypothalamic amenorrhea. Appetite 2009;52(1):184-92.

Schneid JL., De Souza MJ. Menstrual irregularities and energy deficiency in physically active women: the role of ghrelin, PYY and adipocytokines. Med Sci Sports Exerc 2010;55:82-112.

Schoeller DA. How accurate is self-reported dietary energy intake? Nutr Reviews 1990;48(10):373-9.

Schwartz MW., Woods SC., Porte D., et al. Central nervous system control of food intake. Nature 2000;404:661-71.

Scofield KL., Hecht S. Bone Health in Endurance Athletes: Runners, Cyclists, and Swimmers. Curr Sport Med Reports 2012;11(6):328-34.

Shaw D., Gohl K., Basson J. Intestinal mucosal atrophy and adaptation. World J Gastroenterolog 2012;18(44):6357-75.

Singhal V., Misra M., Klibanski A. Endocrinology of anorexia nervosa in young people:recent insights. Curr Opin Endocrinol Diabetes Obes 2014;21:64-70.

Sjödin AM., Forslund AH., Westerterp KR., et al. The influence of physical activity on BMR. Med Sci Sports Exerc 1996;28(1):85-91.

Skaaderud F., Fladvad T., Garthe I. et al. Den dårlig ernærte idrettsutøveren. Vektreduksjon, kroppsmodifikasjon og spiseforstyrrelser i toppidrett. Retningslinjer for holdning og handling. 2012. www.olympiatoppen.no.

Slavin JL. Dietary fibre and satiety - not all fibre is alike:Woodhead Publishing Ltd; 2009;333-40.

Smith DA., Dollman J., Withers RT., et al. Relationship between maximum aerobic power and resting metabolic rate in young adult women. J Appl Physiol 1997;82(1):156-63.

Snead DB., Stubbs CC., Weltman JY., et al. Dietary Patterns, Eating Behaviors, and Bone-Mineral Density in Women Runners. Am J Clin Nutr 1992;56(4):705-11.

Solomon SJ., Kurzer MS., Calloway DH. Menstrual-Cycle and Basal Metabolic-Rate in Women. Am J Clin Nutr 1982;36(4):611-6.

Solomon CG., Hu FB., Dunaif A., et al. Menstrual cycle irregularity and risk for future cardiovascular disease. J Clin Endocrinol Metab 2002;87(5):2013-7.

Soyka LA., Misra M., Frenchman A., et al. Abnormal bone mineral accrual in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 2002;87:4177-85.

Speakman JR., Selman C. Physical activity and resting metabolic rate. Proceed Nutr Society 2003;62(3):621-34.

Steiner H., Pyle RP., Brassington GS., et al. The College Health Related Information Survey (C.h.R.I.s.-73): a screen for college student athletes. Child Psychiatry Hum Dev 2003;34(2):97-109.

Stubbs RJ., Hughes DA., Johnstone AM., et al. Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. Am J Physiol-Regulat Integr Comp Physiol 2004;1:286(2):R350-R358.

Stunkard AJ., Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 1985;29(1):71-83.

Sundgot-Borgen J. Nutrient Intake of Female Elite Athletes Suffering from Eating Disorders. Intern J Sport Nutr 1993;3(4):431-42.

Sundgot-Borgen J. Risk and Trigger Factors for the Development of Eating Disorders in Female Elite Athletes. Med Sci Sports Exerc 1994;26(4):414-9.

Sundgot-Borgen J., Torstveit MK. Prevalence of eating disorders in elite athletes is higher than in the general population. Clin J Sport Med 2004;14(1):25-32.

Sundgot-Borgen J., Torstveit MK. Aspects of disordered eating continuum in elite high-intensity sports. Scand J Med Sci Sports 2010;20:112-21.

Sundgot-Borgen J., Meyer NL., Lohman TG., et al. How to minimise the health risks to athletes who compete in weight-sensitive sports review and position statement on behalf of the Ad Hoc Research Working Group on Body Composition, Health and Performance, under the auspices of the IOC Medical Commission. Brit J Sports Med 2013;47(16):1012-22.

Tenforde AS., Fredericson M. Influence of sports participation on bone health in the young athlete: a review of the literature. Am Acad Phys Med Rehab 2011;3:861-867.

Thein-Nissenbaum JM., Rauh MJ., Carr KE., et al. Menstrual Irregularity and Musculoskeletal Injury in Female High School Athletes. J Athlet Train 2012;47(1):74-82.

Thompson J., Manore MM. Predicted and measured resting metabolic rate of male and female endurance athletes. J Am Diet Assoc 1996 Jan;96(1):30-4.

Thong FS., McLean C., Graham TE. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. J Appl Physiol 2000;88(6):2037-44.

Tomten S.E, Høstmark AT. Energy balance in weight stable athletes with and without menstrual disorders. Scandinavian Journal of Medicine & Science in Sports 2006 Apr;16(2):127-33.

Torstveit MK., Sundgot-Borgen J. Participation in leanness sports but not training volume is associated with menstrual dysfunction: a national survey of 1276 elite athletes and controls. Br J Sports Med 2005a;1:39(3):141-7.

Torstveit MK., Sundgot-Borgen J. The female athlete triad exists in both elite athletes and controls. Med Sci Sports Exercise 2005b;37(9):1449-59.

Torstveit MK., Rosenvinge JH., Sundgot-Borgen J. Prevalence of eating disorders and the predictive power of risk models in female elite athletes: a controlled study. Scand J Med Science Sports 2008;18(1):108-18.

Torstveit MK., Sundgot-Borgen J. Are under- and overweight female elite athletes thin and fat? A controlled study. Med Sci Sports Exerc 2012;44(5):949-57.

Valassi E., Scacchi M., Cavagnini F. Neuroendocrine control of food intake. Nutr Metab Cardiovasc Dis 2008;18:158-68.

Van Heest J., Rodgers CD., Mahoney CE., De Souza MJ. Ovarian Suppression Impairs Sport Performance in Junior Elite Female Swimmers. Med Sci Sports Exerc 2014;46(1):156-66.

Wade GN., Jones JE. Neuroendocrinology of nutritional infertility. Am J Physiol Regul Integr Comp Physiol 2004;287(6):R1277-R1296.

Warren MP., Holderness CC., Lesobre V., et al. Hypothalamic amenorrhea and hidden nutritional insults. J Soc Gynecolog Invest 1994;1(1):84-8.

Warren MP., Voussoughian F., Geer EB., et al. Functional hypothalamic amenorrhea: Hypoleptinemia and disordered eating. J Clin Endocrinol Metab 1999;84(3):873-7.

Warren MP., Perlroth NE. The effects of intense exercise on the female reproductive system. J Endocrinol 2001 Jul;170(1):3-11.

Warren MP., Brooks-Gunn J., Fox RP., et al. Osteopenia in exercise-associated amenorrhea using ballet dancers as a model: A longitudinal study. J Clin Endocrinol Metab 2002;87(7):3162-8.

Warren MP., Brooks-Gunn J., Fox RP., et al. Persistent osteopenia in ballet dancers with amenorrhea and delayed menarche despite hormone therapy: a longitudinal study. Fertil Steril 2003;80(2):398-404.

Warren MP. Endocrine manifestations of eating disorders. J Clin Endocrinol Metab 2011;96(2):333-43.

Westerterp KR., Meijer GA., Janssen EM., et al. Long-Term Effect of Physical-Activity on Energy-Balance and Body-Composition. Br J Nutr 1992;68(1):21-30.

Whybrow S., Hughes DA., Ritz P., et al. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. Br J Nutr 2008;100(5):1109-15.

Wilmore JH., Wambsgans KC., Brenner M., et al. Is There Energy-Conservation in Amenorrheic Compared with Eumenorrheic Distance Runners. J Appl Physiol 1992;72(1):15-22.

Woodruff SJ., Meloche RD. Energy availability of female varsity volleyball players. Intern J Sport Nutr Exerc Metab 2013;23:24-30.

Wrutniak-Cabello C., Casa F., Cabello G. Thyroid hormone action on mitochondria. J Mol End 2001;26:67-77.

Wulff HE., Melin A., Waaddegaard M., Kanstrup IL. BMD in elite female triathletes is related to isokinetic peak torque without any association to sex hormone concentrations. J Sports Med Phys Fitness 2012;52(5):489-500.

Yeager KK., Augustino R, Nattiv A. The female athlete triad – disordered Eating, amenorrhea, osteoporosis. Med Sci Sports Exerc 1993;25(7):775-7.

PAPER I

Energy Availability and the Female Athlete Triad in Elite Endurance Athletes

Melin A., Tornberg ÅB., Skouby S., Møller SS., Sundgot-Borgen J., Faber J., Sidelmann JJ., Aziz M., Sjödin A.

PAPER II

Lower Energy Density and High Fibre Intake are Dietary Concerns in Female Endurance Athletes

Melin A., Tornberg ÅB., Skouby S., Møller SS., Faber J., Sundgot-Borgen J., Sjödin A.

Lower energy density and high fibre intake are dietary concerns in female endurance athletes

Anna Melin[1,2], Åsa B Tornberg [2, 3], Sven Skouby[4], Sara Sig Møller[1], Jens Faber[5], Jorunn Sundgot-Borgen[6], & Anders Sjödin[1]

[1]Department of Nutrition, Exercise and Sports, University of Copenhagen, Frederiksberg, Denmark; [2]Physiotherapy, Department of Health Sciences, Lund University, Lund, Sweden; [3]Genetic & Molecular Epidemiology (GAME) Unit, Lund University Diabetes Centre (LUDC), Clinical Research Centre, Skåne University Hospital, Malmö, Sweden; [4]Endocrinological and Reproductive Unit, Department of Obstetrics/Gynaecology. Herlev Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Herlev, Denmark; [5]Medical and Endocrinological Unit, Herlev Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Herlev, Denmark; [6]Norwegian School of Sport Sciences, Oslo, Norway.

Running title: Energy availability and dietary intake

Contact name: Anna Melin, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, DK-Frederiksberg, aot@nexs.ku.dk, anna.katarina.melin@hotmail.com, fax number +45 3533 2483, cell phone, +46732629714

Abstract

Low/reduced energy availability (EA) is linked to oligomenorrhea/functional hypothalamic amenorrhea (FHA), frequently reported in weight-sensitive sports, making EA a major nutritional concern for female athletes. The aim of this study was to describe dietary characteristics of athletes with low/reduced EA and/or oligomenorrhea/FHA. Endurance athletes (n=45) were recruited from national teams and competitive clubs. Protocols included gynaecological examination, body composition, eating disorder/disordered eating (ED/DE)-evaluation, and 7-day dietary intake and EA assessment. Athletes with ED/DE (n=11), menstrual dysfunction other than oligomenorrhoea/FHA (n=5), and low dietary-record validity (n=4) were excluded. Remaining subjects (n=25) (26.6 ± 5.6 yrs., BMI 20.6 ± 2.0 kg/m^2, exercising 12.0 ± 4.4 h/week) were divided by EA [optimal: ≥188 kJ/kg fat-free mass (FFM)/day (n=11), low/reduced: <188 kJ/kg FFM/day (n=14)] and reproductive function [eumenorrhoea (n=10), oligomenorrhea/FHA (n=15)]. There was no difference in EA between oligomenorrhea/FHA and eumenorrhea subjects. However, subjects with oligomenorrhea/FHA and low/reduced EA shared the same dietary characteristics of lower energy density [(P=0.012), (P=0.020)] and lower fat intake [(P=0.047), (P=0.027), respectively]. Oligomenorrhea/FHA subjects had, furthermore, a higher fibre intake [4.1 ± 0.7 g/MJ/day vs 2.8 ± 0.6, (P<0.001)]. Conclusively, diets lower in energy density and fat content together with higher fibre content, are associated with low/reduced EA and oligomenorrhea/FHA and may constitute targets for dietary intervention in order to prevent/treat these conditions.

Key words: energy availability, amenorrhoea, energy density, dietary fibre

Introduction

Athletes need to maintain sufficient energy and nutrient intake, especially during periods of intense training, in order to avoid fatigue and injury as well as illness (Rodriguez et al. 2009; Burke 2001). Female athletes in sports that require leanness are, however, often reported not to meet energy needs and to have low energy availability (EA) (Horvath et al. 2000). Energy availability in the context of sport is defined as the energy expressed in relation to fat-free mass (FFM) remaining for all physiological functions when exercise energy expenditure has been subtracted from total energy intake (Loucks 2005). In experimental studies, healthy weight-stable eumenorrhoeic women have been reported to have EA of ≥ 188 kJ/kg FFM/day, while suppression of the pulsatility of luteinising hormone (LH) from the pituitary gland, have been shown to occur at EA below 125 kJ/kg FFM/day (Loucks & Thuma 2003), defining low EA. Ovarian function depends on the frequency of LH-pulsatility (Loucks et al. 2011) and persistent low EA with or without an eating disorder (ED) or disordered eating behaviour (DE) is therefore considered to be the primary cause of oligomenorrhoea/ functional hypothalamic amenorrhoea (FHA) in female athletes (Nattiv et al. 2007). Hence, physical active women are recommended EA ≥ 188 kJ/kg FFM/day in order to ensure adequate energy for all physiological functions (De Souza et al. 2014). Athletes aiming to loose body weight or body fat are recommended to follow a diet and training regime providing EA between 125-188 kJ/kg FFM/day (Loucks et al. 2011), defining reduced EA (Gibbs et al. 2013). Low/reduced EA is also related to other endocrine disruptions and conditions such as reduced resting metabolic rate (RMR) and gastrointestinal problems (Mountjoy M et al. 2014). Restricted eating behaviour and menstrual dysfunction (MD) have also been associated with an increased risk of impaired bone health, muscle, skeletal and joint injuries as well as impaired performance (Nattiv et al. 2007; Mountjoy M et al. 2014; De Souza et al. 2014). It is therefore of great concern that low/reduced EA and MD are frequently reported in female athletes (Gibbs et al. 2013). Since weight influences performance in many sports, low/reduced EA can be due to intentional restriction of food intake in order to obtain or maintain a low body weight (Nattiv et al. 2007) and 24% of female élite endurance athletes have been reported to have DSM-IV diagnosed eating disorders (Sundgot-Borgen & Torstveit 2004) which are clinical mental disorders associated with various abnormal eating behaviours and an irrational fear of gaining weight, as well as false beliefs about eating, weight, and shape (Nattiv et al. 2007). Energy requirements for female endurance athletes may vary considerably, but have been documented to reach levels of around 20 MJ/day over several days, corresponding to a physical activity level (PAL) of 3.6 (Sjodin et al. 1996). Studies have, however, reported female endurance athletes to have similar or even lower energy intake compared with sedentary women (Beidleman et al. 1995), while recreationally active women with MD have also been reported to eat a diet low in energy density (Reed et al. 2011). A diet with low energy density will limit energy intake (Rolls 2009) and, therefore, increase the risk for low/reduced EA in female élite endurance athletes, especially in periods with high energy demands. We therefore wanted to investigate dietary characteristics related to low/reduced current EA and/or oligomenorrhea/FHA that might be targeted for nutritional adjustment in female athletes without eating disorders or disordered eating behaviour.

Methods

The methods used in this study have previously been described in detail (Melin A et al. 2014a; Melin A et al. 2014b). The study population was recruited through the Danish and Swedish sport federations and competitive sports clubs involved in endurance sports that demands leanness, such as middle and long-distance running, triathlon and orienteering. Before inclusion, all subjects were informed orally, and in writing, of all study procedures and signed an informed consent form. Permission to undertake the study was provided by the Swedish and Danish Confederation of Sports, Team Denmark, the Data Inspectorate and the Regional Ethical committees, both in Sweden and Denmark (nos. 2011/576 and H-4-2011-096, respectively). Subjects included and defined as endurance athletes were athletes at national team levels or competitive endurance athletes from regional sports clubs, aged between 18 and 38, training a minimum of five times per week. The data collection was performed on two consecutive days followed by a seven-day registration period in the athletes' normal environment. The first day consisted of anthropometric assessment [body weight and height was measured and dual-energy X-ray absorptiometry (DXA) was used to determine fat-free mass (FFM) and fat mass (FM)], and examinations of reproductive function. Menstruating athletes were examined in the early follicular phase (the 3rd to the 5th day of menstruation) by an experienced gynaecologist who performed a transvaginal ultrasound examination, a measurement of sex hormone status and a retrospective history of menstrual function using the Low Energy Availability in Females Questionnaire (LEAF-Q) (Melin A et al. 2014a). Subjects were classified as having eumenorrhoea (menstrual cycles of 28 days (7) and sex hormones within the normal range), oligomenorrhoea: menstrual cycles > 35 days, FHA: primary FHA: no menarche after 15 years of age; or secondary FHA: absence of ≥ 3 consecutive menstrual cycles, polycystic ovarian syndrome (PCOS) or other MD. The second day included examinations of aerobic capacity and assessment of ED/DE.

Figure 1 illustrates the participant flow in the study, showing that of the 45 athletes completing the clinical examination protocol, five were excluded due to other clinically verified MD than oligomenorrhoea/FHA.

Since we wanted to investigate dietary intake and dietary characteristics of female athletes with low/reduced current EA and/or oligomenorrhea/FHA who were not presenting disordered eating behaviour or clinical overt eating disorders, 10 subjects were excluded after meeting the DSM-IV criteria for eating disorders, assessed by the Eating Disorder Examination (EDE-16.0) (Fairburn et al. 2008) and one due to disordered eating behaviour assessed by the Eating Disorder Inventory (EDI-3) (Garner 2004). Four subjects were identified as having low validity of their reported energy intake (corresponding to PAL < 1.6) according the Goldberg cut-off using the equation described by Black (Black 2000) and were excluded and a total of 25 subjects were consequently included in the final analyses of dietary characteristics.
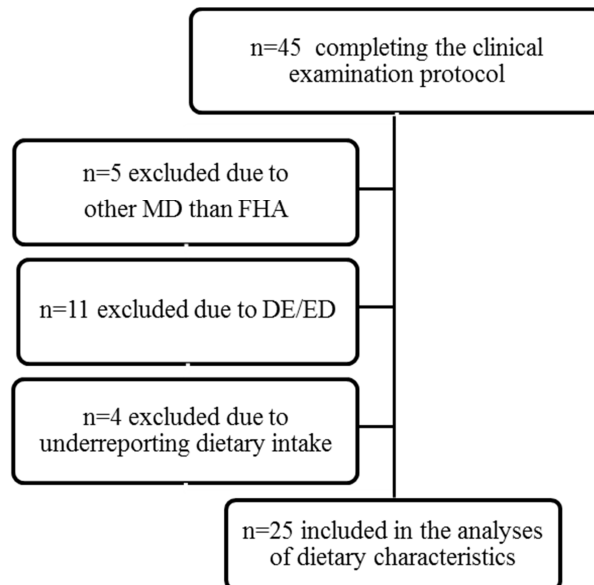
Figure 1 Flow chart

The figure illustrates the exclusive flow chart of the 45 female endurance athletes after finishing the clinical examination protocol. Five subjects were excluded from the dietary analysis after being diagnosed with other menstrual dysfunction than oligomenorrhea/functional hypothalamic amenorrhea, 11 were excluded after being diagnosed with eating disorders or disordered eating behaviour and four subjects were excluded after their dietary records were determined having low validity.

Food intake and training were recorded by the subjects for seven consecutive days to calculate current EA (Loucks 2007). Heart rate monitors and training logs were used to assess exercise energy expenditure and the subjects were instructed to maintain and follow their normal training regime. We calculated EA using the energy intake and exercise energy expenditure determined within the same 7-day period and FFM determined by DXA. Exercise energy expenditure represented only the energy (kJ) attributable to training, so the estimate of the energy expended for RMR and NEAT (non-exercise activity thermogenesis) throughout the duration of training was subtracted from the estimate of exercise energy expenditure. Dietary intake was calculated from prospective weighed (Exido 246030 Kitchen Scale) food records. The nutrient analysis program, Dankost 2000 (Dankost, Copenhagen, Denmark) based on the Food Data Bank from the Danish National Food Institute was used for Danish subjects. Dietist XP (Kost och Näringsdata AB, Bromma, Sweden) based on the database provided from the Swedish National Food Agency was used for Swedish subjects. The subjects provided food labels and recipes, and nutrient content of food items not in the databases was entered manually by the dietician. Subjects were given in-depth verbal and written instructions and a demonstration of how food and drink should be weighed and registered and were instructed to maintain normal food habits and eating patterns. Before entering data in the nutrient analysis program, the same dietician reviewed all completed diet records and asked for supplementary information if needed, i.e., when there was an indication of under or over-reporting of energy

intake as judged by the investigator. Dietary energy density, defined as the amount of energy (kJ) per gram of consumed food (Stubbs & Whybrow 2004), was calculated as all food including dairy products such as butter, cheese, yoghurt, but not milk. Food group variety was analysed by calculating the mean number of servings of nine different food groups over the seven days. The nine food groups in this study were defined as follows: *Fruit and vegetables*, including fresh and dried fruits and all kinds of vegetables including potatoes. One serving of fruit or vegetables was defined as 100 g. *Whole grain products,* including whole grain pasta, brown rice, bulgur wheat, oatmeal, muesli, rye and crisp breads and one serving was defined as 50 g. *Compact carbohydrate-rich foods*: white rice, pasta and bread (< 6 g of dietary fibre/100 g), couscous, cereals such as corn flakes, honey, sugar, cakes, sweets, sweet biscuits, jam and marmalade. One serving was defined as 50 g. *Nuts and seeds*: all nuts and seeds were included and one serving was defined as 20 g. *Meat* included all kinds of meat, fish, poultry and egg. One serving was defined as 100 g. *Dairy products*: milk, yoghurt, cream and all kinds of cheese. One serving of milk and yoghurt was defined as 200 g, one serving of cottage cheese and cream as 100 g and one serving of cheese as 20 g. *Sport products* included energy and protein bars and gels, and one serving was defined as 50 g. *Fats and oils*: salad dressings, butter, margarine, oils and pesto. One serving was defined as 10 g. *Energy-containing drinks*: all drinks containing energy, such as fruit juice, rice and soya milk, sports drinks, soft drinks, beer, wine and alcohol. One serving was defined as 200 g. Combination foods such as pizza and lasagne were separated into main ingredients and placed in the proper food group (e.g. lasagne was divided between compact carbohydrate-rich food, meat and dairy, as well as fruit and vegetables.

Statistics

All statistical procedures were performed using SPSS (version 22.0, IBM, Chicago, Illinois, USA). The dataset was checked for missing data. Normally distributed data were summarised as mean ± SD, and non-normally distributed data as median and interquartile range (IQ 25 and IQ 75 percentiles). Since we wanted to investigate dietary intake and characteristics associated with EA and reproductive function, the subjects were divided by their current EA: optimal EA defined as ≥ 188 kJ/kg FFM/day vs low/reduced EA defined as < 188 kJ/kg FFM/day as well as by their reproductive function: eumenorrhoea vs oligomenorrhoea/FHA. In order to investigate differences between subjects with optimal vs low/reduced EA and between eumenorrhoeic subjects versus subjects with oligomenorrhea/FHA, student's unpaired *t*-test was used for normal distributed data and Wilcoxon rank-sum test for skewed data for comparisons of the mean or median levels of the descriptive details. To explore whether the study groups defined by the combination of current EA and reproductive status were similar with respect to nutritional intake and status, a two-way analysis of variance with the corresponding Bonferroni-adjusted *P*-values was conducted for each outcome. To measure the degree of positive or negative association between continuous outcomes, Pearson's correlation coefficient (*r*) was calculated. Fisher's exact test was applied to determine whether there was a difference between the two kinds of classifications. In order to explore potential nutritional predictors of oligomenorrhea/FHA, a multiple linear regression with backward selection was conducted after evaluating the co-linearity between the predictors by means of the variance inflation factors. A significance level of < 0.05 was used.

114

Results

Description of subjects

The description of the subjects is presented in Table 1. There were no differences in BMI, body composition or exercise capacity between subjects divided by EA, but subjects with low/reduced EA demonstrated a higher training volume and exercise energy expenditure of 25% and 37% respectively, compared with those with optimal EA. Subjects with oligomenorrhea/FHA had a 18% and 14% lower absolute and relative FM (%) respectively, although there were no differences in body weight, BMI or exercise capacity between subjects with oligomenorrhea/FHA and eumenorrhoeic subjects. One subject with secondary FHA and low EA was underweight (BMI < 18.5 kg/m^2). One of the eumenorrheic subjects with optimal current EA reported previously having had periods with DE and secondary FHA. One eumenorrheic subject with reduced current EA reported having been eating a low carbohydrate diet for the last six months. There were no vegetarians or vegans in this group of endurance athletes.

Energy availability, energy intake, energy density and food groups

Energy availability, energy intake and energy density are presented in Table 2. Three subjects had low EA (< 125 kJ/kg FFM/day) (two with secondary FHA) and 11 had reduced EA (< 188 kJ/kg FFM/day) (six with secondary FHA and two with oligomenorrhea). There was, however, no difference in the number of oligomenorrhea/FHA subjects having low/reduced EA (10 of 15) compared with eumenorrhoeic subjects [four of 10, (*P*=0.183)] and there were no differences in energy intake or EA between subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects.

The diet of subjects with low/reduced EA as well as of those with oligomenorrhea/FHA had a 15% and 16% lower energy density compared with subjects with optimal EA and eumenorrhoeic subjects. Energy availability was positively associated with energy intake [*r* = 0.53, (*P*=0.008)] and all macronutrients [carbohydrates (g/kg/day); *r* = 0.43, (*P*=0.034), protein (g/kg/day); *r* = 0.41, (*P*=0.043) and fat (g/kg/day); *r* = 0.57, (*P*=0.003)] and negatively associated to exercise energy expenditure [*r* = - 0.66, (*P*=0.002)]. Energy availability was, furthermore, positively associated with energy density (kJ/g), the intake of food groups from fats and oils and energy-containing drinks, as illustrated in Figure 2. Energy density of the diet was positively associated with the intake of fat and compact carbohydrate-rich foods, as illustrated in Figure 3, and negatively associated with the intake of fibre, protein as well as fruits and vegetables.

115

Table 1 Description of subjects divided by current energy availability and reproductive function

	All (n=25)	Optimal EA (n=11)	Low/reduced EA (n=14)	Eumenorrhoea (n=10)	Oligomenorrhea/FHA (n=15)
Age, years	26.6 ± 5.6	27.9 ± 6.4	25.6 ± 4.9	26.8 ± 4.6	26.5 ± 6.3
Height, cm	169 ± 0.06	168 ± 0.04	170 ± 0.06	170 ± 0.06	168 ± 0.06
Weight, kg	58.8 ± 7.3	57.9 ± 5.3	59.5 ± 8.7	61.2 ± 8.2	57.1 ± 6.47
BMI, kg/m^2	20.6 ± 2.0	20.6 ± 1.6	20.6 ± 2.3	21.2 ± 2.3	20.2 ± 1.6
VO$_{2peak}$, l/min	3.21 ± 0.4	3.21 ± 0.4	3.21 ± 0.4	3.20 ± 0.4	3.22 ± 0.4
VO$_{2peak}$, ml/kg/min	54.5 ± 6.4	54.8 ± 4.6	54.3 ± 7.7	53.0 ± 5.7	55.5 ± 6.9
Exercise, h/week	12.0 ± 4.4	9.7 ± 2.5	13.0 ± 4.8*	11.1 ± 2.9	12.7 ± 5.1
EEE, MJ/day	3.93 ± 1.88	2.95 ± 0.77	4.69 ± 2.16*	3.80 ± 1.11	4.01 ± 2.3
FM, kg	11.8 ± 3.2	11.9 ± 2.4	11.7 ± 3.8	13.4 ± 3.3	10.7 ± 2.7*
FM, %	19.8 ± 3.6	20.3 ± 3.2	19.4 ± 3.9	21.7 ± 3.3	18.6 ± 3.3*
FFM, kg	46.2 [42.8-50.7]	46.2 [42.7-49.5]	46.3 [42.8-51.0]	49.1 [43.9-50.7]	46.0 [42.7-50.7]

Abbreviation: EA: energy availability. FHA: functional hypothalamic amenorrhea. BMI: body mass index. VO2$_{peak}$: maximal oxygen uptake. EEE: exercise energy expenditure. FM: fat mass. FFM: fat free mass. For comparison of mean levels between the groups with optimal vs low/reduced EA and the eumenorrhoeic group vs oligomenorrhea/FHA, an unpaired *t*-test was used for normally distributed variables and Wilcoxon rank-sum test for skewed distributed variables. *$P<0.05$ between subjects with optimal vs low/reduced EA and eumenorrhoeic subjects vs subjects with oligomenorrhea/FHA. Data are presented as mean ± SD for normal distributed data and as median and interquartile range [25-75] for skewed data.

Table 2 Dietary intake in subjects divided by current energy availability and reproductive function

	All (n=25)	Optimal EA (n=11)	Low/reduced EA (n=14)	Eumenorrhoea (n=10)	Oligomenorrhea /FHA (n=15)	*P*-value EA	Reproductive function
EI (MJ/day)	11.57 ± 1.90	12.46 ±1.90	10.86 ±1.64	12.12 ± 2.31	11.19 ± 1.55	0.056	0.475
(kJ/kg/day)	193 ± 51	217 ± 38	174 ± 53	201 ± 48	187 ± 53	0.040	0.833
(kJ/kg FFM/day)	247 ± 40	272 ± 42	228 ± 26	256 ± 54	242 ± 28	0.004	0.832
EA (kJ/kgFFM/day)	177 ± 51	218 ± 31	146 ± 38	189 ± 56	170 ± 48	<0.001	0.977
Energy density (kJ/g food)	6.1 ± 1.0	6.7 ± 0.7	5.7 ± 0.9	6.8 ± 0.7	5.7 ± 0.9	0.020	0.012
CHO (g/day)	369 ± 83	386 ± 83	355 ± 84	355 ± 66	378 ± 94	0.201	0.370
(g/kg/day)	6.4 ± 1.6	6.8 ± 1.7	6.1 ± 1.5	6.0 ± 1.7	6.7 ± 1.6	0.078	0.144
(E %)	53 ± 8	51 ± 10	54 ± 7	50 ± 6	55 ± 9	0.866	0.160
Fibre (g/day)	41 ± 11	39 ± 10	42 ± 13	33 ± 9	46 ± 10	0.971	0.007
(g/MJ)	3.6 ± 0.9	3.2 ± 0.8	3.9 ± 1.0	2.8 ± 0.6	4.1 ± 0.7	0.308	<0.001
Protein (g/day)	116 ± 26	121 ± 28	113 ± 25	116 ± 34	117 ± 21	0.377	0.802
(g/kg/day)	2.0 ± 0.5	2.1 ± 0.5	1.9 ± 0.5	1.9 ± 0.6	2.1 ± 0.4	0.156	0.335
(E %)	17 ± 3	17 ± 4	18 ± 3	16 ± 4	18 ± 3	0.788	0.342
Fat (g/day)	93 ± 32	108 ± 40	81 ± 17	111 ± 34	81 ± 24	0.091	0.047
(g/kg/day)	1.6 ± 0.5	1.9 ± 0.6	1.4 ± 0.2	1.8 ± 0.6	1.4 ± 0.3	**0.027**	0.092
(E %)	30 ± 8	31 ± 10	28 ± 6	34 ± 7	27 ± 8	0.864	0.052

Abbreviations: EA: energy availability. EI: energy availability. FHA: functional hypothalamic amenorrhea. CHO: carbohydrates. Data are presented as mean ± SD. To investigate whether there were differences between subjects with optimal and low/reduced current EA as well as between eumenorrhoeic subjects and subjects with oligomenorrhea/FHA; a two-way analysis of variance was used. There were no group interaction effects in any of the measured variables.
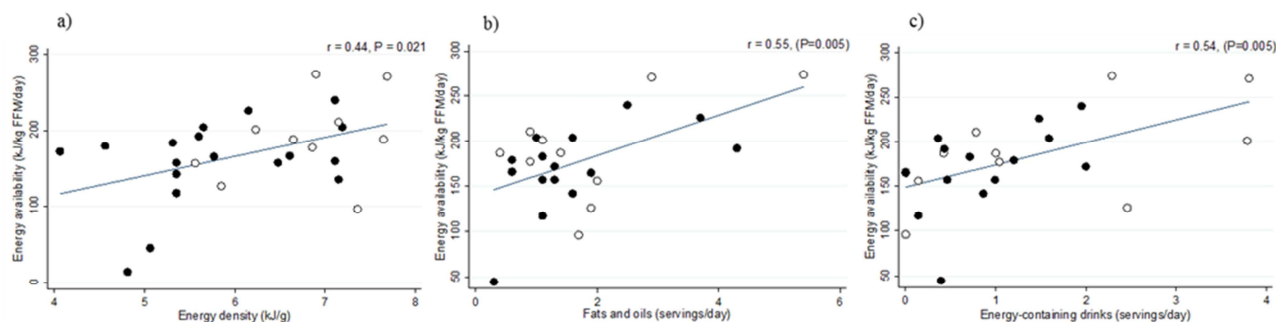
Figure 2 The association between energy availability and energy density as well as intake of different foods

The figure illustrates the positive association between energy availability (kJ/kg FFM/day) and a) energy density of the food, b) the intake of fats and oils and c) the intake of energy-containing drinks. Eumenorrhoeic athletes (open circles) and athletes with oligomenorrhoea/FHA (filled circles).
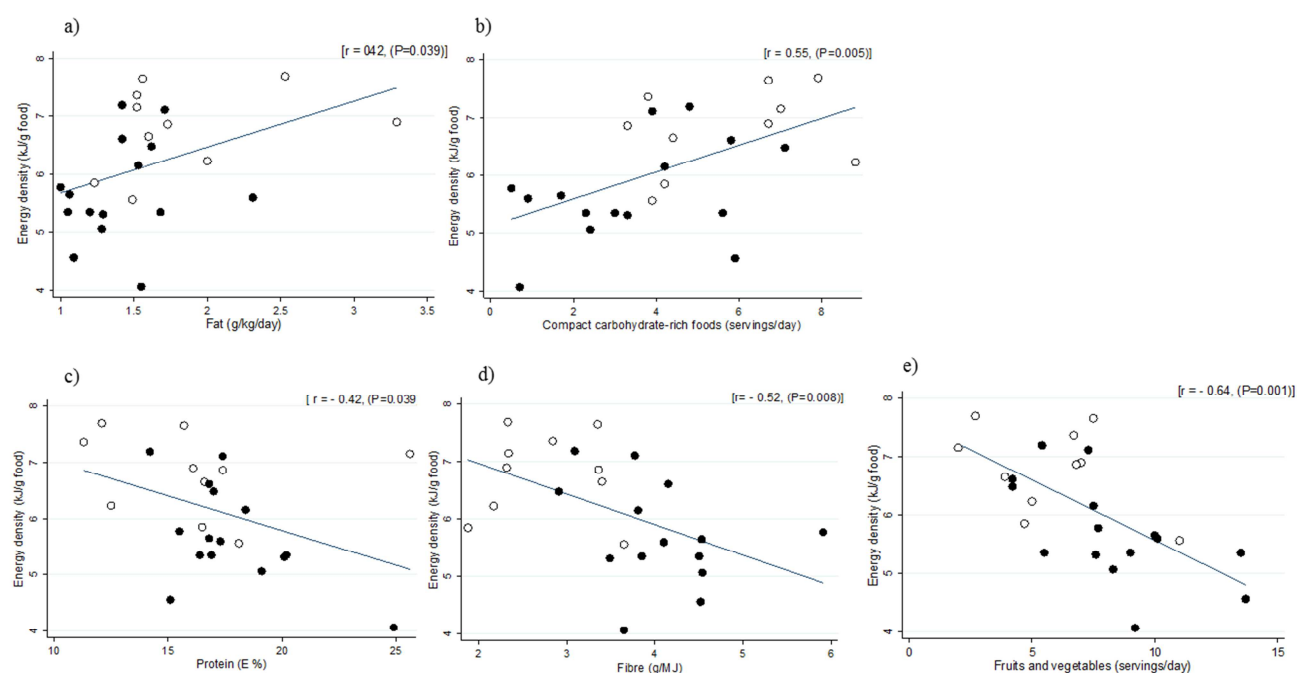

Figure 3 The association between energy density and the intake of nutrients as well as different foods

The figure illustrates the positive associations between energy density and a) fat intake and b) the intake of compact carbohydrate-rich foods as well as the negative associations to c) the relative intake of protein and d) fibre and e) the intake of fruits and vegetables. Eumenorrhoeic athletes (open circles) and athletes with oligomenorrhoea/FHA (filled circles).

Carbohydrate intake

There was no difference in the intake of carbohydrates between groups. Fifty percent of the subjects with the low/reduced EA compared with 73% with optimal EA had a carbohydrate intake ≥ 6 g/kg/day, recommended for endurance athletes training moderate-to-high intensity exercise 1-3 h/day (Burke et al. 2011). Eumenorrhoeic subjects ate more compact carbohydrate-rich foods compared with subjects with oligomenorrhea/FHA [5.7 ± 2.0 servings/day vs 3.5 ± 2.1 servings/day, (P=0.019)], while there was no difference between subjects with optimal EA compared with subjects with low/reduced EA [5.2 ± 2.5 servings/day vs 3.7 ± 1.9 servings/day, (P=0.115)]. There was, however, a group interaction effect (P=0.028). Subjects with oligomenorrhea/FHA had a 23% higher total (g/day) and a 32% higher relative fibre intake (g/MJ) compared with the eumenorrhoeic group and fibre intake exceeding the Nordic nutritional recommendation of 25-35 g/day (Nordic Council of Ministers 2013) was more common among subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects [12 of 15 vs three of 10, (P=0.018)]. There was a trend toward a higher intake of whole grain products in subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects [4.4 ± 2.2 servings/day vs 2.7 ± 1.6 servings/day, (P=0.050)], while no difference was found between subjects with low/reduced EA compared with those with optimal EA [3.6 ± 1.9 servings/day vs 3.8 ± 2.5 servings/day, (P=0.447)]. There was a trend towards a higher intake of fruit and vegetables in subjects with oligomenorrhea /FHA compared with eumenorrhoeic subjects [8.2 ± 2.9 servings/day vs 5.7 ± 2.6 servings/day, (P=0.077)], but there was no difference between subjects with low/reduced EA compared with subjects with optimal EA [8.0 ± 3.1 servings/day vs 6.2 ± 2.6 servings/day, (P=0.239)]. There were no differences in the intake of sports products or the intake of energy-containing drinks between subjects with low/reduced EA compared with subjects with optimal EA [0.4 ± 0.5 servings/day vs 0.5 ± 0.9 servings/day (P=385) and 1.6 ± 1.4 servings/day vs 0.8 ± 0.7 servings/day, (P=0.385)] or between subjects with oligomenorrhea/FHA compared with eumenorrheic subjects [0.4 ± 0.4 servings/day vs 0.6 ± 0.9 servings/day, (P=0.361) and 1.6 ± 1.2 servings/day vs 0.7 ± 0.8 servings/day, (P=0.375)].

Protein intake

There were no differences in protein intake or in the intake of meat, fish, poultry and egg or dairy products between subjects with low/reduced EA compared with optimal EA [2.1 ± 0.6 servings/day vs 2.0 ± 0.9 servings/day, (P=0.780) and 3.4 ± 1.7 servings/day vs 3.6 ± 1.3 servings/day, (P=0.682)] or between subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects [1.9 ± 0.5 servings/day vs 2.2 ± 1.0 servings/day, (P=0.298) and 3.3 ± 1.3 servings/day vs 3.7 ± 1.9 servings/day, (P=0.935)]. There was no difference in the intake of nuts and seeds between subjects with low/reduced EA compared with optimal EA [0.7 ± 0.9 servings/day vs 1.1 ± 1.0 servings/day, (P=0.338)] or between subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects [1.0 ± 0.9 servings/day vs 0.8 ± 1.0 servings/day, (P=0.418)], but there was a group interaction effect (P=0.011).

Fat intake

Subjects with low/reduced EA had a 26% lower relative fat intake (g/kg/day) compared with subjects with optimal EA and subjects with oligomenorrhea/FHA had a 27% lower total fat intake (g/day) compared with eumenorrhoeic subjects.

A multiple regression analysis, including all subjects, with a backward elimination containing EA (kJ/kg FFM/day), energy density (kJ/g), intake of fat (g/day) and fibre (g/MJ/day) as possible predictors of oligomenorrhea/FHA showed a partial correlation coefficient (r^2) for dietary fibre intake of 0.25, $P<0.001$, with an unstandardized regression coefficient (ß) of 0.37 (0.21-0.53, 95 % confidence interval) and a mean variance inflation factor (VIF) of 1.46 (range 1.34-1.65).

Eating behaviour

The total EDI-3 score and the score for drive for thinness (DT) as well as body dissatisfaction (BD) subscales are presented in Table 3.

Table 3 EDI-3 score in subjects divided by current energy availability and reproductive function

	All (n=25)	Optimal EA (n=11)	Low/reduced EA (n=14)	Eumenorrhoea (n=10)	Oligomenor-rhea/FHA (n=15)	*P*-value EA	Repro-ductive func-tion
EDI score	24.5 ± 2.8	24.2 ± 1.0	24.8 ± 2.4	23.0 ± 2.0	25.5 ± 2.8	0.721	**0.047**
DT-score	3.0 [0.5-4.5]	3.0 [0.0-5.0]	3.0 [1.0-4.0]	0.0 [0.0-1.0]	4.0 [2.0-6.0]	0.710	**0.003**
BD-score	3.0 [1.5-7.0]	3.3 [1.0-7.0]	4.0 [2.0-7.0]	3.0 [2.0-6.0]	3.0 [1.0-10.0]	0.789	0.393

Abbreviation: EA: Energy availability. FHA: functional hypothalamic amenorrhea. EDI-3: eating disorder inventory 3. DT: Drive for thinness subscale. BD: body dissatisfaction subscale. Data are presented as mean ± SD for normal distributed data and as median and interquartile range [25-75] for skewed data. To investigate whether there were differences in nutritional status between subjects with optimal vs. low/reduced EA as well as between subjects with oligomenorrhea/FHA and eumenorrhoeic subjects, a two-way analysis of variance was used.

There were no differences in the total EDI-3 or subscale scores between subjects divided by EA. Subjects with oligomenorrhea/FHA had a higher mean total EDI-3 score and a higher DT-score compared with eumenorrhoeic subjects. There were positive associations between DT-score and the intake of dietary fibre (g/MJ) [$r = 0.59$, (P=0.003)], while there was a negative association with energy density (kJ/g food) [$r = - 0.41$, (P=0.045)], the intake of fat (g/kg/day) [$r = - 0.41$, (P=0.046)] as well as compact carbohydrate-rich foods (servings/day) [$r = - 0.65$, (P=0.001)].

Discussion

In the present study we found that EA was positively associated not only with energy intake and all macronutrients, but also with the energy density of the diet and the intake of energy-containing drinks as well as fats and oils. We did not find any differences in energy intake or EA between eumenorrhoeic athletes and athletes with oligomenorrhea/FHA. We did, however, find that athletes

with oligomenorrhea/FHA and athletes with low/reduced EA shared the same dietary characteristics with a lower energy density and a lower fat content. Furthermore, athletes with oligomenorrhea/FHA were more weight concerned and had a diet with a greater amount of dietary fibres and fewer compact carbohydrate-rich foods compared with eumenorrhoeic athletes.

Energy intake, energy availability, energy density and food groups
The mean relative energy intake in the present study was 193 kJ/kg/day and slightly higher compared with the mean intake of 180 kJ/kg/day pooled from a number of studies of female endurance athletes reported by Burke (Burke 2001). A lower energy intake and/or a higher training volume have been reported in endurance athletes with MD compared with eumenorrhoeic athletes (Tomten & Hostmark 2006; Thong et al. 2000). Other studies have, however, found similar energy intakes and training volumes in female endurance athletes with MD as in eumenorrheic athletes (Gremion et al. 2001; Guebels et al. 2014; Schaal et al 2011), just as we found in the present study. In the present study, 44% (n=11) had current EA between 125-188 kJ/kg FFM/day, levels that are recommended to athletes needing to lose weight (Loucks et al. 2011), while 12% (n=3) had current EA <125 kJ/day, levels that in clinical settings have shown to suppress LH pulsatility in healthy sedentary eumenorrhoeic women after only five days (Loucks et al. 2003). We have earlier reported lower resting metabolic rate (RMR) and LH levels in female endurance athletes at a current EA between 125 and 188 kJ/kg FFM/day compared to athletes with a current EA ≥ 188 kJ/kg FFM/day (Melin A et al. 2014b). It is possible that the threshold of low EA established by Loucks and Thuma (2003) in a laboratory setting that predicts the decline in LH pulsatility in eumenorrheic sedentary women may not similarly manifest in athletes under free-living conditions using self-report and field methods to determine EA (Mountjoy M et al. 2013). Furthermore, the threshold of long term EA associated with oligomenorrhea/FHA may be higher or vary among exercising women (Gibbs et al. 2013).

There are different possible reasons for low/reduced EA in female athletes and it could be unintentional, due to difficulties in eating enough during intense training periods, especially if the diet meets the criteria for a healthy diet recommended to the general population: low in energy density and high in bulky fibres (Nordic Council of Ministers 2013). In the present study, low/reduced EA was associated with a combination of higher exercise energy expenditure due to greater training load and a lower energy intake mainly due to a lower intake of fat. We also found that EA was positively associated with the energy density of the diet, the intake of energy-containing drinks and, especially, with the intake of fats and oils. Even though we did not find any differences is energy intake or EA between subjects with oligomenorrhea/FHA compared with eumenorrheic subjects, subjects with oligomenorrhea/FHA followed a lower energy density diet compared with eumenorrhoeic subjects. These results support what has previously been reported in recreationally active women with MD (Reed et al. 2011). The bulking property of dietary fibre lowers the energy density of the diet mainly by binding water, since water contributes to weight but not to energy (Rolls 2009). Furthermore, energy density is increased more by fat than carbohydrate or protein due to its higher energy content per weight (Rolls 2009) and we also found a positive association between the energy density of the diet and fat intake in these endurance athletes. We also found that energy den-

sity was negatively associated with fibre and protein content as well as with a high intake of fruit and vegetables, and that it was positively associated with the intake of compact carbohydrate-rich foods. Diets with low energy density are often recommended for weight control (Nordic Council of Ministers 2013) since they have been shown to increase satiation and prolong the feeling of satiety (Holt et al. 1995). A diet with low energy density is, therefore, likely to increase the risk for low/reduced EA in female athletes, especially during periods with high training loads.

Carbohydrate intake

In the present study, 50% of the athletes with low/reduced EA had a carbohydrate intake of 6-10 g/kg/day recommended to endurance athletes with a training regimen of 1-3 hours/day (Burke et al. 2011). A carbohydrate intake that is adequate to replenish glycogen storage after training is important because reduced carbohydrate availability for the working muscles limits performance during prolonged sub-maximal exercise as well as during repeated bouts of high intensity exercise (Burke et al. 2011). Adequate carbohydrate intake is also important for the effective restoration of liver glycogen in order to maintain normal blood glucose levels and thereby facilitate LH pulsatility (Loucks et al. 1998). Even though there were no differences in the carbohydrate intake between eumenorrhoeic subjects and those with oligomenorrhea/FHA in the present study, the oligomenorrhea/FHA athletes consumed less compact carbohydrate-rich foods such as white rice, pasta, bread and sweets compared with eumenorrhoeic athletes; foods that are important constituents of a carbohydrate-rich diet and that commonly is recommended to athletes in order to enhance performance (Burke et al. 2011).

Diets rich in fibre are recommended for their health-promoting effects, including weight regulation. Recommendations to eat more fibre are primarily based on its protective effect against colorectal cancer, cardiovascular disease, and type II diabetes (Nordic Council of Ministers 2013). In the present study, 64% of the athletes exceeded even the upper level of the recommended daily intake of fibre in the Nordic countries (25-35 g/day) (Nordic Council of Ministers 2013). Even when considering a potentially higher energy intake, 72% exceeded the 3 g/MJ (Nordic Council of Ministers 2013) and this excessive intake of dietary fibre was linked to oligomenorrhea/FHA. The World Health Organisation's (WHO) (2003) recommendation of more than 25 g/day of fibre, without any upper limit, may be interpreted as "the more, the better". The higher fibre intake among subjects with oligomenorrhea/FHA compared with eumenorrhoeic subjects that we found in the present study has been reported previously in endurance athletes by Laughlin and Yen (1996), in recreational active women by Reed et al. (2011). An excessive intake of fibre is a potential nutritional concern in this group of endurance athletes, since it could increase the risk for low/reduced EA by several mechanisms. Energy digestibility is affected by dietary fibre since dietary fibre increases faecal fat excretion and the reported amount of increased fat and energy excreted range from 100 to 300 kJ/day in intervention studies, providing 7-20 g/day of dietary fibre (Kristensen 2009). An excessive dietary fibre intake of > 40 g/day, as seen among the female athletes with oligomenorrhea/FHA in the present study, might therefore reduce energy absorption even further and consequently increase the risk for low/reduced EA. Some dietary fibre such as pectin in fruit and potatoes, and grains such as oats, barley, rye and wheat form a gel-like structure in the stomach and de-

lay the passage of food into the duodenum. This results in prolonged satiety, thus limiting the intake of energy within a meal and delaying the return of hunger (Kristensen 2009). Other types of fibre such as cellulose and hemicellulose in vegetables and cereal bran are not digested but bind with water in the large intestine (Kristensen 2009). Besides increasing satiation due to their bulking effect, an excessive fibre intake may lead to gastrointestinal disorders such as bloating and diarrhoea (Lupton & Trumbo 2006) - common problems among endurance athletes (Melin A et al. 2014a).

Independent of the effect of fibre on energy intake, studies have reported a negative association between fibre intake and oestrogen levels in sedentary premenopausal women (Aubertin-Leheudre et al. 2008; Laughlin et al. 1998). The presumed mechanism involves oestrogens binding to fibre in the colon, which reduces the reabsorption of circulating oestrogen (Aubertin-Leheudre et al. 2008) and hence increases the excretion of oestrogens in the faeces. Oestrogen excreted in bile needs to be hydrolysed before it can be reabsorbed and a presumed indirect oestrogen-lowering effect of fibre is reduced β-glucuronidase activity in the colon which leads to the reduced reabsorption of oestrogen (Aubertin-Leheudre et al. 2008). These results support our findings derived from the multiple regression analysis that a high fibre intake, independently of its effect on EA or energy density, could potentially increase the risk of oligomenorrhea/FHA in female athletes. Even though clinical trials have shown a negative association between dietary fibre intake and oestrogen levels in sedentary premenopausal women (Aubertin-Leheudre et al. 2008; Laughlin et al. 1998), a potential effect needs to be tested in controlled experiments in athletes in order to confirm that the negative associations between dietary fibres and oligomenorrhea/FHA derive from an actual causal relationship.

Protein intake

The recommended protein intake for endurance athletes is 1.2-1.7 g/kg /day (Philips 2011) and periods with low energy and/or carbohydrate intake may increase amino acid oxidation and hence protein requirements. In the present study, although the protein intake was generally high and exceeded the recommended 1.2-1.7 g/kg/day for endurance athletes (Rodriguez et al. 2009), 29 % of the subjects with low/reduced EA did not have a protein intake of 1.8-2.0 g/kg/day recommended during energy deficiency in order to minimise the loss of FFM as well as to optimize glycogen storage (Burke et al. 2011). A high protein intake as observed among the female athletes in the present study (1.9 g/kg/day) is on the other hand considered to result in a less energy dense diet than a diet with more carbohydrates and, in particular, fat (Stubbs et al. 2004) and could thereby increase the risk for low/reduced EA. In the present study 80% of the subjects with oligomenorrhea/FHA had a protein intake > 1.7 g/kg/day and a combined excessive intake of both protein and dietary fibre could, therefore, increase the risk of energy deficiency, especially in periods of large training volumes.

Fat intake

Our finding that endurance athletes with oligomenorrhea/FHA have a lower fat intake than eumenorrhoeic athletes supports the findings of earlier studies of female endurance athletes (Tomten & Hostmark 2006; Laughlin et al. 1996; Thong et al. 2000). We also found a lower fat intake in athletes with low/reduced EA compared with those with optimal EA. In a study by Horvath et al.

(2000), *ad libitum* energy intake from a low fat diet (17 E%) during a month only provided 60% of the energy requirement in female runners, and the higher the contribution of energy intake was from fat, the lower the energy deficiency found. In the present study, however, only one athlete had a lower fat E% than the recommended 20-35 E% for athletes (Rodriguez et al. 2009). The presence of low/reduced EA, despite the recommended fat E%, emphasises the importance of not assessing diet adequacy by the relative macronutrient distribution.

Eating behaviour
The reason for low/reduced EA could be due to intentional restriction of food intake in order to obtain and/or maintain a low body weight or as a result of a range of pathological conditions, from disordered eating behaviour to clinically overt eating disorders (Nattiv et al. 2007). In the present study, we wanted to investigate dietary characteristics related to EA and/or reproductive function without the presence of abnormal and pathological eating behaviour, so subjects diagnosed with disordered eating behaviour and eating disorders were excluded. Interestingly, we still found a higher, although not pathological DT-score, in the athletes with oligomenorrhea/FHA, indicating a heightened concern with dieting and a more entrenched pursuit of thinness compared with the eumenorrhoeic athletes. De Souza et al (2007) used an elevated DT-score as a proxy indicator for energy deficiency in exercising women and found no difference in the current energy intake between exercising women with elevated vs normal DT-levels, while the prevalence of MD was higher in women with an elevated DT-score compared with those with a normal DT-score. These results are in line with our findings of a higher DT-score in female athletes with oligomenorrhea/FHA compared with eumenorrhoeic subjects, despite similar current energy intake or EA obtained. We also found that a higher DT-score was associated with a diet with lower energy density, a higher intake of dietary fibres and a more restricted intake of fat as well as compact carbohydrate-rich food. These combined results indicate that even a slightly increased drive to lose weight or to maintain a low body weight with a diet following the general dietary guidelines increases the risk for persistent low/reduced EA and oligomenorrhea/FHA in athletes.

Practical implications
Early detection and intervention of low/reduced EA with or without disordered eating behaviour/eating disorders and oligomenorrhea/FHA are important in order to prevent long-term health consequences such as impaired bone health, to optimize performance and recovery, and to prevent injury and gastrointestinal problems (Nattiv et al. 2007; Mountjoy M et al. 2014; De Souza et al. 2014). The high prevalence of low/reduced EA and oligomenorrhea/FHA found in this population of female endurance athletes emphasises the importance of specific dietary recommendations for female athletes. The recommended treatment for the endocrine and reproductive disruptions caused by low/reduced EA is to secure adequate EA by modifying the diet and/or the training regime and in order to ensure adequate energy for all physiological functions EA ≥ 188 kcal/kg FFM/day is recommended (De Souza et al. 2014). Dietary recommendations for female athletes with oligomenorrhea/FHA include increasing energy intake with 1.2-2.4 MJ/day (Mountjoy M et al. 2014) in small increments in order to avoid unwanted weight gain. Based on the results from the present study we would like to suggest that the following additional practical considerations could be used

to guide nutritional prevention and treatment of low/reduced EA and oligomenorrhea/FHA in female athletes:

- Calculate nutritional needs based on total energy expenditure and carbohydrate and protein requirements (g/kg/day) derived from the individual's goals and sport-specific training regimen (Rodriguez et al. 2009) and then add the required amount of energy from fat and carbohydrates from compact carbohydrate-rich foods in order to limit the volume of the meals.
- Follow the general dietary guidelines concerning absolute intakes of dietary fibre, but not necessarily in relation to energy content (g/MJ), in order to avoid low energy density.
- Besides ensuring an adequate intake of fat, increase energy density by replacing whole grain products and fruit and vegetables with more compact carbohydrate-rich foods.
- Increase energy and carbohydrate intake by replacing non-energy-containing with energy-containing drinks such as fruit juices, milk and sport drinks.

Furthermore, the fact that 11 out of 45 athletes (24%) participating in the initial clinical examination protocol in the present study were diagnosed with disordered eating behaviour/eating disorders emphasises the importance of prevention and early detection as well as the need for multidisciplinary professional treatment of these disorders in order to address the possible underlying psychological factors (Mountjoy M et al. 2014; Nattiv et al. 2007). Our results also indicate that even a slightly increased drive to lose weight or maintain what is considered an appropriate body weight or body composition in the sport, is associated with a diet characterised by lower energy density and lower fat as well as excessive dietary fibre and thereby an increased risk for persistent low/reduced EA and oligomenorrhea/FHA.

This cross-sectional study cannot demonstrate any causative effect of dietary factors on current EA or oligomenorrhea/FHA. However, our results suggest that diets with lower energy density as well as excessive dietary fibre are nutritional issues in female athletes that need to be prioritised. Several methodological difficulties are involved in quantifying a clinically useful EA, including underreporting or under-eating (Burke 2001), timing of the assessment in order to collect data representative of the athletes' habitual EA, as well as difficulties in capturing the habitual EA because of variations in training and food intake over time. Assessing EA during a single 7-day period, as we have done in this study, does not, therefore, necessarily capture the athletes' habitual EA and measuring EA at a different period could have given a different result. In this group of athletes, moderate weight fluctuations are frequent and even if there were no self-reported changes in weight during this limited observation period, it is a weakness that body weight was not measured during the registration period. Finally, the large number of outcomes evaluated in the present study increases the risk of false positive findings, so the study should primarily be seen as exploratory, with a need for future confirmatory studies that also include the potential effects on performance and recovery.

Perspectives
The present study demonstrates that a diet with a lower energy density, a more restricted intake of fat, compact carbohydrate-rich foods and energy-containing drinks, as well as a high intake of die-

tary fibre, are dietary characteristics associated with low/reduced EA and oligomenorrhea/FHA in female endurance athletes and should therefore be targeted for nutritional intervention and adjustment. Our results indicate that even a slightly increased drive to lose or maintain a low body weight is associated with dietary characteristics likely to increase the risk for low/reduced EA and oligomenorrhea/FHA. Handling the issue of leanness and body weight with care is, therefore, of paramount importance in sport environments. An athlete who needs to lose weight should be provided with professional counselling, ensuring a time-limited nutritional treatment plan with proper and effective guidelines for weight loss that ends with re-establishing optimal EA and weight stability. Controlled intervention trials specifically designed to investigate potential direct and/or indirect causal relationships between excessive dietary fibre intake and oligomenorrhea/FHA in female athletes are needed.

Reference List

Aubertin-Leheudre, M., Gorbach, S., Woods, M., Dwyer, J. T., Goldin, B., & Adlercreutz, H. Fat/fiber intakes and sex hormones in healthy premenopausal women in USA. J Steroid Biochem Molecular Biol 2008: 112: 32-39.

Beidleman, B. A., Puhl, J. L., De Souza, M. J. Energy-Balance in Female Distance Runners. Am J Clin Nutr 1995: 61: 303-311.

Black, A. E. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. Int J Obes Relat Metab Disord 2000: 24: 1119-1130.

Burke L. Energy Needs of Athletes. Can J Appl Physiol-Revue. 2001: 26: 202-219.

Burke ML, Hawley JA, Wong SH, Jeukendrup A. Carbohydrates for training and competition. J Sport Sci 2011: 29: 17-27.

De Souza, M. J., Hontscharuk, R., Olmsted, M., Kerr, G., Williams, N. I. Drive for thinness score is a proxy indicator of energy deficiency in exercising women. Appetite 2007: 48: 359-367.

De Souza, M. J., Nattiv, A., Joy E, Misra, M., Williams NI, Mallinson RJ et al. 2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. Brit J Sports Med 2014: 48: 1-20.

Fairburn, C. G., Cooper, Z., O'Connor, M. Eating Disorder Examination (Edition 16.0D). In Cognitive Behavior Therapy and Eating Disorders, New York, Guilford Press. 2008

Garner, D. M. Eating Disorder Inventory-3. Professional Manual. Par Inc. 2004

Gibbs, J. C., Williams NI, Mallinson RJ, Reed, J. L., Rickard AD, De Souza, M. J. Effect of High Dietary Restraint on Energy Availability and Menstrual Status. Med Sci Sports Exerc 2013: 45: 1790-1797.

Gibbs, J. C., Williams, N. I., De Souza, M. J. Prevalence of individual and combined components of the female athlete triad. Med Sci Sports Exerc 2013: 45: 985-996.

Gremion, G., Rizzoli, R., Slosman, D., Theintz, G., Bonjour, J. P. Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. Med Sci Sports Exerc 2001: 33: 15-21.

Guebels CF, Kam LC, Maddalozzo GF, Manore M.M. Active Women Before/After an Intervention Designed to Restore Menstrual Function: Resting Metabolic Rate and Comparison of Four Methods to Quantify Energy Expenditure and Energy Availability. Int J Sport Nutr Exerc Metab. 2014: 24: 37-46.

Holt, S. H., Miller, J. C., Petocz, P., Farmakalidis, E. A satiety index of common foods. Eur J Clin Nutr 1995: 49: 675-690.

Horvath, P. J., Eagen, C. K., Ryer-Calvin, S. D., Prendergast, D. R. The effects of varying dietary fat on the nutrient intake in male and female runners. J Am Coll Nutr, 2000: 19: 42-51.

Joint WHO/FAO Expert Consultation. Diet, Nutrition and The Prevention of Chronic Diseases (Rep. No. 916). WHO Library Cataloguing-in-Publication Data. 2003

Kristensen M. Wholegrains and dietary fibres. Impact on body weight, appetite regulation ans nutrient digestibility. Department of Human Nutrition, University of Copenhagen. 2009

Laughlin, G. A., Yen, S. S. C. Nutritional and Endocrine-Metabolic Aberrations in Amenorrheic Athletes. J Clin Endocrinol Metab 1996: 81: 4301-4309.

Laughlin, G. A., Dominguez, C. E., Yen, S. S. C. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1998: 83: 25-32.

Loucks, A. B., Verdun, M., Heath, E. M. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J Appl Physiol 1998: 84: 37-46.

Loucks, A. B. Influence of energy availability on luteinizing hormone pulsatility and menstrual cyclicity. In W.J.Kraemer & A. D. Rogol (Vol. Eds.), The Endocrine System in Sports and Exercise (pp. 232-249). Blackwell Publishing. 2005

Loucks, A. B. Low energy availability in the marathon and other endurance sports. Sports Med 2007: 37: 348-352.

Loucks, A. B., Kiens, B., Wright, H. H. Energy availability in athletes. J Sports Sci 2011: 29: S7-S15.

Loucks, A. B., Thuma, J. R. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab 2011: 88: 297-311.

Lupton, J. R., Trumbo, P. R. Dietary Fiber. In M.E.Shils, M. Shike, A. Catharine Ross, B. Caballero, R. J. Cousins (Vol. Eds.), Modern Nutrition in Health and Disease (Tenth edition ed., pp. 83-91). Lippincott Williams & Wilkins. 2006.

Melin A, Tornberg ÅB, Skouby S, Faber J, Ritz C, Sjödin A et al.. The LEAF questionnaire: A screening tool for the identification of female athletes at risk for the female athlete triad . Brit J Sports Med 2014a: 48: 540-545.

Melin A, Tornberg ÅB, Skouby S, Møller SS, Sundgot-Borgen J, Faber J et al. Energy availability and the female athlete triad in elite endurance athletes. Scand J Med Sci Sports. 2014b: doi: 10.1111/sms.12261.

Mountjoy M, Sundgot-Borgen J., Burke L, Carter S, Constantini, N. W., Lebrun, C. et al. The IOC concensus statement: beyond the Female Athlete Triad - Relative Energy Deficiency in Sport (RED-S). Br J Sports Med 2014: 48: 491-497.

Nattiv, A. et al. The female athlete triad. Med Sci Sports Exerc 2007: 39: 1867-1882.

Nordic Council of Ministers. Nordic Nutrition Recommendations 2012, part 1 - summary, principles and use. (5th edition ed.) Norden. 2013

Philips SM. Dietary protein for athletes: From requirements to optimim adaptation. J Sports Sci 2011: 29: 29-38.

Reed, J. L., Bowell, J. L., Hill, B. R., Williams, B. A., De Souza, M. J., Williams, N. I. Exercising women with menstrual disturbances consume low energy dense foods and beverages. Appl Physiol Nutr Metab 2011: 36, 382-394.

Rodriguez, N. R., DiMarco, N. M., Langley, S., Denny, S., Hager, M. H., Manore, M. M. et al. Nutrition and Athletic Performance. Med Sci Sports Exerc 2009: 41: 709-731.

Rolls BJ. The relationship between dietary energy density and energy intake. Physiol Behav 2009: 97: 609-615.

Schaal K, Van Loan MD, Casazza GA. Reduced Catecholamine Response to Exercise in Amenorrheic Athletes. Med Sci Sports Exerc 2011: 43: 34-43.

Sjodin, A. M., Forslund, A. H., Westerterp, K. R., Andersson, A. B., Forslund, J. M., Hambraeus, L. M. The influence of physical activity on BMR. Med Sci Sports Exerc 1996: 28: 85-91.

Stubbs, R. J., Whybrow, S. Energy density, diet composition and palatability: influences on overall food energy intake in humans. Physiol Behav 2004: 81: 755-764.

Sundgot-Borgen, J., Torstveit, M. K. Prevalence of eating disorders in elite athletes is higher than in the general population. Clin J Sport Med 2004: 14: 25-32.

Thong, F. S. L., McLean, C., Graham, T. E. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. J Appl Physiol 2000: 88: 2037-2044.

Tomten S.E, Hostmark AT. Energy balance in weight stable athletes with and without menstrual disorders. Scand J Med Sci Sports 2006: 16: 127-133.

PAPER III

APPENDIX

The LEAF-Q scoring key

Calculations

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Resting metabolic rate was calculated from the mean of VO_2 and VCO_2 during the last twenty minutes of the measurement, using the Weir equation (154): 3.94 (VO_2) + 1.1 (VCO_2) x 1.44.

Predicted RMR was calculated using the Cunningham equation: kJ/day: 500 + (22 x FFM [kg]) (156). Subjects were defined as having low RMR when the ratio between measured RMR (mRMR) and predicted RMR (pRMR) was < 0.90 (31).

The calculation of exercise EE during training was based on individual prediction equations from the measured heart rate and corresponding calculated energy expenditure during the incremental maximal exercise test in the laboratory. The individual equation provided the basis for the calculation of exercise energy expenditure, using the training log and heart rate measurements for each training session (27). Regression lines were calculated for the corresponding values of heart rate and energy expenditure during the incremental maximal exercise test in the laboratory, as well as for the recorded heart rate during all exercise sessions during the seven-day recording period. Heart rate had a high linear correlation with O_2 consumption at increasing workloads ($r = 0.94$), with a 95% confidence interval 0.93–0.96.

Current EA was calculated by subtracting exercise energy expenditure from energy intake. Exercise energy expenditure represented only the energy (kJ) attributable to training, so the estimate of the energy expended for RMR and NEAT throughout the duration of training was subtracted from the estimate of exercise energy expenditure: EA = (energy intake – (exercise energy expenditure - (total energy expenditure - exercise energy expenditure))/kg FFM.

Power generated during cycling (W) was converted to kJ/min. Work efficiency was calculated by subtracting energy expenditure (kJ/min) at 0 W from energy expenditure (kJ/min) at 100 W, divided by the power generated during cycling at 100 W (kJ/min), and expressed as a percentage (work efficiency$_{100W \%}$ = ((energy expenditure$_{100w}$ – energy expenditure$_{0w}$)/power$_{100w}$) x 100).

Dietary energy density, defined as the amount of energy per gram of consumed food (157), was calculated as food only.

In order to identify subjects who provided nutritional data of poor validity, the Goldberg cut-off was calculated using the equation described by Black (130):

$$\frac{Energy\ intake}{RMR} : \ >/< PAL\ x\ \left(\frac{SD_{min/max}\ x\ \left(S/100 \right)}{\sqrt{n}} \right)$$

Physical activity level (PAL is the mean PAL for the study population, SD $_{min/max}$ is +/- 2 for the 95% lower/upper confidence limit, *n* is the number of subjects (n $=$ 40). *S* is the factor that takes account of the variation in energy intake, RMR, and energy requirements:

$$\frac{\sqrt{CV_{EI}^2 \, + \, CV_{RMR}^2 + CV_{PAL}^2}}{7 \; days}$$

CV_{EI} is the within-subject CV in energy intake, CV_{RMR} is the CV of RMR measurements (130), and CV_{PAL} is the total variation in PAL (130).

Anna Melin
Energy Availability and Reproductive Function in Female Endurance Athletes