

# Low-energy density and high fiber intake are dietary concerns in female endurance athletes

A. Melin<sup>1,2</sup>, Å. B. Tornberg<sup>2,3</sup>, S. Skouby<sup>4</sup>, S. S. Møller<sup>1</sup>, J. Faber<sup>5</sup>, J. Sundgot-Borgen<sup>6</sup>, A. Sjödín<sup>1</sup>

<sup>1</sup>Department of Nutrition, Exercise and Sports, University of Copenhagen, Frederiksberg, Denmark, <sup>2</sup>Department of Health Sciences, Division of Physiotherapy, Lund University, Lund, Sweden, <sup>3</sup>Genetic & Molecular Epidemiology Unit, Lund University Diabetes Centre, Clinical Research Centre, Skåne University Hospital, Malmö, Sweden, <sup>4</sup>Endocrinological and Reproductive Unit, Department of Obstetrics/Gynaecology, Herlev Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, <sup>5</sup>Medical and Endocrinological Unit, Herlev Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, <sup>6</sup>Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway  
Corresponding author: Anna Melin, PhD, MSc clinical nutrition, RD, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, DK-Frederiksberg, Denmark. Tel: +46 7326 29714; Fax: +45 3533 2483; E-mail: aot@nexs.ku.dk; anna.katarina.melin@hotmail.com

Accepted for publication 31 May 2015

**Low or reduced energy availability (LEA) is linked to functional hypothalamic oligomenorrhea/amenorrhea (FHA), which is frequently reported in weight-sensitive sports. This makes LEA a major nutritional concern for female athletes. The aim of this study was to describe dietary characteristics of athletes with LEA and/or FHA. Endurance athletes ( $n = 45$ ) were recruited from national teams and competitive clubs. Protocols included gynecological examination, body composition, eating disorder evaluation, and 7-day dietary intake and EA assessment. Athletes with disordered eating behavior/eating disorders ( $n = 11$ ), menstrual dysfunction other than FHA ( $n = 5$ ), and low dietary record validity ( $n = 4$ ) were excluded. Remaining subjects ( $n = 25$ ) were characterized by EA**

**[optimal:  $\geq 45$  kcal (188 kJ)/kg fat-free mass (FFM)/day ( $n = 11$ ), LEA:  $< 45$  kcal (188 kJ)/kg FFM/day ( $n = 14$ ) and reproductive function [eumenorrhea (EUM;  $n = 10$ ), FHA ( $n = 15$ )]. There was no difference in EA between FHA and EUM subjects. However, FHA and LEA subjects shared the same dietary characteristics of lower energy density (ED) [ $P = 0.012$ ;  $P = 0.020$ ], respectively, and fat content [ $P = 0.047$ ;  $P = 0.027$ ], respectively]. Furthermore, FHA subjects had a lower intake of carbohydrate-rich foods ( $P = 0.019$ ), higher fiber content ( $P < 0.001$ ), and drive for thinness score ( $P = 0.003$ ). Conclusively, low ED together with high fiber content may constitute targets for dietary intervention in order to prevent and treat LEA and FHA in female athletes.**

Athletes need to maintain sufficient energy and nutrient intake, especially during periods of intense training, in order to avoid fatigue, injury, and illness (Burke, 2001; Rodriguez et al., 2009). Female athletes in weight-sensitive sports, however, are often reported not to meet energy needs (Horvath et al., 2000) and to have restricted eating behavior or inadvertent energy deficiency (De Souza et al., 2014; Mountjoy et al., 2014). Energy availability (EA) 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, 2014). Suppression of the pulsatility of luteinising hormone (LH) from the pituitary gland has been shown to occur at an EA below 30 kcal (125 kJ)/kg FFM/day in sedentary women (Loucks & Thuma, 2003), defining a low EA. Athletes aiming to lose weight or body fat are recommended to follow a diet and training regimen providing EA between 30 and 45 kcal (125 and 188 kJ)/kg FFM/day (Loucks et al., 2011), defining reduced EA (Gibbs et al., 2013).

To ensure adequate energy for all physiological functions (De Souza et al., 2014), physically active women are recommended to achieve an EA  $\geq 45$  kcal (188 kJ)/kg FFM/day, defining optimal EA (De Souza et al., 2014; Loucks, 2014).

Ovarian function depends on the frequency of LH pulsatility (Loucks et al., 2011), and persistent low EA with or without an eating disorder or disordered eating behavior is therefore considered to be the primary cause of functional hypothalamic oligomenorrhea or amenorrhea (FHA) in female athletes (Nattiv et al., 2007; Mountjoy et al., 2014). Low as well as reduced EA is also related to a lower resting metabolic rate (RMR; Melin et al., 2014a), and gastrointestinal problems in female endurance athletes (Melin et al., 2014b). Restricted eating behavior and FHA 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; De Souza et al., 2014; Mountjoy et al., 2014). Hence, it is of great concern that restricted eating behavior and FHA are frequently

reported in female athletes (Gibbs et al., 2013). Since weight influences performance in many sports, low or 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). Twenty-four percent of female elite endurance athletes have been reported to have DSM-IV (Diagnostic and Statistical Manual of Mental Disorders 4th edition) diagnosed eating disorders (Sundgot-Borgen & Torstveit, 2004), which are clinical mental disorders associated with various abnormal eating behaviors, an irrational fear of gaining weight, and false beliefs about eating, weight, and shape (Nattiv et al., 2007). Inadvertent low or reduced EA could also occur due to a high or increased training load.

Energy requirements for female endurance athletes may vary considerably but have been reported to reach levels of 4000 kcal (16.7 MJ)/day (Manore, 2002) to 4700 kcal (19.7 MJ)/day over several days (Sjödén et al., 1996). However, female endurance athletes have been reported to have energy intakes similar to or even lower than sedentary women (Beidleman et al., 1995). Some studies in which athletes with disordered eating behavior or eating disorders have been excluded have reported a lower energy intake in female endurance athletes with FHA compared with eumenorrheic (EUM) athletes (Nelson et al., 1986; Thong et al., 2000; Tomten & Høstmark, 2006) or an increased training volume and hence a potentially lower EA (Drinkwater et al., 1984). However, several studies have found similar energy intakes and training volumes in female endurance athletes with FHA as in EUM athletes (Deuster et al., 1986; Myerson et al., 1991; Laughlin & Yen, 1996; Schaal et al., 2011).

Recreationally active women with menstrual cycle disturbances have also been reported to eat a diet low in energy density (ED) (Reed et al., 2011). ED is the amount of energy (kcal or kJ) per amount (g) of food (Stubbs & Whybrow, 2004) and can be calculated for dishes, meals, and the total diet (Vernarelli et al., 2013). ED is influenced by different food components, and a low ED diet is typically high in water-rich foods, such as fruits and vegetables, and has a high fiber and low fat content (Stubbs & Whybrow, 2004; Rolls, 2009; Vernarelli et al., 2013). Experimental studies have demonstrated that a diet with a low ED will limit energy intake (Rolls, 2009). Therefore, a diet with a low ED increases the risk for low and reduced EA in female athletes, especially in periods of high energy demand.

Since many studies investigating dietary intake, training regimes, and EA in female endurance athletes without disordered eating behavior or eating disorders have reported similar results in FHA and EUM subjects (Deuster et al., 1986; Myerson et al., 1991; Laughlin & Yen, 1996; Schaal et al., 2011), it is possible that specific dietary characteristics reported in female athletes with FHA, such as lower ED, lower fat, and higher fiber content, might increase the risk of long-term relative

energy deficiency at a degree large enough to maintain the suppression of the hypothalamic–pituitary axis and thereby FHA. We therefore investigated dietary characteristics related to low or reduced EA, operationally defined as LEA, and/or FHA that could be targeted for nutritional adjustment in female elite athletes.

## Methods

The methods used in this study have previously been described in detail (Melin et al., 2014a,b). The study population was recruited through the Danish and Swedish sport federations and competitive sports clubs involved in weight-sensitive endurance sports, 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 were asked to sign an informed consent. 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 in Sweden and Denmark. Subjects included and defined as endurance athletes were athletes at the national team level or competitive endurance athletes from regional competitive sports clubs, aged between 18 and 38 years, training a minimum of five times per week. Data collection was performed on 2 consecutive days followed by a 7-day recording period in the athletes' normal environment. The first day consisted of an anthropometric assessment and examinations of reproductive function. For the anthropometric assessment, body weight and height were measured and dual-energy X-ray absorptiometry (DXA) was used to determine FFM and fat mass (FM). For reproductive function, menstruating athletes were examined in the early follicular phase (third to fifth day of menstruation) by an experienced gynecologist who performed a transvaginal ultrasound examination, a measurement of sex hormone status, and a retrospective history of menstrual function using the Low EA in Females Questionnaire (Melin et al., 2014b). Subjects were classified as follows: EUM – menstrual cycles of  $28 \pm 7$  days and sex hormones within the normal range; FHA – oligomenorrhea (menstrual cycles > 35 days), primary FHA (no menarche after 15 years of age) or secondary FHA (absence of  $\geq 3$  consecutive menstrual cycles); polycystic ovarian syndrome; or other reasons for menstrual dysfunction, such as anatomic or hormonal defects. The second day included examinations of aerobic capacity and assessment of disordered eating behavior and eating disorders. Figure 1 illustrates the participant flow in the study. Of the 45 athletes completing the clinical examination protocol, five were excluded because of clinically verified menstrual dysfunction other than FHA. Since we wanted to investigate dietary intake and dietary characteristics of female athletes with LEA and/or FHA who were not presenting disordered eating behavior or a clinically overt eating disorder, 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 subject was excluded due to disordered eating behavior assessed by the Eating Disorder Inventory (EDI-3; Garner, 2004). Four subjects were identified as having a low validity of their reported energy intake (corresponding to a physical activity level < 1.6), according to the Goldberg cut-off using the equation described by Black (2000), and were excluded. A total of 25 subjects were consequently included in the final analyses of dietary characteristics.

### Energy availability

Dietary intake and training were recorded by the subjects for 7 consecutive days to calculate EA. Heart rate monitors and training logs were used to assess exercise energy expenditure, and the

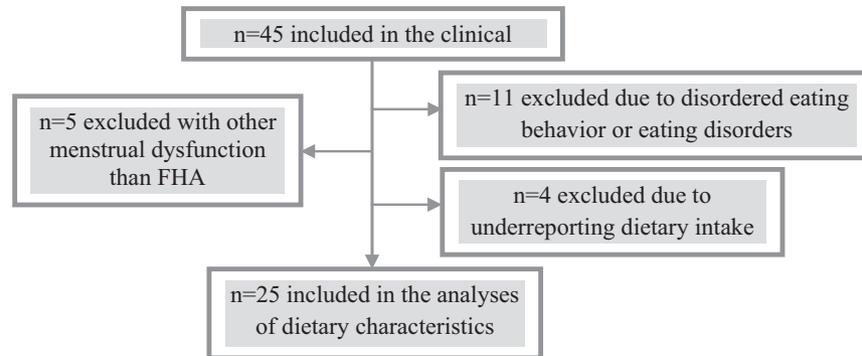


Fig. 1. Flowchart.

subjects were instructed to maintain and follow their normal training regimen. 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 (kcal/kJ) attributable to self-reported training, and the estimate of the energy expended for RMR and non-exercise activity thermogenesis throughout the duration of training was subtracted from the estimate of exercise energy expenditure (Loucks, 2014).

#### Dietary intake

Dietary intake was calculated from 7-day weighed 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. Subjects provided food labels and recipes, and nutrient content of food items not in the databases were entered manually by the dietitian. Subjects were given in-depth verbal and written instructions and a demonstration of how food and drink should be weighed and recorded and were instructed to maintain normal food habits and eating patterns. Before entering data in the nutrient analysis program, the same dietitian reviewed all completed dietary 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.

#### Energy density

Dietary ED was calculated in three different ways by dividing total kcal by total grams of (I) all food and no beverage (II) all food and energy-containing beverages, and (III) all food and beverages, including water (Vernarelli et al., 2013).

#### Food group variety

Food group variety was analyzed by calculating the mean number of servings of nine different food groups over the 7 days. The nine food groups in this study were defined as follows: (1) fruit and vegetables, including fresh and dried fruits and all kinds of vegetables including potatoes. One serving of fruit or vegetables was operationally defined as 100 g; (2) whole-grain products, including whole-grain pasta, brown rice, bulgur wheat, oatmeal, muesli, rye, and crisp breads. One serving was defined as 50 g; (3) carbohydrate-rich foods, including white rice, pasta, and bread (< 6 g of dietary fiber/100 g), couscous, cereals (such as corn flakes), honey, sugar, cakes, sweets, sweet biscuits, jam, and

marmalade. One serving was defined as 50 g; (4) nuts and seeds with all nuts and seeds included. One serving was defined as 20 g; (5) meat, including all kinds of meat, fish, poultry, and egg. One serving was defined as 100 g; (6) dairy products, including milk, yoghurt, dairy-based sports shakes, 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; (7) sport products, including energy and protein bars and gels. One serving was defined as 50 g; (8) fats and oils, including salad dressings, butter, margarine, oils, and pesto. One serving was defined as 10 g; (9) energy-containing drinks, including all non-dairy-based drinks containing energy, such as fruit juice, rice and soya milk, sports drinks, soft drinks, beer, wine and alcohol, and coffee or tea with sugar. One serving was defined as 200 g. A combination of foods, such as pizza and lasagna, was separated into their main ingredients and placed in the relevant food group (e.g. lasagna was divided between carbohydrate-rich food, meat and dairy products, 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 summarized as mean  $\pm$  standard deviation, and non-normally distributed data as median and interquartile range (25 and 75 percentiles). Since we wanted to investigate dietary intake and characteristics associated with EA as well as reproductive function, the subjects were characterized by their EA [optimal EA defined as  $\geq 45$  kcal (188 kJ)/kg FFM/day vs LEA defined as  $< 45$  kcal (188 kJ)/kg FFM/day] and by their reproductive function (EUM vs FHA). In order to investigate differences between subjects with optimal EA vs LEA and between EUM vs FHA subjects, the Student unpaired *t*-test was used for normally distributed data and the 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 EA and reproductive status were similar with respect to nutritional intake and dietary characteristics, 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 for normally distributed data and Spearman's rank correlation coefficient for skewed data. 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 FHA, a multiple linear regression with backward selection was conducted after evaluating the collinearity between the predictors by means of the variance inflation factors. A significance level of  $< 0.05$  was used.

## Results

### Description of subjects

Subject characteristics are presented in Table 1. There were no differences in body mass index (BMI), body composition, or exercise capacity between subjects divided by EA, but subjects with LEA demonstrated a higher training volume and exercise energy expenditure of 25% and 37%, respectively, compared with those with optimal EA. Subjects with FHA had 18% and 14% lower absolute and relative FM (%), respectively, although there were no differences in body weight, BMI, or exercise capacity between FHA subjects and EUM subjects. One subject with secondary FHA and low EA [ $< 30$  kcal (125 kJ)/kg FFM/day] was underweight (BMI  $< 18.5$  kg/m<sup>2</sup>). One of the EUM subjects with optimal EA [ $\geq 45$  kcal (188 kJ)/kg FFM/day] reported previously having had periods with disordered eating behavior and secondary FHA. One EUM subject with reduced EA [30–45 kcal (125–188 kJ)/kg FFM/day] reported eating a low carbohydrate diet for the previous 6 months. There were no vegetarians or vegans in this group of endurance athletes.

### Energy availability and dietary intake

EA and dietary intake are presented in Table 2. Three subjects had low EA [ $< 30$  kcal (125 kJ)/kg FFM/day] (2 with secondary FHA) and 11 had reduced EA [ $< 45$  kcal (188 kJ)/kg FFM/day] (6 with secondary FHA and 2 with oligomenorrhea). We found, however, no difference in the number of subjects with FHA having LEA (10 of 15) compared with EUM [4 of 10 ( $P = 0.183$ )], and there were no differences in energy intake or EA between FHA and EUM subjects.

As expected, EA 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 was negatively associated with exercise energy expenditure [ $r = -0.66$  ( $P = 0.002$ )]. Furthermore, EA was positively associated with ED but only when calculated as (I) all food and no beverage [ $r = 0.43$  ( $P = 0.034$ )], with the intake of food groups from fats and oils [ $r = 0.55$  ( $P = 0.005$ )], and energy-containing drinks [ $r = 0.054$  ( $P = 0.005$ )].

As anticipated, the ED of the diet calculated as (I) all food and no beverage was positively associated with the intake of fat [ $r = 0.42$  ( $P = 0.039$ )], and was negatively associated with the intake of fiber [ $r = -0.52$  ( $P = 0.009$ )] and fruits and vegetables [ $r = -0.64$  ( $P = 0.001$ )]. Furthermore, ED calculated as (I) all food and no beverage was negatively associated with the intake of protein [ $r = -0.42$  ( $P = 0.039$ )], and was positively associated with the intake of carbohydrate-rich foods [ $r = 0.55$  ( $P = 0.005$ )]. ED calculated as (II) all food and energy-containing beverages was negatively associated with the intake of fruits and vegetables [ $r = -0.54$  ( $P = 0.006$ )], and sports products [ $r = -0.49$  ( $P = 0.013$ )]. ED calculated as (III) all food and beverages including water was negatively associated with the intake of fiber [ $r = -0.45$  ( $P = 0.026$ )] and fruits and vegetables [ $r = -0.46$  ( $P = 0.016$ )].

### Dietary features in subjects characterized by energy availability

The diet of subjects with LEA had a 15% lower ED compared with subjects with optimal EA when calculated as (I) all food and no beverage (Fig. 2). We found, however, no differences between EA groups when ED was calculated as (II) all food and energy-containing beverages or as (III) all food and beverages including

Table 1. Description of subjects characterized by energy availability and reproductive function

|                                 | All<br>( <i>n</i> = 25) | Optimal EA<br>( <i>n</i> = 11) | LEA<br>( <i>n</i> = 14) | EUM<br>( <i>n</i> = 10) | FHA<br>( <i>n</i> = 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 <sup>2</sup>          | 20.6 ± 2.0              | 20.6 ± 1.6                     | 20.6 ± 2.3              | 21.2 ± 2.3              | 20.2 ± 1.6              |
| VO <sub>2peak</sub> , L/min     | 3.21 ± 0.4              | 3.21 ± 0.4                     | 3.21 ± 0.4              | 3.20 ± 0.4              | 3.22 ± 0.4              |
| VO <sub>2peak</sub> , 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, kcal/day                   | 940 ± 450               | 705 ± 184                      | 1121 ± 516*             | 909 ± 265               | 959 ± 550               |
| FM, kg                          | 11.8 ± 3.2              | 11.7 ± 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)        |

For comparison of mean levels between the groups with optimal EA vs LEA and EUM vs FHA, the Student unpaired *t*-test was used for normally distributed variables and the Wilcoxon rank-sum test for skewed distributed variables. Data are presented as mean ± standard deviation for normal distributed data and as median and interquartile range (25–75) for skewed data.

\* $P < 0.05$ .

BMI, body mass index; EA, energy availability; EEE, exercise energy expenditure; EUM, eumenorrhea; FHA, functional hypothalamic oligomenorrhea/amenorrhea; FM, fat mass; LEA, low or reduced EA [ $< 45$  kcal (188 kJ)/kg fat-free mass (FFM)/day]; VO<sub>2peak</sub>, maximal oxygen uptake.

Table 2. Dietary intake in subjects characterized by energy availability and reproductive function

|                   | All<br>(n = 25) | Optimal EA<br>(n = 11) | LEA<br>(n = 14) | EUM<br>(n = 10) | FHA<br>(n = 15) | P-value |                       |
|-------------------|-----------------|------------------------|-----------------|-----------------|-----------------|---------|-----------------------|
|                   |                 |                        |                 |                 |                 | EA      | Reproductive function |
| Energy intake     |                 |                        |                 |                 |                 |         |                       |
| (kcal/day)        | 2766 ± 455      | 2980 ± 454             | 2598 ± 392      | 2899 ± 553      | 2677 ± 370      | 0.056   | 0.475                 |
| (kcal/kg/day)     | 46.1 ± 12.2     | 51.8 ± 9.0             | 41.6 ± 12.7     | 48.2 ± 11.5     | 44.7 ± 12.8     | 0.040   | 0.833                 |
| (kcal/kg FFM/day) | 59.2 ± 9.4      | 64.8 ± 9.9             | 54.7 ± 6.3      | 61.1 ± 12.7     | 57.9 ± 6.6      | 0.004   | 0.832                 |
| EA                |                 |                        |                 |                 |                 |         |                       |
| (kcal/kg FFM/day) | 42.5 ± 12.1     | 52.2 ± 7.5             | 34.9 ± 9.1      | 45.1 ± 13.6     | 40.7 ± 11.1     | < 0.001 | 0.977                 |
| Carbohydrates     |                 |                        |                 |                 |                 |         |                       |
| (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                 |
| Fiber             |                 |                        |                 |                 |                 |         |                       |
| (g/day)           | 41 ± 11         | 39 ± 10                | 42 ± 13         | 33 ± 9          | 46 ± 10         | 0.971   | 0.007                 |
| (g/1000 kcal)     | 14.9 ± 3.9      | 13.4 ± 3.4             | 16.1 ± 4.0      | 11.6 ± 2.7      | 17.1 ± 3.0      | 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                 |

Data are presented as mean ± standard deviation. To investigate whether there were differences between subjects with optimal EA and LEA as well as between EUM and FHA subjects, a two-way analysis of variance with Bonferroni correction for each variable was used. There was no group interaction effect in any of the measured variables.

EA, energy availability; EUM, eumenorrhea; FHA, functional hypothalamic oligomenorrhea/amenorrhea; LEA, low or reduced EA [ $< 45$  kcal (188 kJ)/kg fat-free mass (FFM)/day].

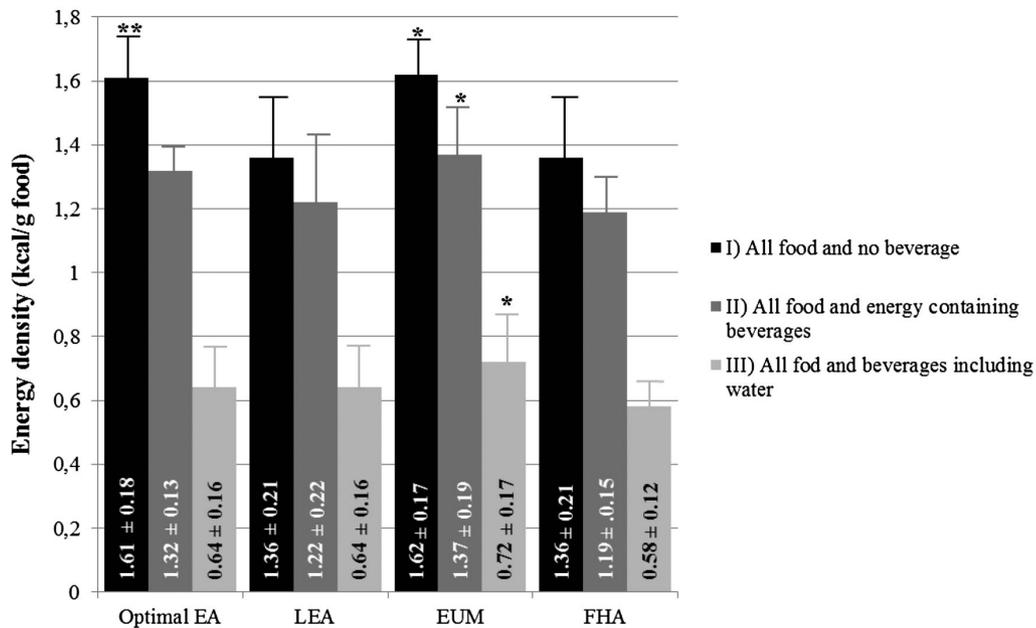


Fig. 2. Energy density in subjects characterized by energy availability and reproductive function.

water, although subjects with optimal EA had a higher intake of energy-containing drinks compared with those with LEA (Table 3).

Fifty percent of the subjects with LEA compared with 73% of those with optimal EA had a carbohydrate intake

$\geq 6$  g/kg/day, which is recommended to endurance athletes training with moderate to high-intensity exercise 1–3 h/day (Burke et al., 2011). We found no differences in the fiber content (Table 2) or in the intake of carbohydrate-rich foods, whole-grain products, or fruit

Table 3. Food group variety in subjects characterized by energy availability and reproductive function

|   | Optimal EA<br>(n = 11) | LEA<br>(n = 14) | EUM<br>(n = 10) | FHA<br>(n = 15) | P-value |                       |                            |
|---|------------------------|-----------------|-----------------|-----------------|---------|-----------------------|----------------------------|
|   |                        |                 |                 |                 | EA      | Reproductive function | EA + reproductive function |
| Fruit and vegetables (100 g/serving)      | 6.2 ± 2.6              | 8.0 ± 3.1       | 5.7 ± 2.6       | 8.2 ± 2.9       | 0.239   | 0.077                 | 0.320                      |
| Whole grain products (50 g/serving)       | 3.8 ± 2.5              | 3.6 ± 1.9       | 2.7 ± 1.6       | 4.4 ± 2.2       | 0.447   | 0.050                 | 0.884                      |
| Carbohydrate-rich foods (50 g/serving)    | 5.2 ± 2.5              | 3.7 ± 1.9       | 5.7 ± 2.0       | 3.5 ± 2.1       | 0.115   | 0.019                 | 0.028                      |
| Nuts and seeds (20 g/serving)             | 1.1 ± 1.0              | 0.7 ± 0.9       | 0.8 ± 1.0       | 1.0 ± 0.9       | 0.338   | 0.418                 | 0.011                      |
| Meat (100 g/serving)                      | 2.0 ± 0.9              | 2.1 ± 0.6       | 2.2 ± 1.0       | 1.9 ± 0.5       | 0.780   | 0.298                 | 0.459                      |
| Dairy products (20–200 g/serving)         | 3.6 ± 1.3              | 3.4 ± 1.7       | 3.7 ± 1.9       | 3.3 ± 1.3       | 0.682   | 0.935                 | 0.236                      |
| Sport products (50 g/serving)             | 0.4 ± 0.4              | 0.6 ± 0.9       | 0.4 ± 0.5       | 0.5 ± 0.9       | 0.516   | 0.908                 | 0.722                      |
| Fats and oils (10 g/serving)              | 2.3 ± 1.6              | 1.2 ± 0.5       | 1.9 ± 1.4       | 1.6 ± 1.1       | 0.035   | 0.966                 | 0.256                      |
| Energy-containing drinks (200 mL/serving) | 1.6 ± 0.4              | 0.7 ± 0.2       | 1.6 ± 1.4       | 0.8 ± 0.7       | 0.039   | 0.218                 | 0.473                      |

Fruit and vegetables included fresh and dried fruits and all kinds of vegetables including potatoes. Whole-grain products included whole-grain pasta, brown rice, bulgur wheat, oatmeal, muesli, rye, and crisp breads. Carbohydrate-rich foods included white rice, pasta, and bread (< 6 g of dietary fiber/100 g), couscous, cereals (such as corn flakes), honey, sugar, cakes, sweets, sweet biscuits, jam, and marmalade. Nuts and seeds with all nuts and seeds included. Meat included all kinds of meat, fish, poultry, and egg. Dairy products included milk, yoghurt, dairy-based sports shakes, cream, and all kinds of cheese. Sport products included energy and protein bars and gels. Fats and oils included salad dressings, butter, margarine, oils, and pesto. Energy-containing drinks included all non-dairy-based drinks containing energy, such as fruit juice, rice and soya milk, sports drinks, soft drinks, beer, wine and alcohol, and coffee or tea with sugar. Data are presented as mean ± standard deviation 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 EA and LEA as well as between subjects with FHA and EUM, a two-way analysis of variance with Bonferroni correction for each variable was used. EA, energy availability; EUM, eumenorrhea; FHA, functional hypothalamic oligomenorrhea/amenorrhea; LEA, low or reduced EA [ $< 45$  kcal (188 kJ)/kg fat-free mass (FFM)/day].

Table 4. EDI-3 score in subjects characterized by energy availability and reproductive function

|                   | All<br>(n = 25) | Optimal EA<br>(n = 11) | LEA<br>(n = 14) | EUM<br>(n = 10) | FHA<br>(n = 15) | P-value |                       |
|-------------------|-----------------|------------------------|-----------------|-----------------|-----------------|---------|-----------------------|
|                   |                 |                        |                 |                 |                 | EA      | Reproductive function |
| EDI-3 total 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                 |

Data are presented as mean ± standard deviation 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 EA and LEA as well as between subjects with FHA and EUM, a two-way analysis of variance with Bonferroni correction for each variable was used. There was no group interaction effect in any of the measured variables. BD, body dissatisfaction subscale; DT; drive for thinness subscale; EA, energy availability; EDI-3, eating disorder inventory 3; EUM, eumenorrhea; FHA, functional hypothalamic oligomenorrhea/amenorrhea; LEA, low or reduced EA [ $< 45$  kcal (188 kJ)/kg fat-free mass/day].

and vegetables or in the intake of sports products between subjects with optimal EA compared with subjects with LEA (Table 3).

All subjects but one met the dietary recommendations for protein ( $\geq 1.2$  g/kg/day) for endurance athletes (Rodriguez et al., 2009; Philips, 2011), and 71% of the subjects with LEA met the dietary protein recommendations during energy deficiency ( $\geq 1.8$  g/kg/day; Burke et al., 2011). There were no differences in protein intake (Table 2) or in the intake of meat, fish, poultry and egg, dairy products, or nuts and seeds between EA groups (Table 3), and the protein intake for the whole group ( $n = 25$ ; g/day) was positively associated with the intake of dairy products [ $r = 0.43$  ( $P = 0.011$ )]. Subjects with LEA had a 26% lower relative fat intake (g/kg/day) compared with subjects with optimal EA (Table 2). The total EDI-3 score and the score for drive for thinness (DT) as well as body dissatisfaction subscales are presented in

Table 4. There were no differences in the total EDI-3 or subscale scores among subjects characterized by EA.

Dietary features in subjects characterized by reproductive function

The diet of subjects with FHA had a 16% lower ED compared with EUM subjects when calculated as (I) all food and no beverage, 13% when calculated as (II) all food and energy-containing beverages, and 19% when calculated as (III) all food and beverages including water (Fig. 2). There was no difference in the intake of carbohydrates between groups (Table 2), although EUM subjects ate more carbohydrate-rich foods compared with FHA subjects (Table 3). There was, furthermore, a group interaction effect, meaning that EUM subjects with optimal EA had a higher intake of carbohydrate-rich foods compared with FHA subjects with optimal EA as

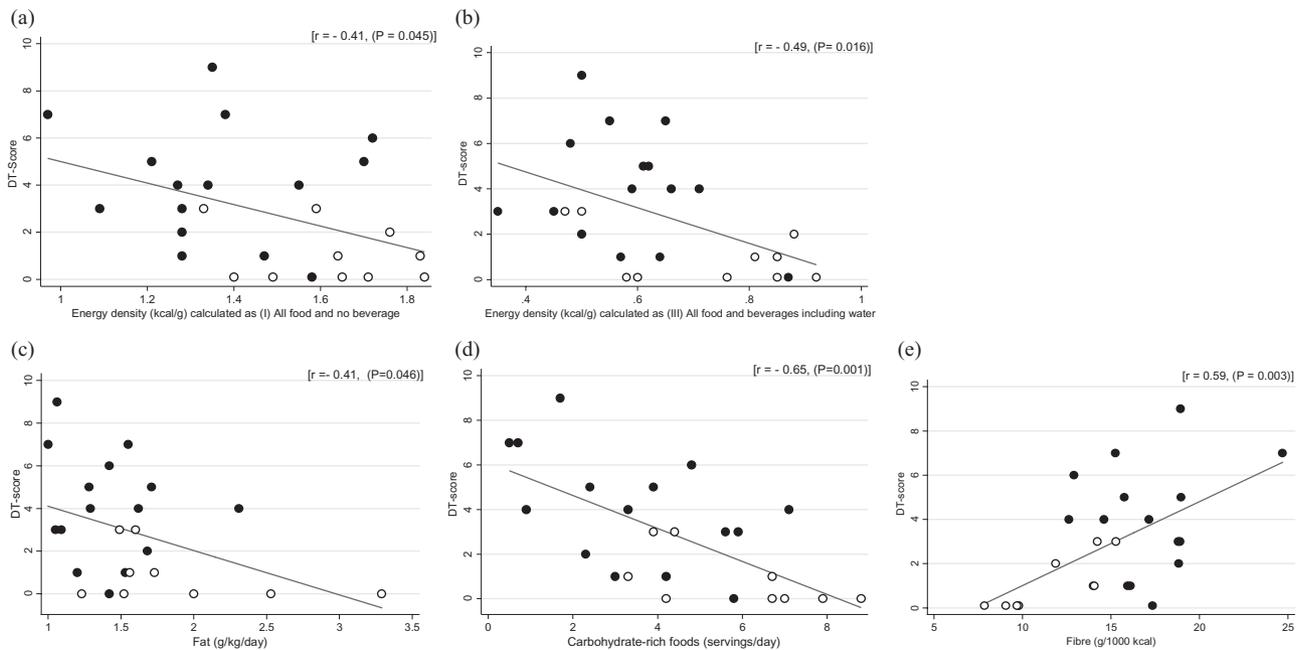


Fig. 3. The associations between drive for thinness score and energy density, the intake of nutrients, and food groups.

well as LEA. Subjects with FHA had a 23% higher total (g/day) and a 32% higher relative fiber intake (g/1000 kcal) compared with the EUM group (Table 2). Fiber intake exceeding the nutritional recommendation of 25–35 g/day (United States and Scandinavia), 3 g/MJ/day (Scandinavia) or 14 g/1000 kcal/day (United States; Institute of Medicine of the National Institute of Science, 2002, Nordic Council of Ministers, 2013) was more common among subjects with FHA compared with EUM subjects [12 of 15 vs 3 of 10 ( $P = 0.018$ ); 13 of 15 vs 4 of 10 ( $P = 0.022$ ), and 13 of 15 vs 2 of 10 ( $P = 0.001$ ), respectively]. There was a trend toward a higher intake of whole-grain products and fruit and vegetables in FHA subjects compared with EUM subjects, while there were no differences in the intake of sports products or the intake of energy-containing drinks between groups characterized by reproductive function (Table 3). As previously mentioned, all subjects but one met the dietary recommendations for protein for endurance athletes (Rodriguez et al., 2009; Philips, 2011), and there were no differences in protein intake (Table 2) or in the intake of meat, dairy products, or nuts and seeds between groups (Table 3). There was, however, a group interaction effect in the intake of nuts and seeds, meaning that the FHA athletes with optimal EA had a higher intake of nuts and seeds compared with FHA athletes with LEA. Subjects with FHA had a 27% lower total fat intake (g/day) compared with EUM subjects.

Multiple regression analysis, including all subjects, with a backward elimination containing EA (kcal/kg FFM/day), ED calculated as (I) all food and no beverage (kcal/g), intake of fat (g/day), and fiber content (g/1000 kcal/day) as possible predictors of FHA, showed a partial correlation coefficient for dietary fiber intake of

$r^2 = 0.25$  ( $P < 0.001$ ), with an unstandardized regression coefficient ( $\beta$ ) of 0.37 (95% confidence interval 0.21–0.53) and a mean variance inflation factor of 1.46 (range 1.34–1.65).

Subjects with FHA had a higher mean total EDI-3 score and a higher DT score compared with EUM subjects (Table 4). There were negative associations between the DT score for the whole group and ED calculated as (I) all food and no beverage (Fig. 3) and as (III) all food and beverages including water, fat content (g/kg/day), and the intake of carbohydrate-rich foods (servings/day). Furthermore, there was a positive association between the DT score and the fiber content of the diet (g/1000 kcal).

## Discussion

The present study is the first to investigate dietary characteristics associated with EA and reproductive function in female endurance athletes. We did not find any differences in energy intake or EA between EUM and FHA athletes. We did find, however, that athletes with FHA and athletes with LEA shared the same dietary characteristics with a lower ED and a lower fat content. Furthermore, athletes with FHA were more weight concerned and had a diet with a greater amount of dietary fiber and fewer carbohydrate-rich foods compared with EUM athletes.

### Energy intake and energy availability

The mean relative energy intake in the present study was 46 kcal (193 kJ)/kg/day, which is similar to the mean intake of 43 kcal (180 kJ)/kg/day reported by Burke

(2001) in female endurance athletes. Although energy intake was 8% lower, exercise energy expenditure 6% higher, and consequently EA 10% lower in subjects with FHA compared with EUM subjects, we found no significant difference between the groups. These findings are in line with most studies reporting energy intake and exercise energy expenditure in female endurance athletes without disordered eating behavior or eating disorders (Deuster et al., 1986; Myerson et al., 1991; Laughlin & Yen, 1996) and support the findings by Schaal et al. (2011) who reported no difference in EA between EUM ( $n = 5$ ) vs menstrual dysfunction ( $n = 5$ ) runners and triathletes. In the present study, 44% ( $n = 11$ ) had EA between 30 and 45 kcal (125 and 188 kJ)/kg FFM/day, levels that are recommended for athletes needing to lose weight (Loucks et al., 2011), while 12% ( $n = 3$ ) had EA < 30 kcal (125 kJ)/day, levels that in clinical settings have shown to suppress LH pulsatility in healthy sedentary EUM women after only 5 days (Loucks & Thuma, 2003). We previously reported lower RMR and LH levels in female endurance athletes with reduced EA [30 to 45 kcal (125 to 188 kJ)/kg FFM/day] compared with athletes with optimal EA (Melin et al., 2014a). These combined results indicate that the threshold for low EA established by Loucks and Thuma (2003) [ $< 30$  kcal (125 kJ)/kg FFM/day] 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). Furthermore, it is possible that the reproductive function in some women is more robust or more sensitive than it is in others (Ciadella-Kam et al., 2014) and that the threshold of long-term EA associated with FHA may vary among exercising women (Gibbs et al., 2013).

### Energy density and food groups

There are different possible reasons for LEA in female athletes, and it could be unintentional because of difficulties in eating enough during intense training periods. This is especially true if the diet meets the criteria for a healthy diet recommended to the general population: low in ED and high in bulky fibers (Nordic Council of Ministers, 2013). In the present study, LEA was associated with a combination of higher exercise energy expenditure because of greater training load and a lower energy intake mainly because of a lower intake of fat. We also found that EA was positively associated with the ED of the diet when calculated as (I) all food and no beverage, the intake of energy-containing drinks, and especially with the intake of fats and oils.

Even though we did not find any differences in current energy intake or EA between subjects with FHA compared with EUM subjects, subjects with FHA evidently followed a lower ED diet compared with EUM subjects. These results support what has previously been reported in recreationally active women with menstrual cycle dis-

turbances (Reed et al., 2011). The bulking property of dietary fiber lowers the ED of the diet mainly by binding water since water contributes to weight but not to energy (Rolls, 2009). Furthermore, ED is increased more by fat than carbohydrate or protein because of its higher energy content per weight (Rolls, 2009); as expected, we found a positive association between the ED of the diet and fat intake and a negative association between ED and fiber content as well as with the intake of fruit and vegetables. In the present study, we also found that ED was negatively associated with protein content and that it was positively associated with the intake of carbohydrate-rich foods, such as white rice and pasta, bread ( $< 6$  g of whole grains/100 g), and sweets. Diets with low ED 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 ED is therefore likely to increase the risk for LEA in female athletes, especially during periods with high training loads.

### Carbohydrate intake and fiber content of the diet

In the present study, 50% of the athletes with LEA had a carbohydrate intake of 6–10 g/kg/day, which is recommended for endurance athletes with a training regimen of 1–3 h/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 submaximal 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, 2014). Even though there were no differences in the carbohydrate intake between EUM and FHA subjects in the present study, the FHA athletes consumed less carbohydrate-rich foods, such as white rice, pasta and bread compared with the EUM athletes; these foods are important constituents of a carbohydrate-rich diet and commonly recommended to athletes in order to enhance performance (Burke et al., 2011).

Diets rich in fiber are recommended for their health-promoting effects, including weight regulation. Recommendations to eat more fiber are primarily based on their protective effect against colorectal cancer, cardiovascular disease, and type II diabetes (Nordic Council of Ministers, 2013). The positive health-promoting actions and periodically the weight regulatory effects of fiber are also important to female athletes. However, in the present study, 64% of the athletes (80% of the FHA athletes) exceeded the upper level of the recommended daily intake of fiber in the United States and the Nordic countries (25–35 g/day; Institute of Medicine of the National Academies, 2002, Nordic Council of Ministers,

2013). Even when considering a potentially higher energy intake, 72% (87% of the FHA athletes) exceeded 3 g/MJ (Nordic Council of Ministers, 2013) while 56% (87% of the FHA athletes) exceeded 14 g/1000 kcal (Institute of Medicine of the National Academies, 2002); the high intake of dietary fiber was linked to FHA. Therefore, while there are good reasons to encourage fiber intake in the majority of the population, there might also be good reasons to be concerned about high fiber intake in some athletic populations. Very few studies investigating dietary intake in female endurance athletes, however, have reported the fiber content of the diet (Deuster et al., 1986; Myerson et al., 1991; Laughlin & Yen, 1996). The higher fiber intake among subjects with FHA compared with EUM subjects that we found in the present study has been reported previously in endurance athletes by Laughlin and Yen (1996) and in recreational active women by Reed et al. (2011). A high fiber content beyond the upper recommended level is a potential nutritional concern in this group of endurance athletes since it could increase the risk for LEA by several mechanisms. (a) Energy digestibility is affected by dietary fiber since dietary fiber increases faecal fat excretion; the reported amount of increased fat and energy excreted is normally small, ranging from 24 to 72 kcal (100–300 kJ)/day in intervention studies providing 7–20 g/day of dietary fiber (Kristensen, 2009). However, a dietary fiber intake as high as > 40 g/day, as seen among the female athletes with FHA in the present study, might reduce energy absorption even further and consequently increase the risk for LEA. (b) Some dietary fiber, 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 delay 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. Other types of fiber, such as cellulose and hemicellulose in vegetables and cereal bran, are not digested but bind with water in the large intestine and increase satiety because of their bulking effect (Kristensen, 2009). These fiber-related effects are normally seen as beneficial. However, in endurance athletes with a high total energy expenditure, they can potentially become problematic since high-intensity exercise also 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 caused by intense exercise does not automatically lead to increased hunger or energy intake within 48 h in healthy young men, and hence a nutritional challenge for athletes could be that there is no strong drive to automatically match energy intake to exercise energy expenditure. Therefore, the combined effects of daily high-intensity exercise and a

high-fiber intake on appetite regulation might increase the risk for LEA in female athletes. (c) Besides increasing satiety because of their bulking effect, an excessive fiber intake may lead to gastrointestinal disorders, such as bloating and diarrhea (Lupton & Trumbo, 2006); these are common problems among female endurance athletes (Melin et al., 2014b). (d) Independent of the effect of fiber on energy intake, studies have reported a negative association between fiber intake and estrogen levels in sedentary premenopausal women (Laughlin et al., 1998; Aubertin-Leheudre et al., 2008). The presumed mechanism involves estrogens binding to fiber in the colon, which reduces the reabsorption of circulating estrogen (Aubertin-Leheudre et al., 2008) and hence increases the excretion of estrogens in the feces. Estrogen excreted in bile needs to be hydrolyzed before it can be reabsorbed, and a presumed indirect estrogen-lowering effect of fiber is reduced  $\beta$ -glucuronidase activity in the colon, which leads to reduced reabsorption of estrogen (Aubertin-Leheudre et al., 2008). These results support our findings derived from the multiple regression analysis that a high fiber intake, independently of its effect on EA or ED, could potentially increase the risk of FHA in female athletes. Even though clinical trials have shown a negative association between dietary fiber intake and estrogen levels in sedentary premenopausal women (Laughlin et al., 1998; Aubertin-Leheudre et al., 2008), a potential effect needs to be tested in controlled experiments in athletes in order to confirm that the negative associations between a high fiber content of the diet and FHA derive from a 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, the protein intake was generally high and exceeded the recommended 1.2–1.7 g/kg/day for endurance athletes (Rodriguez et al., 2009). In addition, 71% of the subjects with LEA reached a protein intake of 1.8–2.0 g/kg/day recommended during energy deficiency in order to minimize the loss of FFM and to optimize glycogen storage (Burke et al., 2011); this might have contributed to the similar FFM found in the LEA group compared with the group with optimal EA. On the other hand, a high protein intake, as observed among the female athletes in the present study (1.9 g/kg/day), is considered to result in a less ED diet than a diet with more carbohydrates and, in particular, fat (Stubbs & Whybrow, 2004) and could increase the risk for LEA. In the present study, 80% of the subjects with FHA had a protein intake > 1.7 g/kg/day, and a combined excessive intake of both protein and dietary fiber could therefore increase the risk of energy deficiency, especially in periods of large training volumes.

### Fat intake

In the present study, athletes with LEA had a lower intake of fat compared with those with optimal EA. The same dietary characteristic was seen in FHA subjects compared with EUM athletes, although no difference in energy intake was found, which supports the findings of earlier studies of female endurance athletes (Deuster et al., 1986; Laughlin & Yen, 1996). Horvath et al. (2000) found that ad libitum energy intake from a low fat diet (17 E%) during a month provided only 60% of the energy requirement in female runners; with a higher contribution of energy intake from fat, energy deficiency was found to be lower. 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 LEA, despite the recommended fat E%, emphasizes the importance of not assessing diet adequacy by the relative macronutrient distribution. In the present study, the mean relative intake was 1.8 g fat/kg/day for the EUM athletes, which is similar to the 1.9 g fat/kg/day reported by Deuster et al. (1986). In comparison, subjects with FHA had a fat intake of 1.4 g/kg/day in the present study just as reported by Deuster et al. (1986).

### Eating behavior

The reason for LEA 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 behavior 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 an abnormal and pathological eating behavior, so subjects diagnosed with disordered eating behavior or eating disorders were excluded. Interestingly, we still found higher, although not pathological, EDI-3 and DT scores in the athletes with FHA, indicating a heightened concern with dieting and a more entrenched pursuit of thinness compared with the EUM 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 FHA compared with EUM subjects despite similar current energy intake or EA obtained. We also found that a higher DT score was associated with a diet with lower ED, a higher content of dietary fiber, and a more restricted intake of fat and 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 LEA and FHA in athletes.

### Practical implications

Early detection and intervention of LEA with or without disordered eating behavior or eating disorders and 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; De Souza et al., 2014; Mountjoy et al., 2014). The high prevalence of LEA and FHA found in this population of female endurance athletes emphasizes the importance of specific dietary recommendations for female athletes. The recommended treatment for the endocrine and reproductive disruptions caused by LEA 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, an EA  $\geq 45$  kcal (188 kJ)/kg FFM/day is recommended (De Souza et al., 2014). Dietary recommendations for female athletes with FHA include increasing energy intake with 300–600 kcal (1.2–2.4 MJ)/day (Mountjoy et al., 2014) in small increments in order to avoid unwanted weight gain. Based on the results from the present study, we suggest that the following additional practical considerations could be used to guide nutritional prevention and treatment of LEA and 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 carbohydrate-rich foods and fat in order to limit the volume of the meals.
- Follow the general dietary guidelines concerning absolute intakes of dietary fiber (e.g. 25–35 g/day), but not necessarily relative to energy intake (g/1000 kcal or g/MJ), in order to reduce the risk of low ED.
- Besides ensuring an adequate intake of fat, increase ED by replacing some of the whole-grain products and fruit and vegetables with more 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 behavior or eating disorders emphasizes the importance of prevention and early detection and the need for multidisciplinary professional treatment of these disorders in order to address the possible underlying psychological factors (Nattiv et al., 2007; Mountjoy et al., 2014). 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 characterized by lower ED and lower fat as well as high dietary fiber content and thereby an increased risk for persistent LEA and FHA.

### Limitations

This cross-sectional study cannot demonstrate any causative effect of dietary factors on EA or FHA. However, our results suggest that diets with lower ED and high dietary fiber are nutritional issues in female athletes that need to be prioritized. Several methodological difficulties are involved in quantifying a clinically useful EA, including under-reporting or under-eating (Burke, 2001), timing of the assessment in order to collect data representative of the athletes' habitual EA, and 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 necessarily capture the athletes' habitual EA, and measuring the 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 observational period, it is a limitation that body weight was not measured during the recording 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.

### Perspectives

The present study demonstrates that a diet with a lower ED, a more restricted intake of fat, carbohydrate-rich foods, and energy-containing drinks as well as a high content of dietary fiber are dietary characteristics associated with LEA and FHA in female endurance athletes and should 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 persistent LEA and 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 counseling, ensuring a time-limited nutritional treatment plan with proper and effective guidelines for weight loss that ends with re-establishing an optimal EA and weight stability. Controlled intervention trials specifically designed to investigate potential direct and/or indirect causal relationships between high dietary fiber content and FHA in female athletes are needed.

### Acknowledgements

We thank Mubeena Azis and Katrine Haahr at Herlev Hospital for performing gynecological examinations; Anders Johansson, Lund University, and Ulla Kjørulff-Hansen, Herlev Hospital, for assisting with blood sampling; Fiona Koivola, Lund University, for assisting during testing; Hanne Udengaard, Herlev Hospital, for logistics assistance; Janni Borg Hoffmeyer for diet record analysis; and Christian Ritz, University of Copenhagen, for statistical advice. We highly appreciate the extraordinary cooperation of the athletes participating in this study and the support of the Swedish and Danish national sports federations and Team Denmark.

### Funding

This study was funded by research grants from the Faculty of Science, University of Copenhagen, World Village of Women Sports Foundation and Arla Foods Ingredients.

### Author contributions

AM, ÅT, SS and AS designed the study, and AM, ÅT, and AS analyzed the data. AM and AS were primarily responsible for writing the manuscript. AM, ÅT, and SSM collected data, while JSB, JF, and SS provided advice in their areas of expertise and together with ÅT and SSM critically revised the manuscript. All authors approved the final version of the manuscript.

**Key words:** Amenorrhea, dietary fiber, energy availability, energy density.

### References

- Aubertin-Leheudre M, Gorbach S, Woods M, Dwyer JT, 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 BA, Puhl JL, De Souza MJ. Energy-balance in female distance runners. *Am J Clin Nutr* 1995; 61: 303–311.
- Black AE. 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* 2001; 26: 202–219.
- Burke ML, Hawley JA, Wong SH, Jeukendrup A. Carbohydrates for training and competition. *J Sport Sci* 2011; 29: 17–27.
- Ciadella-Kam L, Guebels CF, Maddalozzo GF, Manore MM. Dietary intervention restored menses in female athletes with exercise-associated menstrual dysfunction with limited impact on bone and muscle health. *Nutrients* 2014; 6: 3018–3039.
- De Souza MJ, Hontscharuk R, Olmsted M, Kerr G, Williams NI. Drive for thinness score is a proxy indicator of energy deficiency in exercising women. *Appetite* 2007; 48: 359–367.
- De Souza MJ, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, Gibbs JC, Olmsted M, Goolsby M, Matheson G. 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. *Br J Sports Med* 2014; 48: 1–20.
- Deuster PA, Kyle SB, Moser PB, Vigersky RA, Singh A, Schoemaker EB. Nutritional intakes and status of highly trained amenorrheic and eumenorrheic women runners. *Fertil Steril* 1986; 46 (4): 636–643.
- Drinkwater BL, Nilson K, Chesnut CH 3rd, Bremner WJ, Shainholtz S, Southworth MB. Bone mineral content of amenorrheic and eumenorrheic athletes. *N Engl J Med* 1984; 311 (5): 277–281.
- Fairburn CG, Cooper Z, O'Connor M. Eating Disorder Examination (Edition 16.0D). In: Fairburn CG, ed. *Cognitive behavior therapy and eating disorders*. New York: Guilford Press, 2008: 1–49.
- Garner DM. *Eating Disorder Inventory-3. Professional Manual*. Par Inc. 2004.
- Gibbs JC, Williams NI, De Souza MJ. Prevalence of individual and combined components of the female athlete triad. *Med Sci Sports Exerc* 2013; 45: 985–996.
- Holt SH, Miller JC, Petocz P, Farmakalidis E. A satiety index of common foods. *Eur J Clin Nutr* 1995; 49: 675–690.
- 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: 42–51.
- 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; 31 (1): 9–19. Aug.
- Institute of Medicine of the National Institute of Science. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients)*. Washington, DC: National Academies Press, 2002.
- King NA, Lluch A, Stubbs RJ, Blundell JE. High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr* 1997; 51 (7): 478–483.
- Kristensen M. *Wholegrains and dietary fibres. Impact on body weight, appetite regulation and nutrient digestibility*. Department of Human Nutrition, University of Copenhagen, 2009.
- Larson-Meyer DE, Palm S, Bansal A, Austin KJ, Hart AM, Alexander BM. Influence of running and walking on hormonal regulators of appetite in women. *J Obes* 2012; 2012: 1–15. doi: 10.1155/2012/730409
- Laughlin GA, Dominguez CE, Yen SSC. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab* 1998; 83: 25–32.
- Laughlin GA, Yen SSC. Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *J Clin Endocrinol Metab* 1996; 81: 4301–4309.
- Loucks AB. Chapter 5, Sports nutrition. In: Maughan RJ, ed. *Energy balance and energy availability*. 1st edn. Chichester, West Sussex: John Wiley & Sons, Ltd, 2014: 72–87. International Olympic Committee.
- Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci* 2011; 29: S7–S15.
- 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: 297–311.
- Lupton JR, Trumbo PR. Dietary fiber. In: Shils ME, Shike M, Catharine Ross A, Caballero B, Cousins RJ, eds. *Modern nutrition in health and disease*. 10th edn. Baltimore: Lippincott Williams & Wilkins, 2006: 83–91.
- Manore M. Dietary recommendations and athletic menstrual dysfunction. *Sports Med* 2002; 32 (14): 887–901.
- Melin A, Tornberg ÅB, Skouby S, Møller SS, Sundgot-Borgen J, Faber J, Sidelmann JJ, Aziz M, Sjödin A. Energy availability and the female athlete triad in elite endurance athletes. *Scand J Med Sci Sports* 2014a: 1–13. doi: 10.1111/sms.12261
- Melin A, Tornberg ÅB, Skouby S, Faber J, Ritz C, Sjödin A, Sundgot-Borgen J. The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad. *Br J Sports Med* 2014b: 48: 540–545.
- Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, Meyer N, Sherman R, Steffen K, Budgett R, Ljungqvist A. The IOC consensus statement: beyond the Female Athlete Triad – Relative Energy Deficiency in Sport (RED-S). *Br J Sports Med* 2014; 48: 491–497.
- Myerson M, Gutin B, Warren MP, May MT, Contento I, Lee M, Pi-Sunyer FX, Pierson RN Jr, Brooks-Gunn J. Resting metabolic-rate and energy-balance in amenorrheic and eumenorrheic runners. *Med Sci Sports Exerc* 1991; 23 (1): 15–22.
- Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP, American College of Sports Medicine. The female athlete triad. *Med Sci Sports Exerc* 2007; 39: 1867–1882.
- Nelson ME, Fisher EC, Catsos PD, Meredith CN, Turksoy RN, Evans WJ. Diet and bone status in amenorrheic runners. *Am J Clin Nutr* 1986; 43 (6): 910–916.
- Nordic Council of Ministers. *Nordic nutrition recommendations 2012*. 5th edn, Part 1 – summary, principles and use. Copenhagen: Norden, 2013.
- Philips SM. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci* 2011; 29: 29–38.
- Reed JL, Bowell JL, Hill BR, Williams BA, De Souza MJ, Williams NI. Exercising women with menstrual disturbances consume low energy dense foods and beverages. *Appl Physiol Nutr Metab* 2011; 36: 382–394.
- Rodriguez NR, DiMarco NM, Langley S, Denny S, Hager MH, Manore MM. 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.
- Sjödin AM, Forslund AH, Westerterp KR, Andersson AB, Forslund JM, Hambraeus LM. The influence of physical activity on BMR. *Med Sci Sports Exerc* 1996; 28: 85–91.
- Stubbs RJ, 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 MK. Prevalence of eating disorders in elite athletes is higher than in the general population. *Clin J Sport Med* 2004; 14: 25–32.
- Thong FSL, McLean C, Graham TE. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. *J Appl Physiol* 2000; 88: 2037–2044.
- Tomten SE, Høstmark AT. Energy balance in weight stable athletes with and without menstrual disorders. *Scand J Med Sci Sports* 2006; 16: 127–133.
- Vernarelli JA, Mitchell DC, Rolls BJ, Hartman TJ. Methods for calculating dietary energy density in a nationally representative sample. *Procedia Food Sci*. 2013; 2: 68–74.