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## Energy availability and the female athlete triad in elite endurance athletes

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The female athlete triad (Triad), links low energy availability (EA), with menstrual dysfunction (MD), and impaired bone health. The aims of this study were to examine associations between EA/MD and energy metabolism and the prevalence of Triad-associated conditions in endurance athletes. Forty women [ $26.2 \pm 5.5$  years, body mass index (BMI)  $20.6 \pm 2.0$  kg/m<sup>2</sup>, body fat  $20.0 \pm 3.0\%$ ], exercising  $11.4 \pm 4.5$  h/week, were recruited from national teams and competitive clubs. Protocol included gynecological examination; assessment of bone health; indirect respiratory calorimetry; diet and exercise measured 7 days to assess EA; eating disorder (ED) examination; blood analysis. Subjects with low/reduced EA ( $< 45$  kcal/kg FFM/day), had lower resting metabolic rate (RMR) compared with those with optimal

EA [ $28.4 \pm 2.0$  kcal/kg fat-free mass (FFM)/day vs  $30.5 \pm 2.2$  kcal/kg FFM/day,  $P < 0.01$ ], as did subjects with MD compared with eumenorrheic subjects ( $28.6 \pm 2.4$  kcal/kg FFM/day vs  $30.2 \pm 1.8$  kcal/kg FFM/day,  $P < 0.05$ ). 63% had low/reduced EA, 25% ED, 60% MD, 45% impaired bone health, and 23% had all three Triad conditions. 53% had low RMR, 25% hypercholesterolemia, and 38% hypoglycemia. Conclusively, athletes with low/reduced EA and/or MD had lowered RMR. Triad-associated conditions were common in this group of athletes, despite a normal BMI range. The high prevalence of ED, MD, and impaired bone health emphasizes the importance of prevention, early detection, and treatment of energy deficiency.

The female athlete triad (Triad) is a syndrome-linking energy availability (EA) with reproductive function and bone health in exercising women (Drinkwater et al., 2005; Nattiv et al., 2007). Female athletes focusing on leanness have been reported to have an increased risk for low EA (Manore et al., 2007), associated with changes in the endocrine system affecting energy and bone metabolism, as well as in the cardiovascular and reproductive systems (Warren, 2011). Most female athletes have a body weight and body composition within the normal range, independent of their reproductive function (Redman & Loucks, 2005), and body weight seems to be preserved during long-term energy deficiency, involving several metabolic mechanisms, such as a reduction in energy metabolism at rest (RMR) and in nonexercise activity thermogenesis (NEAT) (Redman et al., 2009). Menstrual dysfunctions (MD) are common among female athletes, but are often ignored and regarded as a natural result of intense training, despite the fact that negative health consequences, including an increase in a

number of cardiovascular risk factors and the risk of premature osteoporosis, are well documented (Nattiv et al., 2007). The hormonal synthesis, follicular development, endometrial proliferation and increased luteal phase thermogenesis are energy-consuming processes (Harber, 2004) and RMR changes up to 10% during the menstrual cycle are often seen (Henry et al., 2003). MD, therefore, have energy-preserving effects.

The Triad develops as a continuum of severity starting with optimal EA, eumenorrhea and good bone health. The healthy state is followed by subclinical conditions such as reduced EA with or without disordered eating behavior (DE), short luteal phase defect (LPD) or anovulation, and BMD below the expected range for age. The continuum ends with severe clinically overt conditions associated with low EA [ $< 30$  kcal/kg fat-free mass (FFM)/day], with or without eating disorders (ED), oligomenorrhea/functional hypothalamic amenorrhea (FHA) and osteoporosis as Z-score  $< -2$ , together with secondary risk factors for fracture (e.g., under nutrition,

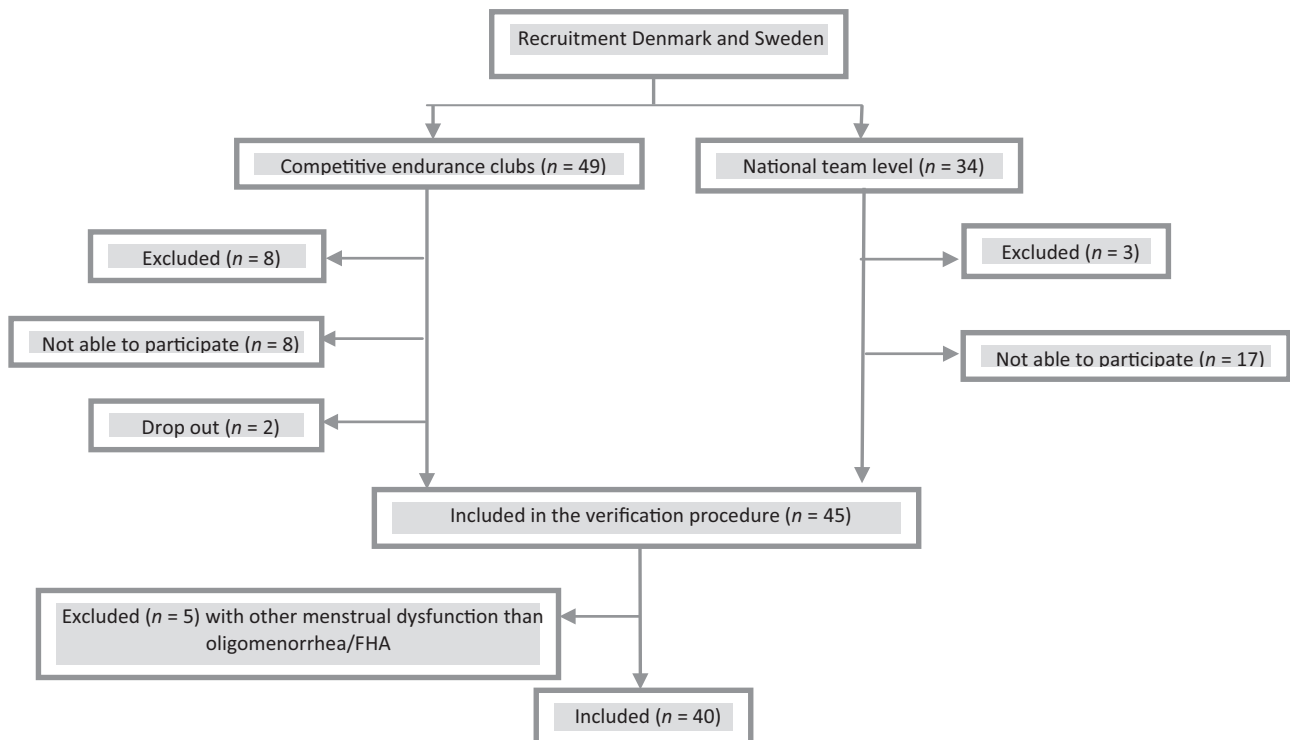
hypoestrogenism, prior fractures) (Nattiv et al., 2007). EA is defined as the ingested energy remaining for all other metabolic processes after the energy cost of training has been subtracted (Loucks & Thuma, 2003). In healthy young female adults, 5 days of EA < 30 kcal/kg FFM/day has been shown to reduce blood glucose levels (and thereby carbohydrate availability) and hypothalamic-pituitary-axis hormones, like triiodothyronine ( $T_3$ ), to suppress the pulsatility of gonadotropin-releasing hormone (GnRH), and luteal hormone (LH), and to elevate cortisol (Loucks & Thuma, 2003). There are several possible reasons for low EA in female athletes. It could be unintentional and primarily expenditure-driven because of difficulties in eating enough during periods of high-intensity training. Since weight influences performance in many sports, low EA can be due to intentional restriction of food in order to obtain a low body weight (Nattiv et al., 2007). This behavioral pattern seems frequent, and 24% of female elite endurance athletes have been reported as having Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnosed ED (Sundgot-Borgen & Torstveit, 2004). However, the prevalence of low EA among female athletes has been little investigated (Gibbs et al., 2013). We therefore performed an observational study with the aims of investigating, the potential effects of EA and reproductive function on

energy metabolism as well as the prevalence of the Triad and related clinical conditions in a group of female endurance athletes.

## Materials and methods

The study population was recruited through the Danish and Swedish sport federations in weight-bearing endurance sports, and in local competitive endurance sports clubs, through flyers, webpage announcements and mailings. Interested athletes received the study protocol and a self-administered questionnaire, assessing age, sport, training regime, and self-assessed menstrual function. Athletes returning the questionnaire and who were interested in further participation were contacted by the research group. 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).

The protocol was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov). Subjects included and defined as elite endurance athletes were athletes at national team levels or competitive endurance athletes from regional sports clubs, 18–38 years of age, training a minimum of five times per week. Exclusion criteria included clinically verified MD other than oligomenorrhea/FHA; pregnancy; chronic illness; smoking; use of forms of contraceptives other than oral, e.g., hormonal coil/patches; inability or being unwilling to stop hormonal contraceptives for at least 6 weeks prior to investigation; or injuries preventing the athlete from training  $\geq 2$  weeks. The recruitment procedure is illustrated in Fig. 1. Eighty-three athletes



**Fig. 1.** Flowchart inclusion. The figure illustrates the recruitment procedures; 83 athletes volunteered, 25 were unable to participate due to illness, competitions, long training camps or studies abroad etc., 11 were excluded, two dropped out, and 45 completed the study protocol. After completing the study protocol, five subjects were diagnosed with a menstrual dysfunction other than oligomenorrhea/functional hypothalamic amenorrhea (FHA), and 40 subjects were included in the final analyses.

volunteered, 25 were unable to participate because of illness, competitions, long training camps, or studies abroad, 11 were directly excluded (two had chronic illness, two had long-term injuries, three used hormonal coil, and four were not willing to stop with their hormonal contraceptives), two dropped out, and 45 completed the study protocol. After completing the study protocol, five subjects were diagnosed with an MD other than oligomenorrhea/FHA; three with poly cystic ovarian syndrome (PCOS) and therefore excluded, and 40 subjects were included in the final analyses; 13 national team level athletes and 27 from competitive endurance sports clubs.

## Methods

Data collection was performed on two consecutive days (Fig. 2) followed by a 7-day registration period in the athletes' normal environment. The first day comprised of examinations involving bone health, blood pressure (BP) and reproductive function. The second day included examinations of energy metabolism, aerobic capacity, as well as assessment of ED. The subjects were instructed not to exercise for more than 30 min at low or moderate intensity on the day before, and on the first day of examination, and to arrive at the clinic in a fasted (from midnight) and rested state.

### Energy availability and energy expenditure

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. Dietary intake and training intensity were recorded by the subjects for seven consecutive days to assess current EA. Energy intake was calculated from a prospective weighed (Exido 246030 Kitchen Scale, Gothenburg, Sweden) food record, using a nutrient analysis program, Dankost 2000 (Dankost, Copenhagen, Denmark) for Danish food items, and Dietist XP (Kost och Näringsdata AB, Bromma, Sweden) for Swedish food items. Subjects were given in-depth verbal and written instructions and a demonstration of how food and beverages should be weighed and registered. Subjects were instructed to maintain their 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, and the mean daily energy intake from the 7-day record was used in the analysis. In order to identify subjects who provided nutritional data of poor validity, the Goldberg cut-off was calculated using the equation described by [Black \(2000\)](#).

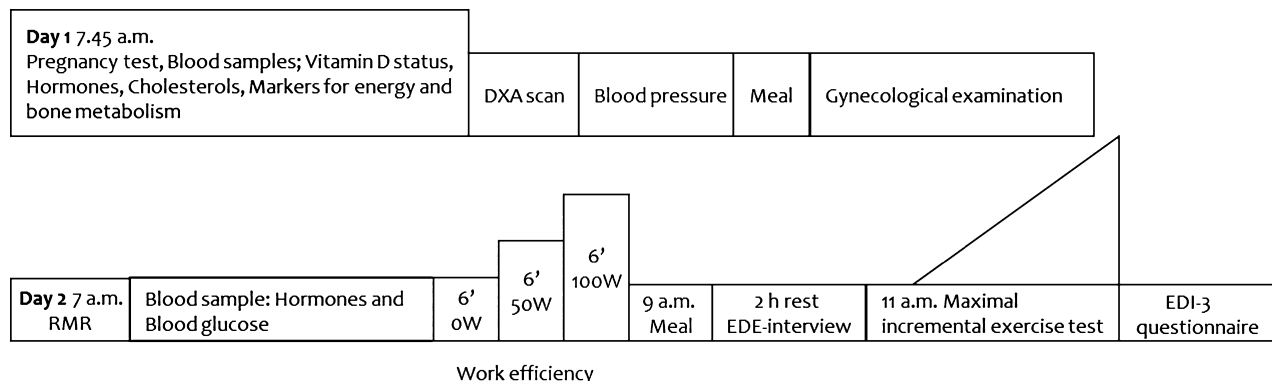
Seven subjects, five with DE/ED, were identified as having low validity of their energy intake [physical activity level (PAL) < 1.6]. Since they all had physiological symptoms of persistent energy deficiency, such as MD and/or a ratio between measured and predicted RMR ( $RMR_{ratio}$ ) < 0.90, we chose not to remove anyone from the analysis based on this cut-off point. Heart rate (HR) monitors (Polar RS400®, Stockholm, Sweden) and training logs were used to assess exercise energy expenditure (EEE). Subjects were instructed to maintain and to follow their normal training regime. They were, furthermore, instructed to describe each session in as much detail as possible, regarding type, duration, and intensity of exercise and to wear the HR monitor at all training sessions (except swimming) and during cycling (training as well as transportation). The mean daily EEE for the sessions described as exercise by the subjects, was used and the method for calculation of current EA is described under Calculations.

### Disordered eating behavior and eating disorders

Eating behavior was assessed using the Eating Disorder Inventory (EDI-3), a questionnaire assessing behavior and attitudes related to DE behavior, as well as to overt ED (12). Subjects were categorized as having DE behavior when the EDI risk subscale score for Drive for Thinness (DT) was  $\geq 14$ , and/or a Body Dissatisfaction (BD) risk score  $\geq 19$ , according to the classification by Garner (2004) and without the presence of DSM-IV diagnosed ED. The Eating Disorder Examination (EDE-16) ([Cooper et al., 1989](#)), a validated semi-structured interview was used to determine whether subjects met the criteria for ED, according to DSM-IV-criteria for anorexia nervosa, bulimia nervosa, and ED not otherwise specified (EDNOS). All interviews were performed by the same EDE-certified member of the research team.

### Energy metabolism

Subjects were transported by car to the clinic on the morning of the second test day in order to minimize physical activity prior to the measurement. RMR was assessed between 7:00 and 8:30 h, after an overnight fast, using a ventilated open hood system (Oxycon Pro 4, Jeager, Germany). The system was automatically calibrated before each test, and again weekly, by an alcohol burning test with coefficients of variability (CV) of 0.7% for  $O_2$ , 1.1% for  $CO_2$ , 0.8 % for the respiratory exchange ratio, and 2% for EE. After voiding, subjects laid down for 15 min before measurements of oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ) over 35 min (the equation is defined under Calculations). Work



**Fig. 2.** Protocol. The figure illustrates the protocol used during the data collection at day 1 at Herlev Hospital, Denmark and day 2 at the Health Clinic Lab at Lund University, Sweden. The subjects arrived in the fasted rested state both days. DXA, dual-energy X-ray absorptiometry; RMR, resting metabolic rate using ventilated hood; EDE-16, Eating Disorder Examination ([Cooper et al., 1989](#)); EDI-3, self-reported questionnaire Eating Disorders Inventory-3 (Garner, 2004).

efficiency was assessed by a standardized test protocol in the fasted state, initiated by the subject seated on the bicycle ergometer (Monark 939E, Monark Exercise AB, Vansbro, Sweden) for 6 min, followed by cycling for 6 min at 0W, 50W and 100W, respectively. An air-tight mask covering the mouth and nose was used in order to measure respiratory gas exchange (the equations for calculations are defined under Calculations). In order to calculate daily total EE, HR monitors (Polar RS400®) were used to assess EE during bicycle transportation, while actigraphy (ActiGraph GT3X®, Pensacola, FL, USA) and the data analysis software ActiLife 5 (ActiGraph) were used for assessment of NEAT. Subjects were instructed to wear an accelerometer on the wrist during sleep, and on the hip from getting up in the morning until bedtime, and only to take it off during showering, swimming, bicycle transportation, and training.

### Exercise capacity

Two hours after a standardized breakfast, an incremental exercise test on the bicycle ergometer was performed, initiated by cycling for 6 min at 50W, followed by an increase in workload of 12–14 W/min until exhaustion. An air-tight mask covering the mouth and nose was used in order to measure  $\text{VO}_{2\text{peak}}$  and respiratory exchange ratio, and HR (Polar RS400®) was measured.

### Reproductive function

Subjects using hormonal contraceptives were requested to stop for a minimum of 6 weeks prior to examination in order to secure a sufficient washout period for exogenous estrogen and progesterone. Subjects not recovering their menstrual bleeding within the 6 weeks were contacted monthly by the research team during a follow-up period of a minimum of 3 months before gynecological assessment. Menstruating athletes were examined in the early follicular phase, on the third to fifth day of menstruation. A pregnancy test was performed on arrival at the hospital, and menstrual function was examined by an experienced gynecologist who performed a transvaginal ultrasound examination (Ultrasound Scanner, Class 1 type B, B-K Medical REF TYPE 2202, Bedfordshire, UK). The maximum number of ovarian follicles present in a single plane was counted, and total volume was assessed. Sex hormone status [estrogen, progesterone, LH, follicle stimulating hormone (FSH), sexual hormone binding globulin (SHBG), prolactin, dehydroepiandrosteron sulfate (DHEA-S), androstendion, and total testosterone] 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 low EA in females questionnaire (LEAF-Q) (Melin et al., 2014). Subjects were then classified with eumenorrhea (menstrual cycles of 28 days  $\pm$  7 days and sex hormones within the normal range); oligomenorrhea (menstrual cycles > 35 days where other causes than hypothalamic suppression had been ruled out); FHA (either primary: no menarche after 15 years of age, or secondary: absence of at least three consecutive menstrual cycles where other causes than hypothalamic suppression had been ruled out) (Nattiv et al., 2007); other MDs (anatomic defects, hyperprolactinemia or other dysfunctional ovarian conditions); PCOS involving at least two of the following: (a) enlarged ovaries with a volume greater than 10 mL and/or  $\geq$  one ovary demonstrating  $\geq$  12 follicles in one plane; (b) irregular or absence of bleeding; and (c) elevated androgen level, or otherwise androgen stigmatized.

### Anthropometry, BP and bone mineral density

Body weight was measured with an accuracy of 0.01 kg in underwear on an electronic scale (Lindeltronic 8000, Samhall Lavi AB, Kristianstad, Sweden). Height was measured without shoes and

standing with legs together against a wall, using a fixed stadiometer (Hultafors AB, Hultafors, Sweden). After resting in a supine position for 7 min, HR and BP were measured three times (the mean was used) using an electronic sphygmomanometer (Microlife BP A100, Widnau, Switzerland). Hypotension was defined as a systolic BP < 90 mmHg and/or diastolic BP < 60 mmHg. Dual-energy X-ray absorptiometry (DXA) (Hologic, Model Discovery 2009, Hologic Inc., Waltham, MA, USA) was used to determine fat-free, fat, and bone mass, respectively. BMD was determined for whole body, lumbar spine (L1-L4) and hip. All measurements and scans were performed in the fasted and resting state between 7:30 and 9:00 h, and were assessed by the same technician, and performed on the same scanner. Calibration of the Hologic Discovery 2009 was performed weekly, using a phantom provided by the manufacturer. Subjects were classified as having normal BMD: Z-scores > -1 in all measured sites, low BMD: Z-score -1 to -2 in at least one site, and osteoporosis: Z-score < -2 in at least one site together with minimum one secondary risk factor such as low EA, ED and oligomenorrhea/FHA (Nattiv et al., 2007).

### Blood sampling

Blood samples were drawn following an overnight fast from an antecubital vein on the first as well as on the second day, between 8:30 and 8:50 h, in a rested state. Sex hormones and cholesterol were analyzed directly. The remaining blood samples were stored for a maximum of 1 h at 5 °C, centrifuged at 4 °C, 1500 g for 15 min, and serum was stored at -80 °C until analyzed. Capillary blood glucose was analyzed using Biosen C Line (EKF Diagnostic, Barleben, Germany) with a measurement range between 0.5–50 mmol/L and a CV of 1.5% at 5 mmol/L. Total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC) and triglyceride (TAG) were analyzed using VITROS Chemistry Products DT slides (Ortho-Clinical Diagnostics, Buckinghamshire, UK). Analytical sensitivity for TC, LDLC, HDLC and TAG were 1.3–8.4 mmol/L, 0.78–9.05 mmol/L, 0.13–2.84 mmol/L and 0.17–4.52 mmol/L, respectively. The intra- and inter-assay precision CV were 2.3%, 2.2%, 1.4%, 2.6% and 2.1%, 3.5%, 1.8%, 2.3%, respectively. Cortisol and IGF-1 were analyzed using Roche Electro Chemiluminescence Immunoassay (ECLI; Roche Diagnostic, Bromma, Sweden). Analytical sensitivity for the cortisol assay was 0.5–1750 nmol/L and for IGF-1, 0.03–50 µg/L. Assay precision CV was 1.3–2.1% and 2.0–2.5%, respectively. Insulin was analyzed using Access Ultrasensitive Insulin assay (Beckman Coulter, Bromma, Sweden) with an analytical sensitivity of 0.03–50 µg/L and an assay precision CV of 7–8%. Leptin was analyzed using Quantikine® ELISA (R&D Systems® Europe Ltd., Abingdon, UK). The lower analytic limit for leptin was 1.56 ng/mL; lower concentrations are associated with some uncertainty and therefore not automatically calculated. Seven subjects had results < 1.56 ng/mL, and in order to calculate their concentrations, the concentration was extrapolated using the formula for the standard curve:  $\log_{10}(\text{ABS}) = A * \log_{10}(\text{concentration}) - B \rightarrow \text{concentration} = 10^{(\log_{10}(\text{ABS}) + B)/A}$ . The intra- and inter-assay precision CV for leptin was 3.0–3.3% and 3.5–5.4%, respectively.  $T_3$  was analyzed using ARCHITECT system assay (Abbott Laboratories, Longford, Ireland) with an analytical sensitivity of  $\leq$  0.25–8 ng/mL and an intra-inter-assay precision CV of 4.1% and 6.0%, respectively. Estrogen, FSHLH, prolactin, progesterone, SHBG, androstendion, DHEA-S, and total testosterone were analyzed using ADVIA Centaur Immunoassay Systems (Siemens Healthcare Diagnostics Products GmbH, Siemens A/S, Ballerup, Denmark). The analytic sensitivity for estrogen (estradiol), FSH, LH, prolactin, progesterone, SHBG, androstendion, DHEA-S, and total testosterone were 43.6–11 010 pmol/L, 0.3–200 IU/L, 0.07–200 IU/L, 6.4–4240 µIU/mL, 0.1–100 nmol/L, 0.1–250 nmol/L, 0.3–100 nmol/L, 35–15 000 nmol/L, and 0.1–100 nmol/L, respectively. The intra- inter-assay precision CV for



estrogen was 2.6% and 4.1%; for FSH, 1.2% and 2.0%; for LH, 2.9% and 2.3%; and for prolactin, 2.0% and 2.3%. The intra–inter assay precision CV for progesterone was 9% and 10%; for androstendion, 8% and 9%; for DHEA-S, 9% and 10%; and for total testosterone, 9% and 10%. SHBG levels were determined by immunofluorimetric assays. For SHBG, the sensitivity and the intra- and inter-assay coefficients of variation were 0.1 nmol/L, 4% and 6%, respectively.

## 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  $\pm$ SD or as mean and 95% CI, and non-normally distributed data by median and interquartile range (IQ 25 and IQ 75). In order to investigate the interrelationship between Triad conditions, but also with regard to energy metabolism and other Triad-related conditions, the subjects were divided according to their current level of EA: low EA ( $< 30$  kcal/kg FFM/day) (Loucks & Thuma, 2003) and reduced EA was defined as EA between 30.0 and 44.9 kcal/kg FFM/day since it is the level recommended to athletes during weight reduction (Loucks et al., 2011). Healthy eumenorrheic sedentary women have been reported to be in energy balance at EA  $\geq 45$  kcal/kg FFM/day (Loucks & Thuma, 2003), defining optimal EA in this study in order to compare results earlier reported by Hoch et al. (2009). For comparison of the mean levels of the descriptive details between the groups, a one-way analysis of variance (ANOVA), with Bonferroni correction for multiple group comparisons was used. To measure the degree of positive or negative association between continuous outcomes, Pearson's correlation coefficient ( $r$ ) was calculated. To test whether there was a difference between the two kinds of classification, e.g., the number of eumenorrheic subject vs subjects with oligomenorrhea/FHA having DE/ED; Fisher's exact test was applied. For comparison of means between subjects with and without DE/ED, as well as with or without MD, Student's paired  $t$ -test was used. To estimate the within-subject variability for energy intake, EEE and EA the pooled variance was calculated. Prior to analysis, the skewed data were logarithmically transformed, and the statistical significance was defined as  $P < 0.05$ .

## Calculations

Body mass index (BMI) was calculated as weight (kg) divided by height squared ( $m^2$ ). RMR was calculated from mean  $VO_2$  and  $VCO_2$  during the last 20 min of the measurement, using the Weir equation (Weir, 1990):  $3.94 (VO_2) + 1.1 (VCO_2) \times 1.44$ . The ratio between measured RMR and predicted RMR was calculated using Cunningham's equation (Cunningham, 1980). Subjects were defined as having low RMR when  $RMR_{ratio}$  was  $< 0.90$  (De Souza et al., 2008). Calculation of EEE during training was based on individual prediction equations from measured HR and corresponding EE during the incremental maximal exercise test in the laboratory. The individual equation provided the basis for the calculation of EEE, using the training log and HR measurement for each training session (Tomten & Hostmark, 2006). Regression lines were calculated for corresponding values of HR and EE during the exercise test in the laboratory, and for the recorded HR during all exercise sessions. HR were strongly linearly correlated with  $O_2$  consumption at increasing workloads ( $r = 0.94$ ), 95% CI 0.93–0.96 and EE from 7 days of training was averaged to determine EEE for each subject. NEAT was derived by calculating EE from accelerometers monitoring bodily movements providing activity counts (counts/min) using the data analysis software ActiLife 5 (ActiGraph) and EE during cycling (exercise as well as transportation) was calculated in the same way as EEE. EA was

calculated by subtracting mean EEE from the mean energy intake then calculated relative to FFM. In order not to overestimate EEE and thereby underestimate EA, EEE was corrected for the mean total EE without EEE during the equivalent time period. Power generated during cycling (W) was converted to kcal/min. Work efficiency was calculated by subtracting EE (kcal/min) at 0 W from EE (kcal/min) at 100 W, divided by the power generated during cycling at 100 W (kcal/min) and expressed as a percentage (work efficiency<sub>100W</sub> % =  $[(EE_{100W} - EE_{0W}) / \text{power}_{100W}] \times 100$ ).

## Results

### Energy availability, energy expenditure and eating disorders

Fifteen subjects had optimal current EA, while 17 had reduced EA, and eight had low EA. Subject characteristics divided by current EA are presented in Table 1. There were no differences in age, anthropometric measures, or maximal aerobic capacity ( $VO_{2peak}$  l/min or mL/kg/min) between the three groups divided by EA. There was a trend towards more training hours/week ( $P = 0.06$ ) in the group with low current EA compared with the group with optimal current EA. Subjects with low current EA had 22% and 32% lower energy intake compared with the groups with reduced EA and optimal EA, respectively. Furthermore, subjects with low EA had 79% higher EEE when compared with subjects with optimal EA and there was a negative association between EA and daily exercising duration (h/week;  $r = -0.38$ ,  $P = 0.019$ ). Subjects with low EA had 50% and 63% lower EA compared with the groups with reduced EA and optimal EA, respectively (Table 2). No differences in total EE or NEAT between the three EA groups were found. The within-subject variability for daily energy intake, EEE and EA was  $\pm 348$  kcal/day,  $\pm 521$  kcal/day and  $\pm 13.2$  kcal/kg FFM/day, respectively, for subjects with low EA. We found this to be  $\pm 427$  kcal/day,  $\pm 539$  kcal/day and  $\pm 13.2$  kcal/kg FFM/day for subjects with reduced EA, and similarly  $\pm 409$  kcal/day,  $\pm 386$  kcal/day  $\pm 12.4$  kcal/kg FFM/day for subjects with optimal current EA.

Ten subjects were diagnosed with ED (one anorexia nervosa, one bulimia nervosa, and eight EDNOS) and one was additionally characterized as having DE. There were no differences in the EDI-3 DT or BD risk score between the groups (Table 1), and a sub-analysis of EA between subjects with or without DE/ED revealed no difference [ $36.7 \pm 13.2$  kcal/kg FFM/day vs  $40.7 \pm 13.5$  kcal/kg FFM/day ( $P = 0.51$ )].

### Energy metabolism

Twenty-one subjects (53%) had low RMR ( $RMR_{ratio} < 0.90$ ). RMR (kcal/kg FFM/day) and  $RMR_{ratio}$  were lower in the subjects with low/reduced current EA compared with those with optimal current EA [ $28.4 \pm 2.0$  kcal/kg FFM/day vs  $30.5 \pm 2.2$  kcal/kg FFM/day ( $P = 0.004$ ), and  $0.87 \pm 0.07$  vs  $0.93 \pm 0.07$  ( $P = 0.017$ )]. RMR was

Table 1. Descriptive details and Triad conditions in all subjects and divided by current energy availability

Variable	All ( <i>n</i> = 40)	Optimal EA ( <i>n</i> = 15)	Reduced EA ( <i>n</i> = 17)	Low EA ( <i>n</i> = 8)
Age (year)	26.3 ± 5.7	26.9 ± 6.0	26.2 ± 6.3	25.5 ± 3.9
Height (cm)	168.9 ± 0.05	166.7 ± 0.04	169.6 ± 0.06	171.4 ± 0.03
Weight (kg)	58.4 ± 6.9	56.8 ± 6.3	58.7 ± 7.3	60.7 ± 7.1
BMI (kg/m <sup>2</sup> )	20.5 ± 1.9	20.4 ± 1.7	20.4 ± 2.1	20.6 ± 2.1
Body fat (kg) <sup>†</sup>	11.9 ± 3.2	11.5 ± 2.6	11.9 ± 3.0	12.7 ± 4.7
Relative FM (%) <sup>†</sup>	20.0 ± 3.5	20.0 ± 3.0	19.8 ± 3.4	20.5 ± 5.1
FFM (kg)	46.1 (43.1–50.7)	44.1 (41.1–49.5)	46.4 (42.9–50.7)	47.9 (45.5–51.7)
Heart rate (beats/min)	46.3 ± 8.0	47.0 ± 9.0	45.8 ± 8.5	45.8 ± 8.2
BP systolic (mmHg)	113.1 ± 11.2	115.2 ± 9.0	114.8 ± 11.7	105.7 ± 11.7
BP diastolic (mmHg)	67.5 ± 9.5	70.8 ± 9.6	67.1 ± 9.2	62.6 ± 8.7
Exercise (h/week)	11.4 ± 4.5	9.6 ± 2.8	11.8 ± 4.6	14.1 ± 5.8(*)
VO <sub>2peak</sub> (L/min)	3.16 ± 0.4	3.15 ± 0.4	3.15 ± 0.4	3.20 ± 0.5
VO <sub>2peak</sub> (mL/kg/min)	55.4 (49.1–59.0)	55.7 (51.4–58.8)	55.0 (50.0–57.2)	52.1 (45.6–63.0)
DT risk score	4.0 (1.0–9.0)	4.0 (0.0–9.0)	3.0 (2.0–7.0)	17.0 (2.0–22.0)
BD risk score	5.0 (2.0–10.0)	5.0 (2.0–10.0)	5.5 (2.5–8.0)	12.5 (3.0–31.0)

Data are presented as mean ±SD for normal distributed data and as median and interquartile range (25–75) for skewed data.

(\*)*P* = 0.06 compared with subjects with optimal current EA.

<sup>†</sup>Determined by DXA.

BD, body dissatisfaction from Eating Disorder Inventory-3; BMI, body mass index; BP, blood pressure; DT, drive for thinness; EA, energy availability; FFM, fat-free mass; FM, fat mass; VO<sub>2peak</sub>, maximal oxygen uptake

Table 2. Energy intake, expenditure, and metabolism in all subjects and divided by current energy availability

Variable	All ( <i>n</i> = 40)	Optimal EA ( <i>n</i> = 15) mean (95% CI)	Reduced EA ( <i>n</i> = 17) mean (95% CI)	Low EA ( <i>n</i> = 8) mean (95% CI)
Energy intake (kcal/day)	2580 [2398–2761]	2943 [2718–3168]	2540 [2311–2769]	1983 [1511–2454]**
EA (kcal/kg FFM/day)	39.6 [35.3–43.9]	51.7 [48.1–55.3]	38.5 [36.3–40.5]**	19.10 [11.6–26.6]***
Energy balance (%)	87.5 [81.8–93.3]	102.5 [97.2–107.9]	87.3 [82.6–91.9]**	60.1 [51.5–68.6]***
Total EE (kcal/day)	2972 [2817–3126]	2865 [2702–3028]	2927 [2659–3195]	3266 [2755–3778]
NEAT (kcal/day)	462 [409–515]	459 [380–538]	457 [380–534]	478 [275–680]
EEE (kcal/day)	879 [743–1015]	711 [599–822]	867 [642–1093]	1222 [768–1675]*
RMR (kcal/day)	1372 [1319–1426]	1401 [1319–1483]	1349 [1253–1444]	1368 [1225–1512]
RMR (kcal/kg FFM/day)	29.2 [28.5–30.0]	30.5 [29.3–31.8]	28.5 [27.5–29.6]*	28.3 [26.5–30.0](*)
RMR <sub>ratio</sub>	0.89 [0.87–0.92]	0.93 [0.89–0.97]	0.87 [0.84–0.91](*)	0.87 [0.81–0.94]
Work efficiency 100W (%)	20.3 [20.0–20.6]	20.2 [19.7–20.7]	20.2 [19.7–20.8]	20.4 [20.0–20.9]

\**P* < 0.05 and \*\**P* < 0.01, (\*) *P* = 0.06, (\*) *P* = 0.08 compared with subjects with optimal current EA. #*P* < 0.05 and ##*P* < 0.01 compared with subjects with reduced current EA.

EA, energy availability; EE, energy expenditure; EEE, exercise energy expenditure; FFM, fat-free mass; energy balance, (energy intake/total energy expenditure × 100); NEAT, non-exercise activity thermogenesis; RMR, resting metabolic rate; RMR<sub>ratio</sub>, the ration between the predicted and measured RMR using the Cunningham equation.

7% and RMR<sub>ratio</sub> 6% lower for the groups with low and reduced EA, as compared with the group with optimal EA, and there was a positive association between EA and RMR<sub>ratio</sub> (*r* = 0.31, *P* = 0.047). There were no differences in RMR or RMR<sub>ratio</sub> between subjects with or without DE/ED [29.2 ± 2.3 kcal/kg FFM/day vs 29.2 ± 2.4 kcal/kg FFM/day (*P* = 0.94) and 0.91 ± 0.09 vs 0.89 ± 0.07 (*P* = 0.43), respectively], while subjects with MD had lower RMR [28.6 ± 2.4 kcal/kg FFM/day vs 30.2 ± 1.8 kcal/kg FFM/day (*P* = 0.029)] and RMR<sub>ratio</sub> [0.87 ± 0.08 vs 0.93 ± 0.06, (*P* = 0.007)], compared with the eumenorrheic subjects. Subjects with MD and optimal current EA (*n* = 8) had higher RMR and RMR<sub>ratio</sub> compared with subjects with MD and reduced/low current EA (*n* = 16) [30.5 ± 2.4 kcal/kg FFM/day vs 27.6 ± 1.9 kcal/kg FFM/day (*P* = 0.004), and 0.92 ±

0.08 vs 0.84 ± 0.06, (*P* = 0.013)]. No difference in work efficiency between the three EA groups was found but there was a trend towards a higher work efficiency in subjects with MD compared with eumenorrheic subjects [20.5 ± 1.0 % vs 19.9 ± 0.7%, (*P* = 0.059)].

## Reproductive function

Sixty percent (*n* = 24) were diagnosed with MD: oligomenorrhea (*n* = 6), primary FHA (*n* = 4), and secondary FHA (*n* = 14). Twenty-five percent of the subjects with MD (six of 24) had DE/ED and 67% (16 of 24) had low/reduced current EA. A sub-analysis showed no difference in current EA between subjects with MD and eumenorrhea [38.6 ± 13.0 kcal/kg FFM/day vs 41.1 ± 14.2 kcal/kg FFM/day (*P* = 0.56)].

### Bone health

Forty-five percent ( $n = 18$ ) had impaired bone health. Three subjects were diagnosed with osteoporosis in the lumbar spine and 15 had low BMD, 14 in the lumbar spine, and three subjects had additional low BMD at the whole-body level, and two had additional low BMD in the hip. One subject had low BMD at whole-body level and in the hip. Sixty-seven percent of the subjects with impaired bone health had MD, and 33% had DE/ED. There was a negative association between exercise (h/week) and whole body, as well as lumbar spine BMD and lumbar spine Z-scores [ $r = -0.32$  ( $P = 0.035$ ),  $r = -0.33$  ( $P = 0.037$ ),  $r = -0.36$ , ( $P = 0.023$ ), respectively]. There was no difference in current EA between subjects with normal or impaired bone health [ $39.6 \pm 14.1$  kcal/kg FFM/day vs  $39.5 \pm 12.8$  kcal/kg FFM/day ( $P = 0.97$ )].

### Biomarkers for energy deficiency

Potential indicators of energy deficiency are presented in Table 3. Subjects with low/reduced current EA had lower LH compared with the group with optimal EA [ $3.5 \pm 2.3$  IU/L vs  $6.6 \pm 3.6$  IU/L ( $P = 0.009$ )]. Thirty-one subjects (78%) had low leptin ( $< 3.88$  ng/mL), and there were positive associations between leptin and total and relative fat mass [ $r = 0.86$  ( $P < 0.001$ ),  $r = 0.81$  ( $P < 0.001$ ), respectively]. There were no differences in leptin, or leptin related to fat mass, between the three EA groups. A sub-analysis showed no difference in leptin levels between subjects divided by eumenorrhea and MD or with and without DE/ED. One subject, with low EA, EDNOS, and FHA, had a low  $T_3$  ( $< 1.2$  nmol/L). One subject, with reduced EA, FHA, and low BMD had elevated cortisol ( $> 800$  mmol/L), and 15 subjects had hypoglycemia ( $< 4$  mmol/L). There were no differences

in  $T_3$ , cortisol or glucose levels between the three EA groups, or between subjects with or without DE/ED. Subjects with MD had lower  $T_3$  ( $1.53 \pm 0.05$  nmol/L vs  $1.69 \pm 0.07$  nmol/L,  $P < 0.046$ ), lower fasting glucose ( $3.94 \pm 0.47$  mmol/L vs  $4.39 \pm 0.35$  mmol/L,  $P = 0.003$ ) and higher cortisol ( $504 \pm 126$  mmol/L vs  $400 \pm 140$  mmol/L,  $P = 0.021$ ), compared with eumenorrheic subjects.

Fifteen subjects (38%) had elevated TC levels ( $\geq 5$  mmol/L) and 10 (25%) had elevated LDLC ( $\geq 3$  mmol/L). There was a trend ( $P = 0.053$ ) toward a higher TC level in the group with low current EA compared with the group with optimal EA, but all ratios between LDLC and HDLC were within the normal range (Table 3). There was a trend ( $P = 0.071$ ) toward a higher TC in subjects with DE/ED compared with subjects without DE/ED, while there was no differences in TC levels between eumenorrheic subjects and subjects with MD.

Seven subjects had hypotension and more subjects with low current EA (four of eight) as compared with those with reduced EA (two of 17) and optimal EA (one of 15), had hypotension ( $P = 0.022$ ), although there were no differences in BP between the three EA groups (Table 1). Subjects with MD and optimal current EA had higher systolic and diastolic BP compared with those with MD and low/reduced current EA [ $117 \pm 8$  mmHg vs  $107 \pm 11$  mmHg ( $P = 0.026$ ) and  $74 \pm 10$  mmHg vs  $64 \pm 8$  mmHg ( $P = 0.013$ )].

### Triad and associated conditions

The prevalence of Triad conditions, low RMR, hypoglycemia, hypercholesterolemia, and hypotension are illustrated in Fig. 3. Thirteen subjects had no clinical Triad condition. When merging clinical and subclinical

Table 3. Potential biomarkers of energy deficiency in all subjects and divided by current energy availability

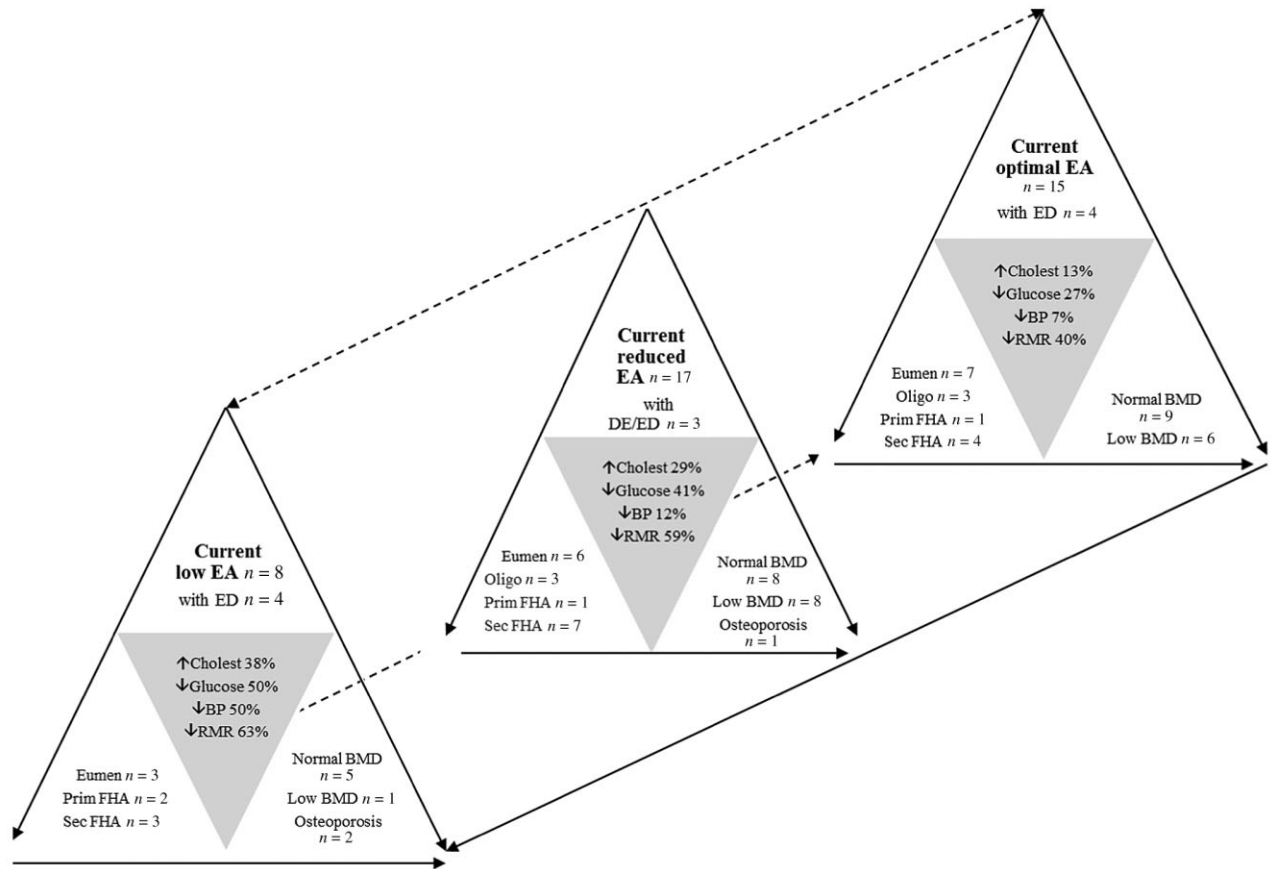
Variable	All ( $n = 40$ )	Optimal EA ( $n = 15$ )	Reduced EA ( $n = 17$ )	Low EA ( $n = 8$ )	Normal range provided by the analytic laboratory
Luteal hormone (IU/L)	4.05 [2.50–6.10]	5.30 [4.90–9.70]	3.40 [1.40–4.90]*	2.50 [2.30–3.65](*)	1.6–9.0
Leptin (ng/mL)	2.38 [1.46–3.63]	1.97 [1.48–5.18]	2.14 [1.43–3.70]	3.06 [1.47–3.17]	3.88–77.27
Leptin (ng/mL/kg FM)	0.20 [0.15–0.29]	0.22 [0.15–0.34]	0.18 [0.15–0.29]	0.21 [0.15–0.26]	
$T_3$ (nmol/L)	$1.60 \pm 0.26$	$1.64 \pm 0.29$	$1.59 \pm 0.17$	$1.51 \pm 0.35$	1.2–2.8
Cortisol (mmol/L)	$464 \pm 139$	$445 \pm 110$	$456 \pm 173$	$514 \pm 103$	200–800
Insulin (mIE/L)	3.0 [2.4–4.4]	3.2 [2.6–4.6]	3.2 [2.4–4.4]	2.7 [2.1–3.1]	$< 25$
IGF-1 ( $\mu$ g/L)	224 [197–262]	215 [194–257]	245 [202–262]	217 [176–265]	114–480
Fasting glucose (mmol/L)	$4.12 \pm 0.48$	$4.08 \pm 0.38$	$4.22 \pm 0.43$	$3.99 \pm 0.72$	$\geq 4.0$
TC (mmol/L)	4.5 [3.9–5.3]	4.0 [3.8–5.1]	4.6 [4.1–5.9]	5.1 [4.7–6.2](*)	$< 5.0$
LDLC (mmol/L)	2.3 [1.9–3.0]	2.2 [1.8–2.5]	2.3 [1.9–3.0]	2.5 [2.3–3.7]	$< 3.0$
HDLC (mmol/L)	$1.8 \pm 0.3$	$1.7 \pm 0.4$	$1.8 \pm 0.3$	$2.0 \pm 0.2$	1.0–2.7
LDLC/HDLC cholesterol ratio	1.3 [1.1–1.8]	1.3 [1.0–1.8]	1.4 [1.1–1.7]	1.3 [1.1–1.9]	$< 3.0$
Triglycerides (mmol/L)	0.66 [0.56–0.83]	0.59 [0.55–0.72]	0.67 [0.61–0.81]	0.75 [0.56–1.01]	0.4–2.6

Data are presented as mean  $\pm$  SD for normal distributed data and as median and interquartile range (25–75) for skewed data.

\* $P < 0.05$  and (\*)  $P = 0.05$  difference compared with subjects with optimal EA.

EA, energy availability; FM, fat mass; HDLC, high-density lipoprotein cholesterol; IGF-1, insulin growth factor-1; LDLC, low-density lipoprotein cholesterol;  $T_3$ , triiodothyronine; TC, total cholesterol.





**Fig. 3.** Current energy availability, Triad and related conditions in female endurance athletes. The figure illustrates the prevalence of low, reduced and optimal current energy availability (EA) and the distribution of disordered eating behavior (DE) or eating disorders (ED), reproductive function, bone health, hypercholesterolemia ( $\uparrow$ Cholest), hypoglycaemia ( $\downarrow$ Gluc), hypotension ( $\downarrow$ BP), and low RMR<sub>ratio</sub> ( $\downarrow$ RMR). Eumen, eumenorrhea; Oligo, oligomenorrhea; Prim FHA, primary functional hypothalamic amenorrhea; Sec FHA, secondary FHA; BMD, bone mineral density. Among the 15 subjects with optimal current EA; four subjects with ED also had oligomenorrhea/FHA and/or low BMD; 2 with hypercholesterolemia also had secondary FHA and at least one other condition; four with hypoglycemia also had oligomenorrhea/FHA and/or low BMD; one with hypotension also had ED and low BMD; four of six with low RMR also had oligomenorrhea/FHA. Among the nine eumenorrheic subjects with low/reduced current EA; three had low BMD, three had hypercholesterolemia and one had low RMR.

Triad conditions, four subjects had no Triad condition. Four subjects had no other Triad condition than low/reduced current EA and they also had normal RMR, BP, glucose, and TC levels. Twenty subjects had one clinical condition (low current EA with or without ED/MD/osteoporosis), six displayed two conditions, while one subject displayed all three clinical Triad conditions. The presence of subclinical MD was not assessed in this study, but of the subclinical conditions investigated (reduced current EA with or without DE or low BMD), 15 subjects had one, and eight subjects had both conditions. When merging clinical and subclinical conditions, 14 subjects (35%) had one condition, 13 (32%) had two, and nine (23%) displayed all three Triad conditions. All subjects with hypercholesterolemia, hypotension and/or hypoglycemia had at least one Triad condition, while all but two subjects with low RMR had at least one Triad condition.

## Discussion

This study is the first to examine the prevalence of low/reduced EA assessed during a single week and its interrelationship with the additional Triad and related conditions, simultaneously with potential adaptations in energy metabolism in a group of elite female endurance athletes. Sixty-three percent of the women in this study were found to have low or reduced current EA, while 28% were diagnosed with DE/ED. Sixty percent had MD, and 45% had impaired bone health. Subjects, with low/reduced current EA, as well as those with MD, showed signs of metabolic adaptation in the form of lower RMR. Triad and associated conditions such as low RMR, hypoglycaemia, hypotension, and hypercholesterolemia were common in this group of endurance athletes, despite a normal BMI range. The high prevalence of ED, MD, and impaired bone health emphasizes the

importance of prevention, early detection, and treatment of any of these associated conditions.

### Energy availability and eating disorders

The prevalence of low/reduced current EA ( $< 45$  kcal/kg FFM/day) was high in this group of endurance athletes (63%), compared with the 36% found in high school athletes from a variety of sports, and the 39% in sedentary controls as reported by Hoch et al. (2009), and our results indicate both intake-related and expenditure-driven causes. 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 & Torstveit, 2004) using the same method of careful diagnostic workout. EDNOS was the most common ED in the present study (20%), compared with 10% in Norwegian endurance athletes, when merging the number of athletes diagnosed with EDNOS (5%) and anorexia athletica (5%) in the study by Sundgot-Borgen and Torstveit (2004). Among the Norwegian endurance athletes, the more severe diagnose bulimia nervosa was the most common (10%; Sundgot-Borgen & Torstveit, 2004), compared with only 2.5% in the present study, while the reported prevalence of anorexia nervosa was low in Norwegian endurance athletes (4%; Sundgot-Borgen & Torstveit, 2004), as well as in the Swedish and Danish endurance athletes in the present study (2.5%). In the present study, only one out of 10 athletes with EDNOS (one with low current EA) used to vomit in order to regulate her weight, while no other pathological techniques such as diuretics or laxatives were used. Normally, one would assume that athletes diagnosed with EDNOS would have persistent reduced or low EA unless they reported better diets or practiced less restricted eating behavior during the registration period because they were being monitored. The fact that only one of the athletes met the criteria for anorexia nervosa might contribute to the explanation of the reported EA results, and we know that also athletes with EDNOS and bulimia nervosa might differ from day to day when it comes to energy intake (Torstveit and Sundgot-Borgen, 2012). Compulsive exercise was, furthermore, a more pronounced diagnostic characteristic trait for these four subjects, than, e.g., restricted eating behavior and dissatisfaction with body weight, which could explain why they would “allow” themselves a higher dietary intake as long as they felt that they exercised “enough.” Only seven of the 25 subjects with reduced or low EA had DE/ED in the present study. This observation indicates that diagnosis of DE/ED is not a sufficiently sensitive clinical indicator of current energy deficiency.

There are methodological dilemmas associated with assessment of EA in female athletes, such as the ability to discriminate between DE or restricted eating behavior and underreporting when performing nutritional surveys, and there could also be problems associated with

calculating EEE. Seven subjects, four with ED, were identified as having low validity of their energy intake according the Goldberg cut-off, using the equation described by Black (2000). We chose, however, not to remove anyone from the analysis based on this cut-off point since they all had physiological symptoms of persistent energy deficiency.

The lack of difference in BMI and body composition between the three groups of EA could potentially be explained by the fact that measurements over one week only provide a “snapshot” and not necessarily the long-term EA. In this study, however, 72% of the subjects with low or reduced EA had low RMR and or MD indicating energy-preserving adaptations that are coupled to the present EA status. Similar findings regarding lack of difference in BMI has also been reported in recreationally active women categorized as either energy replete or energy deficient using the  $RMR_{ratio}$  (De Souza et al., 2008). Furthermore, athletes with ED have been found to be underweight, have normal weight or even be overweight (Torstveit and Sundgot-Borgen, 2012) and most studies have found no difference in body composition between eumenorrheic athletes and athletes with MD (Loucks, 2006).

In the clinical study by Loucks and Thuma (2003), investigating the dependence of LH pulsatility on EA in eumenorrheic sedentary women, the habitual mean energy intake in these weight stable subjects was  $48 \pm 7$  kcal/kg FFM/day, and therefore, an EA of 45 kcal/kg FFM/day was administered as an estimation of an average energy intake in order to reach energy balance. There is no consensus regarding the cut-off for optimal EA in female athletes. Since athletes are recommended to follow a diet and exercise regimens that provide EA of 30-45 kcal/kg FFM/day in order to lose weight (Loucks et al., 2011), we chose to define the level of optimal EA as  $\geq 45$  kcal/kg FFM/day in the present study as in the study by Hoch et al. (2009). The cut off for the clinical level of low EA in the Triad has been defined as  $< 30$  kcal/kg FFM/day (Loucks & Thuma, 2003) and it has been shown that there are both age-dependent and individual differences in the impact of low current EA on the endocrine function (Loucks, 2006). In the present study, subjects with reduced current EA had lower LH levels compared with those with optimal current EA, and as many as 59% had low RMR, indicating that these changes may occur already at EA  $\geq 30$  kcal/kg FFM/day in free-living subjects.

### Energy metabolism

The mean RMR in subjects with optimal current EA ( $30.5 \pm 2.2$  kcal/kg FFM/day) and in the eumenorrheic subjects ( $30.2 \pm 2$  kcal/kg FFM/day) were similar to the level earlier reported in eumenorrheic endurance athletes (O'Donnell et al., 2009), even though the subjects in the present study were asked to restrain from hard and/or

prolonged exercise for approximately 60 h prior to the RMR assessment. The group with optimal current EA had 7% higher RMR than the groups with reduced or low EA and similar to the 6% higher RMR found in the eumenorrheic subjects compared with those with MD. De Souza et al. (2008) reported 7% higher RMR in eumenorrheic recreationally active women compared with those with secondary FHA, diagnosed by consistently suppressed estrogen levels across the study period. These findings are similar to ours, despite the fact that the group of athletes with MD in the present study consisted of a mix of oligomenorrhea, primary and secondary FHA. O'Donnell et al. (2009) reported a 12% higher RMR in eumenorrheic endurance athletes measured in the follicular phase compared with athletes with MD. The relatively small difference in RMR between subjects with or without MD in the present study could, therefore, be due to the fact that 50% of the eumenorrheic subjects had low or reduced EA, while 33% of the subjects with MD had current optimal EA. 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 (Goldsmith et al., 2010). We did not find any difference in work efficiency between the three groups divided by current EA. We did, however, find a trend toward increased work efficiency in athletes with MD, indicating a potential energy-preserving effect not only during rest.

### Menstrual dysfunction

MD is common among female athletes, and ranges from anovulation and LPD to oligomenorrhea/FHA (Nattiv et al., 2007). The 60% prevalence of clinical MD in our study is similar to the 64% among endurance athletes reported by Pollock et al. (2010), and to the 54% reported in high school athletes by Hoch et al. (2009). Hormonal contraceptives are commonly used as treatment for MD, but the effectiveness for improving BMD is inconclusive (Manore et al., 2007). Restoring bleeding with hormonal contraceptives may therefore give the illusion of normality, while underlying adverse endocrine factors partly remain. Fifteen (38%) of our subjects used hormonal contraceptives before inclusion in the study. Eight of them had MD, and two of these were not aware of their MD. We have earlier reported that self-reported MD is a valid predictor for verified clinical MD in this group of female endurance athletes (Melin et al., 2014) and according to self-reported data, 44% of the presently eumenorrheic subjects had previously experienced MD. Consequently, in total, 77% of all women in this study had at one point or another experienced MD. Anovulation and LPD have been reported to be as high as 50% in exercising women (De Souza et al., 2010). Research by De Souza et al. (2010) has revealed that in order to accurately detect these subclinical MD, sex hormones must be measured serially throughout the men-

strual cycle. The lack of such repeated measurements is a limitation in this study, and some of our eumenorrheic subjects could have undetected, subclinical MD (De Souza et al., 2010). Menstrual cycles longer than 35 days are not unusual for women in the first decade after menarche and may, therefore, not necessarily constitute oligomenorrhea (Redman & Loucks, 2005). In the present study, however, only two of the oligomenorrheic subjects had a gynecological age  $\leq 10$  years and both reported not having experienced abnormal menstrual cycles earlier.

In the present study, MD occurred at all three levels of current EA and we did not find any differences in current EA between subjects with MD compared with eumenorrheic subjects. We did, however, find lower LH in subjects with low/reduced current EA compared with subjects with optimal current EA, a finding supported by results reported from the clinical study by Loucks which showed that only 5 days with low EA reduces LH pulsatility in eumenorrheic subjects (Loucks & Thuma, 2003). Subjects with MD and optimal current EA had higher RMR compared with subjects with MD and reduced and low current EA, supporting our findings of optimal current EA despite MD. Potential fluctuations in EA that can be due to fluctuations in energy intake as well as in EEE probably occur during the month, the season, and the year. Short-term effects of EA are more likely to affect outcomes such as RMR (Redman et al., 2009) and LH (Loucks & Thuma, 2003). For outcomes such as MD and bone health, it is clearly not well coupled to the 7-day EA provided in this study, but instead to the long-term EA effect.

### Impaired bone health

In the present study, 45% of the female athletes had impaired bone health. These alarming results are in line with the finding of Pollock et al. (2010) showing 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 compared with nonathletes (Nattiv et al., 2007). We found a negative association between training h/week and whole body, as well as lumbar spine BMD, also reported in other studies with female elite athletes from weight-bearing endurance sports (Barrack et al., 2010; Pollock et al., 2010). The endurance athletes in the present study were mostly runners and triathletes, and repetitive low impact training, such as middle and long-distance running, and nonimpact 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 & Fredericson, 2011). Endurance athletes, with low EA and MD may therefore be at an even greater risk for low BMD (Barrack et al., 2010; Tenforde & Fredericson, 2011) compared with eumenorrheic athletes from sports with

mainly high- or odd-impact training. Low EA, malnutrition, and MD have all been associated with rapid bone loss (Nattiv et al., 2007), and the effects of low EA on BMD are reported to be mediated by endocrine factors, including an estrogen-dependent pathway affecting bone resorption (Barrack et al., 2010), as well as an estrogen-independent pathway (Warren, 2011). The estrogen-independent pathway involves the suppression of insulin, IGF-1, and leptin (Warren, 2011), and these factors affecting bone formation are reported to be more sensitive to low EA, and to occur at 30 kcal/kg FFM/day, but perhaps even at higher levels of EA (Ihle & Loucks, 2004). We did not find any association between earlier or present MD and BMD and as could be expected, current EA was not associated with differences in BMD. BMD reflects a lifelong history of mechanical load, EA and by reproductive function, and declines with the number of menstrual cycles missed since menarche (Lloyd et al., 1988), and even though some of this relevant information was collected in the present study, the history in order to estimate the effect on bone health was inadequate. 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.

#### Biomarkers for energy deficiency

An easier and less time consuming method for estimating the level of energy status, instead of EA could be the use of validated biomarkers. Biomarkers suggested to be linked to the Triad, include low leptin and  $T_3$  levels, high levels of cortisol (Warren, 2011), and low RMR<sub>ratio</sub> (De Souza et al., 2008) as well as altered diurnal patterns of several hormones (Laughlin et al., 1998). Low levels of leptin, a marker for low body fat and restricted food intake (Warren, 2011), have been reported among athletes with MD, and have been suggested as an important modulator of ovulatory function (Barrack et al., 2010). Low leptin levels were 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. A sub-analysis revealed no difference in leptin levels between subjects with MD and eumenorrheic subjects, which supports earlier findings that leptin levels *per se* may not be associated with MD in exercising women (Corr et al., 2011). Regarding the other suggested Triad biomarkers; we found only one subject with hypothyroidism and one with elevated levels of cortisol. However,  $T_3$  was lower and cortisol higher in subjects with MD, and we cannot exclude the possibility of altered diurnal patterns since we only analyzed samples taken in the morning. The subjects with MD had, furthermore, lower blood glucose compared with eumenorrheic subjects, and earlier research has suggested that GnRH neuron activity and LH pulsatility are regulated by brain glucose availability (Loucks et al., 1998; Loucks, 2006). We found lower

RMR in subjects with low and reduced current EA, compared with subjects with optimal EA supporting earlier findings that current energy deficiency leads to metabolic adaptations, such as lower RMR, in an attempt to restore energy balance (Redman et al., 2009).

Estrogen is known to affect lipid metabolism by decreasing LDLC and increasing HDLC (Schnaper et al., 2000), and an unfavorable lipid profile has been reported in athletes with MD (Rickenlund et al., 2005). Twenty-five percent of the athletes in the present study had hypercholesterolemia, but with normal LDLC/HDLC ratio, because of a simultaneous high HDLC. Thirty-eight percent of the athletes in this study had total cholesterol  $\geq 5$  mmol/L. High TC is also a common finding in patients with anorexia nervosa (Meczekalski et al., 2013), and has been reported to decrease after weight gain (Ohwada et al., 2006). Most subjects with increased TC in this study had current low/reduced EA and/or DE/ED (73%), while 33% 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, however, only one of the seven subjects with hypotension in the supine position had anorexia nervosa. Even though hypotension was more frequent among subjects with low current EA compared with those with reduced and optimal EA, and subjects with MD and low/reduced current EA had lower supine BP compared with those with MD and optimal current EA, most subjects with hypotension had a BMI within the normal range ( $\geq 18.5$ ).

#### Triad-associated conditions

The prevalence of athletes in this study displaying at least one clinical condition of the Triad was high (50%). Most previous studies have merged subclinical and clinical Triad conditions, and a prevalence of 16–60%, having one condition has been reported (Gibbs et al., 2013), as compared with 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, supporting earlier findings of a low prevalence of all three extreme endpoints of the Triad (Torstveit & Sundgot-Borgen, 2005; 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 MD, reduced or low current EA with or without DE/ED, and impaired bone health are common among female elite endurance athletes. Furthermore, in the present study, 83% of those with impaired bone health, 79% of those with MD, and all subjects with high cholesterol levels, hypotension,



and hypoglycaemia, as well as all but two subjects with low RMR, had at least one additional Triad condition, confirming that these conditions are closely related.

The International Olympic Committee (IOC) has recently published a new Consensus Statement, introducing a new concept, Relative Energy Deficiency in Sports (RED-S; Mountjoy et al., 2014), that replaces the previous statement concerning the Triad (Drinkwater et al., 2005). While the Triad focuses on the interrelationship between EA, reproductive function, and bone health in women, RED-S broadens the concept and acknowledges that energy deficiency affects both men and women. Furthermore, RED-S describes the complexity involved and that more aspects of physiological function, health, and performance are affected. Examples are reduced energy metabolism, impaired glucose homeostasis, and dyslipidemia (Mountjoy et al., 2014), as we also have shown in the present study, as well as gastrointestinal problems and injuries (Mountjoy et al., 2014), as we have reported earlier in this group of female endurance athletes (Melin et al., 2014). In the present study, Triad and associated conditions existed at all three levels of current EA. Findings from clinical laboratory settings might, therefore, not fully apply to free-living elite athletes and it is possible that other factors seen in free-living populations such as within-day variations in EA and dietary characteristics (Mountjoy et al., 2014), as well as long-term reduced EA and high NEAT also may alter the effects of EA.

### 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. However, our results suggest that assessment of BP and fasting blood glucose, as well as RMR and cholesterol, in addition to eating behavior, 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 examinations and objective methods have been used to assess DE/ED, reproductive function, bone health, and energy metabolism. A limitation is the lack of repeated measurements of RMR and sex hormones throughout the menstrual cycle. Assessment of EA at one point in time, in a mixed population of women with ED, non-ED, and underreporters, can provide results that may not represent the “true” habitual individual food intake. However, even though this study only provided a “snapshot” of EA, it demonstrated that current EA was associated with physiological measures of RMR and LH. The lack of objective measurements of body weight

fluctuations during the 7-day recording period is also a limitation of this study. Another limitation is the lack of well-matched nonathletic control subjects in order to assess whether there is an increased prevalence of these conditions in a population of elite female endurance athletes. 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.

### Perspectives

This study demonstrated that female endurance athletes with low/reduced EA and MD had reduced RMR. Furthermore, we found that hypotension, hypoglycemia, and hypercholesterolemia were common clinical features together with the traditional Triad conditions. Conclusively, the high prevalence of coexisting features in this study confirms the complexity involved and that more aspects of physiological function and health than MD and impaired bone health are affected. The findings of either one of these related conditions, therefore, should implicate a careful and expanded assessment. Prevention, early detection, and treatment of energy deficiency and MD is important to prevent health consequences and injuries, but also to optimize performance and recovery, as suggested in the new IOC Consensus Statement.

**Key words:** Energy metabolism, eating disorders, osteoporosis, amenorrhea, hypercholesterolemia.

*Conflicts of interest:* None of the authors have any conflict of interest in the present results.

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