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RUNNING HEAD: Within-day energy deficiency

ABSTRACT

We aimed to estimate and compare within-day energy balance (WDEB) in athletes with eumenorrhea and menstrual dysfunction (MD) with similar 24-hour energy availability/energy balance (EA/EB). Furthermore, to investigate whether within-day energy deficiency is associated with resting metabolic rate (RMR), body-composition, S-cortisol, estradiol, T_3, and fasting blood glucose. We reanalyzed 7-day dietary intake and energy expenditure data in 25 elite endurance athletes with eumenorrhea (n=10) and MD (n=15) from a group of 45 subjects where those with disordered eating behaviors (n=11), MD not related to low EA (n=5), and low dietary record validity (n=4) had been excluded. Besides gynecological examination and disordered eating-evaluation, the protocol included RMR-measurement; assessment of body-composition by dual-energy X-ray absorptiometry, blood plasma analysis, and calculation of WDEB in 1-hour intervals. Subjects with MD spent more hours in a catabolic state compared to eumenorrheic athletes; WDEB <0 kcal: 23.0 hour (20.8–23.4) vs 21.1 hour (4.7–22.3), *P*=0.048; WDEB <-300 kcal: 21.8 hour (17.8–22.4) vs 17.6 hour (3.9–20.9), *P*=0.043, although similar 24-hour EA: 35.6 (11.6) vs 41.3 (12.7) kcal/kg FFM/day, (*P*=0.269), and EB: -659 (551) vs -313 (596) kcal/day, (*P*=0.160). Hours with WDEB <0 kcal and <-300 kcal were inversely associated with RMR_{ratio} (r=-0.487, *P*=0.013, r=-0.472, *P*=0.018), and estradiol (r=-0.433, *P*=0.034, r=-0.516, *P*=0.009), and positively associated with cortisol (r=0.442, *P*=0.027, r=0.463, *P*=0.019). In conclusion, although similar 24-hour EA/EB, the reanalysis revealed that MD athletes spent more time in a catabolic state compared to eumenorrheic athletes**.** Within-day energy deficiency was associated with clinical markers of metabolic disturbances.

KEY WORDS: Energy availability, within-day energy balance, relative energy deficiency, amenorrhea, catabolism, RMR.

INTRODUCTION

Athletes need an adequate and balanced food and fluid intake that provides nutritional support for optimal adaption to training and optimal performance, but also for preventing illness and injuries. An appropriate energy intake is the cornerstone of the athlete's diet because it supports optimal body function, determines the capacity for intake of macronutrient and micronutrients, and assists in manipulating body composition[1,2]. Female athletes focusing on leanness, however, have been reported to have an increased risk of developing restricted eating behaviors and low energy availability (EA)[3,4,5]. Low EA with or without disordered eating (DE) behavior is related to endocrine alterations leading to several health and performance impairing conditions including menstrual dysfunction (MD), gastrointestinal problems, impaired bone health, and increased injury risk[4,5,6] . The hormonal synthesis and increased luteal phase thermogenesis are energy consuming processes[7], and the energy metabolism at rest (RMR) therefore changes up to 10% during the menstrual cycle, with peaks during the late luteal phase or the early follicular phase[8]. MD may consequently result in lower energy needs in a physiological adaptation to insufficient energy intakes. Most female athletes with long-term energy deficiency are reported to maintain a steady body weight and body composition within the normal range, independent of their reproductive function[9]. Therefore, other metabolic mechanisms may be involved, such as a reduction in RMR and/or non-exercise activity thermogenesis (NEAT)[10], as well as in increased work-load efficiency[11,12].

Traditionally, the energy status of athletes is evaluated in blocks of 24-hours as either energy balance (EB) (energy intake – total energy expenditure) or EA (energy intake – exercise energy

expenditure)[13]. Experimental studies have demonstrated a causal relationship between low EA and the endocrine perturbations related to MD[14,15,16]. However, consistent with many other studies[6,17,18] we have previously reported similar 24-hour EA and EB in this group of elite athletes with MD vs eumenorrheic athletes[12,19].

The traditional 24-hour view on human thermodynamics has been criticized for failing to account for the endocrine responses that act on real-time changes in energy intake and expenditure[20]. Therefore, a new view on EB, where energy intake and energy expenditure are assessed in 1-hour intervals, has been proposed to be more appropriate[20]. Within-day energy deficiency (WDED) has previously been associated with an unfavorable body composition in female elite gymnasts and runners, presumably related to both an adaptive reduction in RMR[21], and endocrine responses that favor muscle catabolism and fat gain[20,21]. Therefore, a restrictive eating behavior, resulting in more hours spent in energy deficiency, may have the opposite of the desired effect on athletes' body composition. A desirable range of EB of ± 300 kcal has been suggested, since 300 kcal corresponds to the predicted amount of liver glycogen for female athletes[21]. Exceeding the threshold of EB below -300 kcal, could potentially accelerate biochemical pathways associated with energy deficiency[20] and compromise brain glucose availability and thereby normal gonadotropin-releasing hormone (GnRH) neuron activity and luteinizing hormone (LH) pulsatility[7,13].

According to the International Olympic Committee consensus statement, WDED is a possible contributor to the reproductive and metabolic alterations associated with relative energy deficiency[4]. However, these links have not been demonstrated in athletes. Hence, we wanted to investigate if female elite endurance athletes with MD with similar reported 24-hour EB and EA as eumenorrheic athletes spend more time in a catabolic state and have a larger magnitude of

WDED compared to eumenorrheic athletes. Furthermore, it was our intent to investigate if WDED is associated with suppressed RMR and endocrine alterations in these athletes.

METHODS

Permission to undertake the study was provided by the Swedish and Danish Confederation of Sports, Team Denmark, the Data Inspectorate, and the Regional Ethical committees, both in Sweden and Denmark (nos. 2011/576 and H-4-2011-096, respectively). The protocol was registered at www.clinicaltrials.gov.

We reanalyzed data concerning 7-day energy intake and energy expenditure from a previous study[19], and the recruitment and methods used have previously been described in detail[12]. Athletes recruited and included in the subject pool were endurance athletes on a national team or competitive club level between the age of 18 to 38 years, and training a minimum of 5 times per week. All subjects had been informed orally, and in writing, of all study procedures and signed an informed consent form. Data were collected on 2 consecutive days that were followed by a 7-day recording period in the athletes' normal environment. The timing of the examination and registration period was planned individually for each subject to choose a period that reflects their habitual food habits and exercise regimes. Menstruating athletes were examined in the early follicular phase.

The first day consisted of anthropometric assessment [body weight and height, dual-energy X-ray absorptiometry (DXA) to determine fat free mass (FFM) and fat mass (FM)], and examinations of reproductive function (a transvaginal ultrasound examination by an experienced gynecologist, sex hormone status, and a retrospective menstrual history using LEAF-Q[22]). Subjects were classified with eumenorrhea (menstrual cycles of 28 days ±7 days and sex hormones within the normal range), functional hypothalamic oligomenorrhea (menstrual cycles

>35 days where other causes than hypothalamic suppression had been ruled out), amenorrhea (either primary: no menarche after 15 years of age, or secondary: absence of at least 3 consecutive menstrual cycles where other causes than hypothalamic suppression had been ruled out), or other MD not related to energy deficiency. The second day included examinations of aerobic capacity and assessment of DE assessed by the Eating Disorder Inventory[23]. The incremental test reflected heart rates being linearly correlated with O_2 consumption at increasing workloads (r = 0.94, 95% CI 0.93-0.96), providing the basis for the individual regression lines and later calculations of exercise-associated energy expenditure. RMR was assessed after an overnight fast, using a ventilated open hood system (Oxycon Pro 4, Jeager, Germany). Blood was drawn from an antecubital vein in fasted subjects between 8:30 and 8:50 by a qualified bio-technician[12].

After the 2 examination days, subjects weighed and registered food and beverage intake during a consecutive 7-day period using a digital kitchen scale (Exido 246030 Kitchen Scale, Gothenburg, Sweden). Heart rate monitors (Polar RS400®,Kempele, Finland) and training logs were used to assess energy expenditure during exercise and bicycle transportation, while subjects wore an accelerometer (ActiGraph GT3X®, Pensacola, FL, USA) on the hip (except during showering, swimming, bicycle transportation, and training) for the assessment of NEAT. Exercise energy expenditure for swim sessions was calculated based on mean heart rate obtained from the remaining training sessions. NEAT was calculated for each waking hour using the data analysis software ActiLife 6 (ActiGraph). The nutrient analysis program MadlogVita (Madlog ApS, Kolding, Denmark) and Dietist XP (Kost och Näringsdata AB, Bromma, Sweden) were used to analyze food records. The daily numbers of meals and snacks were counted. Dietary analysis was performed in 25 subjects after excluding 5 due to clinically verified MD other than functional

hypothalamic amenorrhea/oligomenorrhea, 11 due to DE, and 4 due to low validity using the equation described by Black[24].

EA was calculated as total energy intake minus exercise energy expenditure (mean/24hour) expressed in relation to FFM. The athletes themselves defined and registered every training session.

The hourly within-day energy balance (WDEB) was calculated as follows: energy intake – total energy expenditure, where total energy expenditure represents exercise energy expenditure + predicted exercise post oxygen consumption (EPOC) + diet induced thermogenesis (DIT) + NEAT + RMR. WDED variables were used according to Deutz, et al.[21]: total hours with energy deficit (unadapted WDEB < 0 kcal), hours spent in energy deficit exceeding 300 kcal (unadapted WDEB < -300 kcal), and largest single-hour energy deficit (Figure 1). DIT was defined as 10% of energy intake and the equation $175.9 \cdot T \cdot e^{-T/1.3}$ presented by Reed and Hill[25] was used to calculate DIT the first 6 hours after each meal/snack. Based on results from studying young active eumenorrheic women[26], EPOC was calculated as 5% of exercise energy expenditure the first hour post-exercise plus 3% of exercise energy expenditure the second hour post-exercise.In order to control for the problem of potential underestimation of energy requirements, the unadapted/predicted RMR, instead of measured RMR, was used when calculating total energy expenditure. Hence, the aim was to calculate the unadapted EB. The hourly predicted RMR was calculated using the Cunningham equation: RMR, kcal/hour = (500 + (22· FFM [kg]) kcal/day)/24 hours/day. The RMR_{ratio} was calculated as measured RMR/predicted RMR. Sleeping metabolic rate was calculated as 90% of RMR and used instead of RMR during sleeping hours. The starting point for the calculation of WDEB was at midnight on the first day of food recording and was calculated as the mean energy intake of the last daily meal or snack

minus mean total energy expenditure in the time interval following the mean meal/snack consumption. WDEB was calculated continuously for the 7 days of registration.

Statistics

Statistical calculations were performed using RStudio™ (version 0.99.879) with a two-tailed significance level of < 0.05. All data sets were tested for normality and homogeneity of variance before statistical hypothesis tests were performed. Normally distributed data were summarized as mean and standard deviation (SD), and non-normally distributed data as median and interquartile range (IQ 25 and IQ 75 percentiles). Differences between MD and eumenorrheic subjects were investigated using unpaired Student's t-test for normally distributed data and the Wilcoxon rank-sum test for non-parametric data. Pearson's correlation coefficient and Spearman's rank correlation coefficient were calculated to investigate associations between WDED variables and continuous outcomes for normally and non-normally distributed data, respectively.

RESULTS

Subject characteristics are presented in Table 1. As earlier reported[19] 15 of 25 subjects (60%) were diagnosed with MD and there were no differences in age, height, body weight or BMI between subjects characterized by reproductive function. MD subjects had 19% lower FM and 14% lower relative FM compared to eumenorrheic subjects, although there were no differences in training volume or exercise capacity.

There were no differences in any of the energy expenditure components between eumenorrheic and MD subjects, but subjects with MD had lower RMR_{ratio} compared to eumenorrheic subjects (Table 2).

There was no difference in 24-hour EA [35.6 (11.6) vs 41.3 (12.7) kcal/kg FFM/day, $P = 0.269$)] or EB [-659 (551) vs -313 (596) kcal/day, $P = 0.160$] between subjects with MD vs those with eumenorrhea. Subjects with MD spent more time with EB < 0 kcal ($P = 0.048$) and < -300 kcal ($P = 0.043$) compared to eumenorrheic subjects (Table 3). Subjects with MD had significantly more meals/snacks per day compared to eumenorrheic subjects [6.5 (1.0) vs 5.5 (0.8) meals/day, $P = 0.014$]. We found no association between meal frequency and energy intake, but time spent in energy deficiency was positively associated with meal frequency (r = 0.398, $P = 0.0485$).

A sub analysis excluding oligomenorrheic subjects, showed a more pronounced difference between amenorrheic (n = 11) and eumenorrheic subjects in EB < 0kcal [23.3 (22.7 – 23.6) hours vs 21.1 (4.7 – 22.3) hours, ($P = 0.009$)] and < -300 kcal [22.3 (21.6 – 22.7) hours vs 17.6 (3.9 – 20.9) hours ($P = 0.007$)].

The regression analysis showed that the more hours spent with EB < 0 kcal and < -300 kcal, the lower the RMR_{ratio} and estrogen and the higher the cortisol levels (Table 4). In addition, smaller magnitudes of hourly energy deficits (closer to 0 kcal) were associated with higher estrogen level.

DISCUSSION

Although assessed athletes had similar 24-hour EA and EB, this reanalysis revealed that those with MD spent significantly more time in a catabolic state compared to eumenorrheic athletes**.** In addition, the more time the athletes spent in a catabolic state the lower the RMR_{ratio} and estrogen, and the higher the cortisol levels.

Previously, Deutz and colleges[21] investigated WDEB in female elite athletes. The present study is, however, the first to include its relationship with reproductive function and metabolic

adaptations. In contrast to Deutz and collaborators, we found no association between WDED and body composition.

The menstrual cycle is an energy demanding process that relies on adequate availability of energy and glucose for optimal LH pulsatility[13]. Randomized controlled trials have demonstrated a disruption of the normal sex hormone secretion in regularly menstruating women[14,15,16] when manipulating EA, and it is well established that relative energy deficiency increases the risk of MD[4]. However, in line with many earlier studies[6,17,18] we could not demonstrate significant differences in 24-hour EB or EA between MD and eumenorrheic athletes. Several researchers have suggested that a plausible difference among women is their susceptibility to energy restriction[3,17,18,19,27], which might contribute to the explanation for similar 24-hour EB and EA in athletes with and without MD. An additional factor could be that the 24-hour assessment of energy status masks periods with energy deficiency substantial enough to maintain reproductive dysfunction. When calculating the traditional 24-hour EB or EA light training days may have a compensatory effect on the mean 24-hour value. In contrast, the WDEB calculation is cumulative and takes into account potential energy deficiencies or access from previous days. During the follicular phase, pulses of GnRH, responsible of the release of LH and follicle stimulating hormone from the anterior pituitary, occur at hourly intervals[7], and animal studies suggest that the reproductive function is responsive to hourly changes in metabolic fuel oxidation[28]. Both energy expenditure and energy intake fluctuate greatly during waking hours, providing a natural within-day variation in EA and EB in athletes[13]. Although the athletes in the present study, independently of reproductive function, spent the majority of the day in an energy deficient state, the athletes with MD spent 24% more hours in EB < -300 kcal compared to eumenorrheic athletes, providing a potentially more profound catabolic state in female athletes

with MD. It is possible that amenorrheic athletes spend a greater proportion of time during the day without sufficient blood glucose available, which would negatively impact normal functioning of the hypothalamic-pituitary-gonadal-axis. There was however, no difference in the largest hourly deficit between groups, which may indicate that large energy deficit if transient do not disturb reproductive function. The large variations within the eumenorrhea group illustrate that some athletes classified with regular menstrual cycles also had prolonged deficits. Loucks and Thuma[15] demonstrated that women with shorter luteal phase (11 days) are more susceptible to energy deficiency in terms of endocrine alterations compared to women with longer luteal phases (12-14 days). As discussed by the authors, women that by nature have a slightly shorter luteal phase may be of an increased risk of developing MD when exposed to energy deficiency[15]. Thus, the eumenorrheic athletes with a high number of catabolic hours in the present study may be women with a more robust reproductive function. On the other hand, it is unknown whether these athletes may have had subclinical MDs associated with energy deficiency such as anovulation or luteal phase abnormality (luteal length < 10 days or inadequate/low progesterone concentration[6]). This condition is diagnosed by measuring daily ovarian steroid hormones in blood or urine over a full menstrual cycle[9]. Indeed, an incidence of 79% of luteal phase deficiency has been reported among recreational, regularly menstruating runners in a 3-months sample[6]. In the sedentary control group, 90% of all menstrual cycles were ovulatory, whereas in the runners only 45% were ovulatory. Another possible explanation is related to the cross-sectional design where measurements only provide a snapshot over one week and not necessarily the long-term consequences of energy deficiency, which is necessary to develop MD. In this perspective, it is important also to evaluate more acute consequences of energy deficiency such as suppressed RMR as we did in the present study, where we found WDED to be associated with

lower RMR_{ratio}. Several studies have reported reduced RMR in female athletes with MD compared to eumenorrheic athletes[12,29,30,31], and our results support previous studies suggesting that the RMR_{ratio} may be used as a useful marker for identifying athletes with energy deficiency[5,12,18,27]. Consequently, when calculating EB in athletes with insufficient energy intake and MD, using measured RMR might result in a more neutral EB, underestimating the degree of energy deficiency[13,32].

We found WDED to be associated with higher cortisol and lower estrogen levels and a trend suggesting lower T_3 levels, as signs of biological stress. LH pulsatility, T_3 and cortisol levels are regulated by brain glucose availability[13] hence extensive periods in an energy deficient state are likely to cause these hormonal alterations.

The present study found no association between body composition and WDED. This is in contrast to an earlier study assessing elite female runners and gymnasts, where hours with EB < 0 kcal, and with EB < -300 kcal were positively associated with relative fat mass ($r = 0.285$, $P = 0.03$ and $r = 0.407$, $P < 0.01$, respectively), and the largest energy deficit was negatively associated with relative fat mass ($r = -0.378$, $P < 0.01$)[21]. It has been suggested that an increased meal frequency[33], and an increased protein intake of 1.8–2.0 g/kg/day[34] during hypocaloric conditions might have an anti-catabolic effect on FFM in athletes. Hence, the high meal frequency and high protein intake [2.1 (0.4) g/kg/day][19] observed among these MD athletes might potentially explain the maintenance of FFM despite profound WDED.

Limitations

Since this is an observational study, it is not able to demonstrate a causal relationship between WDED and reproductive function or metabolic adaptations. As reviewed by Burke[1], studies using self-reported food diaries are always challenged by errors of validity and reliability. Although subjects with DE behavior and suspected under reporters were excluded, there is a risk for not identifying all subjects miss-reporting dietary intake[24]. Despite possible systemic errors, these are presumed to be equally distributed between groups and the differences found are therefore still valid even if energy expenditure has been overestimated and/or energy intake has been underestimated. Due to the small sample size and several correlation analyses made, our results should primarily be seen exploratory. Nevertheless, our findings that MD athletes spent significantly more time in a catabolic state compared to eumenorrheic athletes although similar 24-hour EA and EB assessment is interesting and was associated with clinical markers of energy suppression e.g. lower RMR. Finally, the energy deficiency cut-off -300 kcal, suggested by Deutz, et al.[21], is theoretically founded and may vary depending on different individual factors, including body weight[35]. Lack of identification of subclinical MD are also a limitation of the study. As other calculation methods evaluating athletes' energy status, the WDEB calculation entails uncertainties. Nevertheless, the 24-hour assessments, although more practical, may be too simple to detect the differences in energy deficiency between MD and eumenorrheic athletes.

Practical implications

Based on the results from the present and other studies[6,17,18], a continuous view on energy status evaluated in smaller time blocks may be more appropriate than the 24-hour assessments.

However, the method is time-consuming and requires equipment capable of assessing energy expenditure in minor blocks, and participants must carefully note the timing of energy intake and energy expenditure. Nonetheless, our results emphasize important considerations for practitioners of the potential limitations of the 24-hour assessment. A high meal frequency may be necessary for athletes to ensure adequate energy intake[35,36] and to improve WDEB. However, the paradoxical finding of a positive association between WDED and meal frequency, suggests that a high meal frequency does not improve WDEB in athletes eating mainly low energy dense foods as earlier reported in this population, especially among MD athletes[19]. Therefore, other dietary characteristics, behavioral attitudes and taboos towards certain foods, including carbohydrate-rich foods, fats, and energy containing beverages needs to be evaluated when counseling athletes at risk for energy deficiency.

Perspectives

The present study demonstrates that the traditional 24-hour assessment of EB and EA may not be sufficient for detecting athletes in an energy deficient state and a continuous view on EB may be more appropriate. This is the first study to investigate the relationship between WDED and clinically verified MD as well as metabolic suppression related to energy deficiency. Therefore, more studies are needed, preferably with larger sample size in order to be able to differentiate between subjects with oligomenorrhea and amenorrhea, but also subjects with subclinical MD (anovulation and short luteal phase defect).

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CONFLICTS OF INTEREST

The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. Dan Benardot is the inventor and scientific advisor of NutriTiming®, a software package that analyzes hourly EB. This software program, however, was not used for this study.

REFERENCES

1. Burke LM. Energy needs of athletes. *Can J Appl Physiol.* 2001;26:S202-19.
2. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *J Acad Nutr Diet.* 2016;116:501-28.
3. Manore MM. Dietary recommendations and athletic menstrual dysfunction. J *Sports Sci.* 2002;32:887-901.
4. Mountjoy M, Sundgot-Borgen J, Burke L, et al. The IOC consensus statement: beyond the Female Athlete Triad – Relative Energy Deficiency in Sport (RED-S). *Brit J Sports Med.* 2014;48:491-7.
5. Nattiv A, Loucks AB, Manore M, Sanborn CF, Sundgot-Borgen J, Warren MP. The female athlete triad. *Med Sci Sports Exer.* 2007;39:1867-82.
6. De Souza MJ, Miller BE, Loucks AB, et al. High Frequency of Luteal Phase Deficiency and Anovulation in Recreational Women Runners: Blunted Elevation in Follicle-Stimulating Hormone Observed during Luteal-Follicular Transition 1. *J Clin Endocrinol Metab.* 1998;83:4220-32.
7. Harber VJ. Energy balance and reproductive function in active women. *Can J Appl Physiol.* 2004;29:48-58.
8. Henry CJK, Lightowler HJ, Marchini J. Intra-individual variation in resting metabolic rate during the menstrual cycle. *Br J Nutr.* 2003;89:811-17.
9. Redman LM, Loucks AB. Menstrual disorders in athletes. *Sports Med.* 2005;35:747-55.

10. Redman LM, Heilbronn LK, Martin CK, et al. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. *PloS One.* 2009;4:e4377.
11. Goldsmith R, Joanisse DR, Gallagher D, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. *Am J Physiol Regul Integr Comp Physiol.* 2010;298:R79-88.
12. Melin A, Tornberg ÅB, Skouby S, et al. Energy availability and the female athlete triad in elite endurance athletes, *Scand J Med Sci Sports.* 2014;25:610-22.
13. Loucks AB. Energy balance and energy availability. In: Maughan, RJ, editor. *The Encyclopedia of Sports Medicine, Energy and Macronutrients*. International Olympic Committee. 1 ed. John Wiley & Sons, Ltd; 2014. p. 72-87.
14. Loucks AB, Heath EM. Dietary restriction reduces luteinizing hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. *J Clin Endocrinol Metab.* 1994;78:910-15.
15. 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.
16. Olson BR, Cartledge T, Sebring N, Defensor R, Nieman L. Short-term fasting affects luteinizing hormone secretory dynamics but not reproductive function in normal-weight sedentary women. *J Clin Endocrinol Metab.* 1995;80:1187-93.
17. Guebels CP, Kam LC, Maddalozzo GF, Manore MM. Active women before/after an intervention designed to restore menstrual function: Resting metabolic rate and

comparison of four methods to quantify energy expenditure and energy availability. *Int J Sport Nutr Exerc Metab.* 2014;24:37-46.

18. Schaal K, Van Loan MD, Casazza GA. Reduced Catecholamine Response to Exercise in Amenorrheic Athletes. *Med Sci Sports Exerc.* 2001;43:34-43.
19. Melin A, Tornberg ÅB, Skouby S, et al. Low-energy density and high fiber intake are dietary concerns in female endurance athletes. *Scand J Med Sci Sports.* 2015;26:1060-71.
20. Benardot D. Energy Thermodynamics Revisited: Energy intake strategies for optimizing athlete body composition and performance. *Pensar en Movimiento.* 2013;11:1-13.
21. Deutz RC, Benardot D, Martin DE, Cody MM. Relationship between energy deficits and body composition in elite female gymnasts and runners. *Med Sci Sport Exerc.* 2000;32:659-68.
22. Melin A, Tornberg ÅB, Skouby S, et al. The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad, Brit J Sports Med. 2014;48:540-5.
23. Garner DM. Eating Disorder Inventory-3. Professional Manual. *Par. Inc.* 2004. p. 1-213.
24. 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;24:1119-30.
25. Reed GW, Hill JO. Measuring the thermic effect of food. *Am J Clin Nutr.* 1996;63:164-9.
26. Phelain JF, Reinke E, Harris MA, Melby CL. Postexercise energy expenditure and substrate oxidation in young women resulting from exercise bouts of different intensity. *J Am Coll Nutr.* 1997;16:140-6.

27. Gibbs JC, Williams NI, Scheid JL, Toombs RJ, De Souza MJ. The association of a high drive for thinness with energy deficiency and severe menstrual disturbances: confirmation in a large population of exercising women. *Int J Sport Nutr Exerc Metab.* 2011;21:280-90.

28. Wade GN, Jones JE. Neuroendocrinology of nutritional infertility. *Am J Physiol Regul Integr Comp Physiol. 2004;*287:R1277-96.

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

30. Scheid JL, Williams NI, West SL, VanHeest JL, De Souza MJ. Elevated PYY is associated with energy deficiency and indices of subclinical disordered eating in exercising women with hypothalamic amenorrhea. *Appetite.* 2009;52:184-192.

31. Vanheest JL, Rodgers CD, Mahoney CE, De Souza MJ. Ovarian suppression impairs sport performance in junior elite female swimmers. *Med Sci Sports Exerc.* 2014*;*46:156-66.

32. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci.* 2011;29:S7-15.

33. Iwao S, Mori K, Sato Y. Effects of meal frequency on body composition during weight control in boxers. *Scand J Med Sci Sports.* 1996;6:265-72.

34. Phillips SM, Van Loon LJ. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci.* 2011;29:S29-38.

35. Benardot D. Timing of energy and fluid intake: New concepts for weight control and hydration. *ACSM's Health & Fitness Journal.* 2007;11:13-19.

36. Hawley J, Burke L. Effect of meal frequency and timing on physical performance, *Br J Nutr*. 1997;77:S91-103.

Figure 1. Graphical depiction of how WDEB values were determined. Assessed values include number of hours > 300 kcal; hours < 300 kcal; hours > 0 kcal; hours < 0 kcal

Modified from Benardot[20]. In contrast to the traditional method comparing energy intake with total energy expenditure or exercise energy expenditure in 24-hour intervals, WDEB assesses time and magnitude deviations from the predicted EB. Horizontal axis: energy status (kcal); vertical axis: time course throughout the day (hours). The given example illustrates 5 hours with WDEB > 0 kcal, 19 hours with WDEB < 0 kcal, 2 hours with WDEB > 300 kcal, and 7 hours with WDEB < -300. Largest surplus is +426 kcal and largest deficit is -829 kcal. The 24-hour energy balance is +19 kcal. Abbreviation: WDEB: within-day energy balance.

Table 1. Description of subjects characterized by reproductive function

	All (n=25)	Eumenorrhea (n=10)	MD (n=15)	*P*-value[1]
Age (years)	26.6 (5.6)	26.8 (4.6)	26.5 (6.3)	0.910
Height (cm)	169.9 (5.8)	170.0 (5.8)	168.1 (5.9)	0.081
Body weight (kg)	58.8 (7.3)	61.2 (8.2)	57.1 (6.4)	0.173
BMI (kg/m^2)	20.6 (2.0)	21.2 (2.3)	20.2 (1.6)	0.228
Body fat (kg)	11.8 (3.1)	13.3 (3.1)	10.8 (2.8)	**0.049**
Body fat (%)	19.8 (3.6)	21.7 (3.3)	18.6 (3.3)	**0.034**
Fat free mass (kg)	46.0 (43.0 – 50.7)	49.2 (44.3 – 50.5)	46.0 (42.9 – 49.8)	0.482
Exercise (hours/week)	12.0 (4.4)	11.1 (2.9)	12.7 (5.1)	0.363
VO_{2peak} (ml/kg/min)	54.5 (6.4)	53.0 (5.7)	55.5 (6.9)	0.354

Data are presented as mean (SD) for normally distributed data and as median and interquartile range (25-75) for non-normally data. Abbreviations: BMI: body mass index, MD: menstrual dysfunction, VO_{2peak}: maximal oxygen uptake. [1)] Difference between eumenorrheic and MD subjects.

Table 2. Energy expenditure characterized by reproductive function

	All (n=25)	Eumenorrhea (n=10)	MD (n=15)	*P*-value[1]
Total EE[2] (kcal/day)	3247 (553)	3205 (443)	3276 (628)	0.761
DIT (kcal/day)	271 (44)	287 (54)	261 (34)	0.189
Exercise EE (kcal/day)	968 (571)	942 (295)	985 (568)	0.828
EPOC (kcal/day)	80 (38)	75 (24)	83 (46)	0.653
NEAT (kcal/day)	446 (147)	400 (74)	446 (177)	0.156
pRMR (kcal/hour)	64 (5)	65 (4)	63 (5)	0.498
pSMR (kcal/hour)	58 (5)	58 (4)	57 (5)	0.516
mRMR (kcal/hour)	57 (7)	60 (6)	55 (7)	0.063
RMR_{ratio}	0.89 (0.07)	0.93 (0.05)	0.87 (0.07)	**0.033**

Data are presented as mean (SD). Abbreviations: DIT: diet induced thermogenesis, EE: energy expenditure, EPOC: excess post-exercise oxygen consumption, MD: menstrual dysfunction, mRMR: measured resting metabolic rate, pRMR: predicted resting metabolic rate, pSMR: predicted sleeping metabolic rate. [1)] Difference between eumenorrheic and MD subjects. [2)] Unadapted total energy expenditure.

Table 3. Within-day energy deficiency characterized by reproductive function

	All (n=25)	Eumenorrhea (n=10)	MD (n=15)	*P*-value[1]
WDEB < 0 kcal (hours/day)	22.1 (19.7 – 23.3)	21.1 (4.7 – 22.3)	23.0 (20.8 – 23.4)	**0.048**
WDEB <-300 kcal (hours/day)	21.3 (15.7 – 22.3)	17.6 (3.9 – 20.9)	21.8 (17.8 – 22.4)	**0.043**
Largest hourly deficit (kcal)	-2626 (2352)	-1793 (2360)	-3181 (2253)	0.159

Data are presented as mean (SD) for normally distributed data and as median and interquartile range (25-75) for non-normally distributed data. Abbreviations: MD: menstrual dysfunction, WDEB: within-day energy balance. [1)] Difference between eumenorrheic and MD subjects.

Table 4. Associations between within-day energy deficiency and markers for catabolic state

	Hours with WDEB < 0 kcal		Hours with WDEB <-300 kcal		Largest hourly deficit[1]	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
RMR_{ratio}	-0.487	**0.013**	-0.472	**0.018**	0.310	0.132
Body fat (%)	-0.313	0.128	-0.337	0.100	0.023	0.913
Cortisol	0.442	**0.027**	0.463	**0.019**	-0.297	0.111
Estradiol	-0.433	**0.034**	-0.516	**0.009**	0.505	**0.012**
T_3	-0.360	0.078	-0.264	0.203	0.287	0.164
Glucose	-0.350	0.086	-0.365	0.073	0.202	0.333

All subjects (n=25) were included in the correlation analysis. Abbreviations: RMR: resting metabolic rate, WDEB: within-day energy balance. [1)]values recorded as negative numbers.