Understanding anthropometry, metabolism and menstrual status of Japanese college female long-distance runners

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Abstract

Aim: To understand the anthropometry, metabolism, and menstrual status of Japanese collegiate female long-distance runners. Methods: This was a cross-sectional study of 29 Japanese college female long-distance runners divided into three menstruation status groups: 1) Regular; 2) Irregular; and 3) Amenorrhea. The amenorrhea group was further divided into participants who consulted a gynecologist and those who did not. Metabolism was measured using resting metabolic rate, total energy expenditure, and physical activity level. Energy was measured using energy intake, exercise energy expenditure, and energy availability (EA). Anthropometric measurements were performed following standardized techniques from the International Society for the Advancement of Kinanthropometry. Results: Of the 29 runners, 68% had menstrual dysfunction. The amenorrhea group who had consulted a gynecologist had lower body mass index (BMI) and lower skinfolds than the other groups. All groups had more than 30 kcal/kg fat free mass/day for EA with no difference between the groups. Conclusions: There was high prevalence of menstrual dysfunction in the female Japanese college long-distance runners. Runners with amenorrhea had lower BMI and lower skinfolds than the other groups, despite having consulted a gynecologist for medical support. All the runners had an energy deficiency higher than the published threshold; therefore, this threshold did not differentiate the amenorrhea or irregular menstruating athletes from regular menstruating athletes. Longitudinal tracking of athletes should include measurement of height, body mass, and skinfolds to enable evaluation of BMI, the sum of eight skinfold sites, and leanness ratio score as possible indicators over time for menstrual dysfunction.

Keywords: Female long-distance runners, Metabolism, Energy availability, Menstrual dysfunction, Anthropometry.

Resumen

Objetivo: Comprender la antropometría, el metabolismo y el estado menstrual de las corredoras de larga distancia universitarias japonesas. Métodos: Este fue un estudio transversal de 29 corredoras de larga distancia universitarias japonesas divididas en tres grupos de estado de menstruación: 1) Regular; 2) Irregular; y 3) amenorrea. El grupo de amenorrea se dividió en participantes que consultaron a un ginecólogo y los que no lo hicieron. El metabolismo se midió utilizando la tasa metabólica en reposo, el gasto energético total y el nivel de actividad física. La energía se midió utilizando la ingesta de energía, el gasto energético del ejercicio y la disponibilidad de energía (EA). Las mediciones antropométricas se realizaron siguiendo técnicas estandarizadas de la Sociedad Internacional para el Avance de la Cineantropometría. Resultados: De las 29 corredoras, el 68% tenía disfunción menstrual. El grupo de amenorrea que había consultado a un ginecólogo tenía un índice de masa corporal (IMC) más bajo y pliegues cutáneos más bajos que los otros grupos. Todos los grupos tenían más de 30 kcal / kg de masa libre de grasa / día para EA sin diferencias entre los grupos. Conclusiones: Hubo una alta prevalencia de disfunción menstrual en las corredoras de larga distancia universitarias japonesas. Las corredoras
con amenorrea tenían un IMC más bajo y pliegues cutáneos más bajos que los otros grupos, a pesar de haber consultado a un ginecólogo para recibir apoyo médico. Todos los corredores tenían una deficiencia energética superior al umbral publicado; por lo tanto, este umbral no diferenciaba la amenorrea o los atletas con menstruación irregular de los atletas con menstruación regular. El seguimiento longitudinal de los atletas debe incluir la medición de la altura, la masa corporal y los pliegues cutáneos para permitir la evaluación del IMC, la suma de ocho sitios de pliegues cutáneos y la puntuación del índice de delgadez como posibles indicadores a lo largo del tiempo de la disfunción menstrual.

Palabras Clave: Corredoras de larga distancia, Metabolismo, Disponibilidad de energía, Disfunción menstrual, Antropometría.

Introduction

The female athlete triad (FAT) symptoms of eating disorder, amenorrhea, and osteoporosis (Otis 1997. Drinkwater et al. 1997) have been reported for decades (Loucks 2007, Manore et al. 2007, Loucks et al. 2011, Goodwin et al. 2014, Melin et al. 2015, Joy et al. 2016).

Sports that promote leanness for success, such as a long-distance running, carry an increased risk of low energy availability (EA) (Manore et al. 2007). This is the result of too light body mass and low body fat which can affect running performance (Brandon and Boileau 1992). Menstrual disorders in female long-distance runners have been reported globally since 1979 and in Japan since 1986 (Abe et al. 1986). Long-distance running events such as Ekiden (long-distance relay) have been popular in Japan; however, there have been few studies focused on long-distance runners in Japan.

Studies have reported that long-distance runners with low body fat percentage had diminishing hypothalamic or pituitary hormone secretion (Dale et al. 1979, Dale et al. 1979, 1981). Eumenorrheic runners with higher body fat percentages have been reported to complete less running than oligomenorrheic and amenorrhoeic runners (Souza, Maresh et al. 2007). The exact mechanism(s) responsible for this remain unknown. Testosterone levels are suppressed in high-volume trained male runners; therefore, a specific volume threshold of endurance training may have an effect on reproductive function (De Souza and Miller 1997).

Laboratory studies of healthy, young, women who have regular menstruation have shown reduced blood glucose levels, lower triiodothyronine (T3) to suppress the pulsatility of gonadotropin-releasing hormone and luteal hormone (LH), and also elevated cortisol that comes from energy deficiency of less than 30 kcal/kg free fat mass (FFM)/day within five days (Loucks 2003, Loucks et al. 2011). From this evidence, an EA below a threshold of 30 kcal/kg FFM/day increases the risk for FAT (Souza et al. 2014). Consequently, there is increasing evidence that energy deficiency plays a causal role in the induction of amenorrhea in exercising women.

Low T3, reduced insulin secretion and elevated cortisol, growth hormone and ghrelin levels may induce amenorrhea. However, hyperandrogenism, such as that observed in the phenotype of polycystic ovarian syndrome, may be associated with oligomenorrhea in exercising women, and may not always represent hypothalamic inhibition secondary to an energy deficiency (Awdishu et al. 2009).

EA is energy intake (EI) minus exercise energy expenditure (ExEE) (Loucks 2007, Manore et al. 2007). To provide EA, an experienced sports dietitian must provide accurate assessment of dietary intake and an exercise physiologist must provide estimation of energy expenditure using a heart rate monitor and accelerometer (Loucks 2007, Manore, Kam et al. 2007). Accurate FFM is needed from dual-energy X-ray absorptiometry (DXA) (Toombs et al. 2012). Measuring EI, ExEE, and EA accurately for athletes undergoing long-term training is not easy given the resources needed; therefore, there have been few reports about EA for long-distance runners in Japan.

The hypothesis was that a higher percentage of Japanese collegiate female long-distance runners would have menstrual dysfunction, lower BMI, and lower metabolism, including EA, than long-distance runners from Western countries. Therefore, the purpose of this study was to understand the anthropometry, metabolism, and menstrual status of Japanese college female long-distance runners.

Material and methods

Study design

This cross-sectional study was part of medical screening during a longitudinal project (December 2017 to March 2020). Participants were exposed to all conditions and eligible for free laboratory checks. During the data collection, all participants lived in the same university dormitory and were provided with the same breakfast and
dinner from the team dietitian on weekdays. After being informed about the research procedures, all participants provided written and oral informed consent; however, all participants had rights to cancel the signed contract at any time.

The study design was in accordance with the Declaration of Helsinki revised in 2013 and was approved by the Ethics Committee of the Toyo University (TU2017-010TU2017-H0052018-H002).

**Participants**

A total of 29 Japanese collegiate female long-distance runners (Ekiden Championship long-distance team) participated in the research.

**Menstrual status**

Menstruation status was determined using a questionnaire that asked the age of menarche and details of the menstrual cycle for the past year. Participants were divided into three menstruation status groups: 1) regular menstrual cycle = 25 to 38 days; 2) irregular menstrual cycle = continually falls outside of regular range with intervals of less than 25 days or 38 to 90 days; and 3) amenorrhea = missed at least three months of menstrual period. Research institutes associated with the Japan Institute of Sports Sciences, including the university hospital for female athletes, provided information on the FAT concept to the coaches and athletes. Therefore, some female athletes who were suffering from menstrual disorders were able to receive advice and treatment from gynecology specialists. For that reason, the amenorrhea group was further divided into participants who consulted a gynecologist (G) or those who had not consulted a gynecologist (NG).

**Measurements**

Morning fasting blood collections were analyzed for total protein, hemoglobin, hematocrit, serum iron, and ferritin (LSI Medience Corporation Japan).

Physiological characteristics were assessed using VO$_2$ max at sitting and during treadmill running. A gas mask (AMA104: Minato Medical Science Co., Ltd. Japan) and a wireless transmitter (ZB-823P: Nihon Kohden Co., Ltd. Japan) were attached to the runner. During 5 minutes of sitting, VO$_2$ and volume of carbon dioxide (VCO$_2$) were measured using a breath gas analyzer (AE-310S: manufactured by Minato Medical Science Co., Ltd.) using the breath-by-breath method every 30 seconds per minute. The participants then completed a 5-minute warm-up run at a self-selected speed on the treadmill (TechnoGym: The Wellness Company Italy) with an 8.5% gradient. Treadmill speed was gradually increased every 3 minutes until the target heart rate was attained. After 12 minutes, the load was increased every 2 minutes until the participant reached exhaustion. The oxygen uptake for the last minute at each load was defined as the oxygen uptake for that load. The rate of perceived exertion (RPE) at each loading speed was reported by the participants using the Borg scale (Borg 1973) during the last 30 seconds of each loading.

Anthropometric measurements were performed following standardized techniques from the International Society for the Advancement of Kinanthropometry. Height was measured on a stadiometer (GPM, Seritex, Inc., Carlstadt, New Jersey, USA). Body mass was recorded on a portable scale (WB-150 Tanita Corporation, Tokyo, Japan). Thickness of eight skinfolds (biceps, subscapular, triceps, iliac crest, supraspinal, abdominal, thigh and calf) was measured using a calibrated caliper (Baty International Ltd, Burgess Hill, UK). Body mass index (BMI: kg/m$^2$) of each runner was calculated using the formula of weight (kg) ÷ (height (m) × height (m)). The sum of eight skinfold sites (∑8SF) and leanness ratio score (LRS) (body mass in kg ÷ ∑8SF in mm) were calculated. Somatotype was calculated using standard procedures (Heath and Carter 1967). Body composition was measured from DXA (Hologic Discovery A, Hologic, Bedford, MA) by a technician with extensive measurement experience at medical institutions (Ebara clinic, Tokyo, Japan) to provide percentage of body fat, fat mass, and FFM.

Resting metabolic rate (RMR) was measured in the post-absorptive state of at least 12 hours and performed after 30 minutes rest in a supine position. Expired air was collected in Douglas bags, and O$_2$ and CO$_2$ concentrations were analyzed using a gas analyzer (Arco System, AR-1, Kashiwa, Japan). The volume of expired air was determined using a dry gas volume meter (Shinagawa, DC-5, Tokyo, Japan). RMR (kcal/d) was calculated using Weir’s equation (Weir 1949).

Total energy expenditure (TEE) was measured using the doubly labelled water method, as described in a previous study (Park et al. 2011). In brief, the participants were asked to collect a baseline urine sample before oral administration of a single dose of doubly labelled water. In the subsequent week, they were asked to collect urine
samples at the same time of day on four occasions. The isotopic ratios of the urine samples were analyzed using isotope ratio mass spectrometry (Hydra 20-20 Stable Isotope Mass Spectrometers; SerCon Ltd, Crewe, UK). TEE was calculated using the A6 equation (Schoeller 2009) and Weir’s equation (Weir 1949). Physical activity level (PAL) was calculated as TEE/RMR.

Total EI was measured for three days (including one day on a weekend), by a photography weighing method. Participants were asked to list the weight of all foods and beverages consumed. As it was not possible to weigh all foods, a set of standard amounts was given such as “1 plate” or “1 piece.” All food names were recorded. A registered dietitian calculated the nutritional EI value using Standard Tables of Food Composition in Japan (Ministry of Education 2015).

ExEE was calculated from the VO\(_2\) and VCO\(_2\) at each stage of the VO\(_2\)\(_\text{max}\) test using the Weir formula. For each participant, from the energy consumption and RPE at each stage of the exercise load test, a regression equation of RPE and energy consumption was created. The energy consumption during training was calculated from the RPE in the activity record of each participant during training and the regression equation of RPE and energy consumption. When RPE was six, the average resting and standing energy consumption was used (Yoshida et al. 2015).

EA was calculated by the formula: EA = EI - ExEE/FFM, where ExEE = Total exercise energy expenditure (TExEE) - (1.3 × duration exercise), and TExEE = (Total Energy Expenditure) - (RMR × duration exercise).

Statistical analysis

Results were calculated as group means ± standard deviation. The Kruskal-Wallis test was used as a nonparametric test, and the Mann-Whitney U test with Bonferroni correction was used as a post hoc comparison calculated using SPSS (Ver 11.5 IBM Corp, USA). Significance was set at p<0.05.

Results

Of the 29 participants, 69% had menstrual dysfunction (27.6% irregular; 41.4% amenorrhea of which 50.0% were G and 50% NG) and 31% had regular menstruation. There were no differences between the groups for age of menarche, VO\(_2\) max, or blood measures (Table 1). However, there were differences between groups for BMI (p=0.029), Σ8SF (p=0.013), LRS (p=0.034), and fat mass (p=0.037). The amenorrhea G group had a smaller BMI than the irregular group (p=0.032). The amenorrhea G group had significantly less Σ8SF than the regular (p=0.030) and irregular groups (p=0.027). The amenorrhea G group had a smaller LRS (p=0.033) and smaller fat mass than the regular group (p=0.038). For somatotype endomorphy, there were differences between the regular and amenorrhea G group (p=0.008) and the irregular and amenorrhea G group (p=0.012).

For the metabolic variables, there were differences between groups for RMR (p=0.003), RMR/kg (p=0.023), and PAL (p=0.004). The amenorrhea G group had a lower RMR than the irregular group (p=0.004). The amenorrhea NG group had lower RMR than the irregular group (p=0.039). The amenorrhea NG group had a lower RMR/kg than the irregular group (p=0.026). The amenorrhea NG group had a lower PAL than the irregular group (p=0.004). The amenorrhea G group had a lower EI/kg (p=0.020) than the irregular group (p=0.048).

Discussion

In the 29 female Japanese college long-distance runners, the prevalence of menstrual dysfunction was 68%. The amenorrhea G group had lower BMI and lower skinfolds than the other groups. All groups had more than 30 kcal/kg FFM/day for EA with no differences between the groups.

Menstrual dysfunction

Our study showed a high percentage of menstrual dysfunction (69%). Japanese researchers have previously reported 47.8% to 68.5% menstrual dysfunction in long-distance runners (Abe et al. 1986, Kajiwara and Shimeki 1993, Suzuki 2001, Kikuchi et al. 2008). These percentages were higher than those found for athletes in other countries. Kenyan distance runners had menstrual dysfunction from 32.7% to 40.0% (Goodwin et al. 2014, Muia et al. 2016). Females engaging in endurance sports in western countries such as Canada, Australia, USA, Denmark, and Sweden had menstrual dysfunction from 37.0% to 60.0% (Barrow and Saha 1988, Melin, Tornberg Å et al. 2015, Heikura, Uusitalo et al. 2018).
**Table 1.** Anthropometric, metabolic, energy, and menstrual status of the 29 Japanese female college runners.

| Background                  | All (Number) | Regular (9) | Irregular (8) | Amenorrhea (G) (6) | Amenorrhea (NG) (6) | p-value | Kruskal-Wallis |
|-----------------------------|--------------|-------------|---------------|--------------------|---------------------|---------|---------------|
| Age (year)                  | 19.1 ± 1.1   | 18.8 ± 1.3  | 19.8 ± 1.0    | 19.5 ± 0.5         | 18. 3± 0.5          | 0.032*  | 0.405 0.948 1.000 1.000 0.143 0.364 |
| Running history (year)      | 6.6 ± 1.9    | 7.2 ± 1.5   | 6.3 ± 2.1     | 6.0 ± 2.2          | 6.7 ± 2.3           | 0.524   |               |
| Age of menarche (year)      | 13.2 ± 1.1   | 13.1 ± 1.1  | 12.8 ± 1.3    | 13.8 ± 1.3         | 13.6 ± 0.5          | 0.429   |               |
| VO2 (mL/kg/min)             | 58.0 ± 6.1   | 59.9 ± 7.1  | 55.6 ± 6.4    | 59.6 ± 7.4         | 57.0 ± 3.4          | 0.449   |               |
| TPE (g/dL)                  | 7.1 ± 0.4    | 7.1 ± 0.3   | 7.3 ± 0.5     | 7.1 ± 0.3          | 7.0 ± 0.3           | 0.453   |               |
| Fe (μg/dL)                  | 81.6 ± 29.1  | 80.6 ± 25.2 | 77.1 ± 27.7   | 70.0 ± 26.6        | 98.2 ± 37.6         | 0.528   |               |
| Hb (g/dL)                   | 13.2 ± 0.8   | 13.2 ± 0.6  | 13.5 ± 1.1    | 12.8 ± 0.8         | 13.3 ± 0.6          | 0.432   |               |
| Ferritin (ng/mL)            | 41.9 ± 2.6   | 45.8 ± 23.6 | 47.7 ± 41.8   | 28.3 ± 15.9        | 40.7 ± 14.7         | 0.651   |               |
| Practice time (min/day)     | 187.5 ± 58.9 | 191.4 ± 62.9| 192.5 ± 40.7  | 207.2 ± 80.4       | 156.7 ± 53.5        | 0.516   |               |
| RPE                         | 10.9 ± 1.2   | 11.1 ± 1.2  | 10.4 ± 1.0    | 10.9 ± 1.1         | 11.2 ± 1.7          | 0.634   |               |
| Height (cm)                 | 158.4 ± 6.2  | 155.6 ± 6.7 | 160.9 ± 5.2   | 158.7 ± 7.2        | 158.8 ± 5.2         | 0.396   |               |
| Body mass (kg)              | 46.3 ± 4.9   | 45.7 ± 5.4  | 48.9 ± 4.0    | 42.4 ± 4.4         | 47.9 ± 3.5          | 0.076   |               |
| BMI (kg/m2)                 | 18.5 ± 1.5   | 18.8 ± 1.1  | 18.9 ± 1.4    | 16.8 ± 1.3         | 19.0 ± 12.1         | 0.029*  | 1.000 0.095 1.000 0.032* 1.000 0.151 |
| Σ 8SF (mm)                  | 61.8 ± 17.7  | 67.7 ± 21.3 | 66.1 ± 13.8   | 43.1 ± 5.9         | 66.1 ± 13.9         | 0.013*  | 1.000 0.030* 1.000 0.027* 1.000 0.111 |
| LRS                         | 0.80 ± 0.19  | 0.73 ± 0.21 | 0.76 ± 0.11   | 1.00 ± 0.17        | 0.75 ± 0.14         | 0.034*  | 1.000 0.033* 1.000 0.134 1.000 0.165 |
| Endomorph                   | 2.4 ± 0.6    | 2.6 ± 0.7   | 2.6 ± 0.4     | 1.8 ± 0.1          | 2.5 ± 0.6           | 0.006*  | 1.000 0.008* 1.000 0.012* 1.000 0.096 |
| Mesomorph                   | 2.9 ± 0.9    | 3.1 ± 0.6   | 3.0 ± 1.0     | 2.2 ± 1.1          | 3.1 ± 0.7           | 0.292   |               |
| Ectomorph                   | 3.7 ± 1.0    | 3.3 ± 0.7   | 3.7 ± 1.0     | 4.8 ± 1.1          | 3.5 ± 0.8           | 0.062   |               |
| Body fat (%)                | 19.6 ± 4.5   | 19.9 ± 4.6  | 21.9 ± 2.5    | 15.8 ± 3.4         | 20.6 ± 5.4          | 0.088   |               |
| FM (kg)                     | 8.2 ± 2.3    | 8.1 ± 2.1   | 9.7 ± 1.6     | 6.0 ± 1.4          | 9.1 ± 2.6           | 0.037*  | 1.000 0.491 1.000 0.038* 1.000 0.145 |
| FFM (kg)                    | 32.1 ± 3.3   | 31.0 ± 3.4  | 33.1 ± 3.2    | 31.1 ± 3.8         | 33.5 ± 2.9          | 0.265   |               |
| RMR (kcal/day)              | 1035.2 ± 145.5 | 1050.1 ± 99.3 | 1172.3 ± 100.7 | 917.3 ± 82.7 | 947.8 ± 159.5 | 0.003* | 0.402 0.424 1.000 0.004* 0.039* 1.000 |
| RMR/kg (kg/day)             | 22.7 ± 3.0   | 23.5 ± 3.2  | 24.5 ± 2.6    | 21.8 ± 1.5         | 19.9 ± 2.4          | 0.023*  | 1.000 1.000 0.193 0.335 0.026* 1.000 |
| RMR/FFM (kcal/FFMkg/day)    | 28.4 ± 4.3   | 29.5 ± 4.5  | 31.0 ± 4.4    | 26.8 ± 1.7         | 24.9 ± 3.3          | 0.079   |               |
| TEE (kcal/day)              | 2583.9 ± 300.3 | 2667.4 ± 335.6 | 2625.5 ± 257.5 | 2371.6 ± 261.2 | 2615.2 ± 305.0 | 0.233   |               |
| TEE (kg/kcal/kg/day)        | 56.4 ± 5.7   | 59.5 ± 6.1  | 54.3 ± 6.4    | 54.6 ± 4.4         | 54.8 ± 4.2          | 0.237   |               |
| TEE/FFM (kcal/FFMkg/day)    | 70.7 ± 8.0   | 74.3 ± 8.8  | 69.3 ± 9.4    | 69.3 ± 6.3         | 68.8 ± 5.9          | 0.550   |               |
| PAL                         | 2.5 ± 0.3    | 2.6 ± 0.4   | 2.2 ± 0.2     | 2.6 ± 0.2          | 2.8 ± 0.3           | 0.004*  | 0.112 1.000 1.000 0.084 0.004* 1.000 |
| EI (kcal/day)               | 2190.1 ± 230.0 | 2239.9 ± 189.3 | 2118.9 ± 239.9 | 2300.3 ± 273.1 | 2108.6 ± 213.9 | 0.371   |               |
| EI/kg (kcal/kg/day)         | 48.0 ± 7.2   | 50.4 ± 6.5  | 43.8 ± 7.7    | 54.4 ± 4.1         | 44.1 ± 4.7          | 0.011*  | 0.549 0.616 0.048* 1.000 0.066 |

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Anthropometry

Our study showed that the average BMI for all runners was 18.5 ±1.5 kg/m². However, the BMI ranged from 16.8 ±1.3 kg/m² to 19.0 ±1.2 kg/m², which was still considered to be under the amenorrhea threshold. Interestingly, the NG group had high BMI and yet still suffered amenorrhea. Long-distance running is a lean sport because anthropometric factors including body weight, body composition, and BMI can affect running economy (Brandon and Boileau 1992). Therefore, theoretically, slenderness is advantageous for long-distance running performances (Black et al. 2020). Runners aim to be light and lean by strictly controlling their body weight (Kinoshita and Fukuda 2016). However, focusing on reducing body weight alone can put runners at an increased risk of energy deficiency (Torstveit et al. 2008, Sundgot-Borgen and Torstveit 2010).

Energy deficiency

All groups had EA more than 30 kcal/FFM kg/day, including the amenorrhea groups (G and NG) in our study. FAT statements have explained that energy deficiency causes menstrual disorders (Nattiv et al. 2007, Souza et al. 2014, Joy et al. 2016). EA less than 30 kcal/FFM kg/day has been associated with reduced LH plasticity (Loucks and Thuma 2003), therefore, FAT researchers have been using this threshold value for evaluating FAT risk (Melin et al. 2015). However, in our study, the results did not support the threshold value of 30 kcal/FFM kg/day. For this reason, energy deficiency is unlikely to be the sole cause of amenorrhea in this Japanese cohort.

It is difficult to say that energy deficiency (calculating EA over weeks) leads to menstrual disorders (often over months or years) in female athletes using cross-sectional research even when there is some evidence of it (Loucks and Thuma 2003, Mountjoy et al. 2018). Such evidence has been mixed in FAT research, especially for menstrual disorders (Awdishu et al. 2009). There is a gap in basic research that captures the phenomenon that causes menstrual disorders due to less EA and clinical research (Loucks and Thuma 2003) targeting athletes in specific sports. FAT is an athlete’s health issue that needs to be prevented; therefore, it is important to consider the health issues of athletes in conjunction with EA. The current use of dietary supplements among long-distance runners needs to be clarified and considered in conjunction with EA, and athlete’s health issues can be prevented by considering athletic characteristics (Barrack et al. 2021). Interestingly, the amenorrhea G group had the highest EI/kg of all the groups. This could possibly be explained by participants’ eating attitudes being affected after having received advice from the gynecologist to eat more. It seems that the gynecologist's advice had a positive effect on eating behaviors.

| El/FFM (kcal/FFMkg/day) | 60.7 ± 9.5 | 63.9 ± 9.1 | 56.3 ± 10.7 | 67.3 ± 7.0 | 55.6 ± 5.8 | 0.076 |
|-------------------------|------------|------------|-------------|------------|------------|-------|
| ExEE (kcal/day)         | 885.7 ± 382.8 | 904.4 ± 317.2 | 781.3 ± 334.4 | 1070.6 ± 591.7 | 783.9 ± 196.8 | 0.740 |
| EA (kcal/FFM/kg/day)    | 37.0 ± 10.3 | 38.3 ± 8.0 | 38.0 ± 10.4 | 35.7 ± 17.0 | 35.3 ± 2.5 | 0.858 |

*P<0.05. TP = Total protein, Fe = serum iron, Hb = hemoglobin, RPE = rate of perceived exertion, BMI = body mass index, 8SF = sum of eight skinfolds, LRS = leanness ratio score, FM = fat mass, FFM = fat free mass, RMR = resting metabolic rate, TEE = total energy expenditure, PAL = physical activity level, EI = total energy intake, ExEE = exercise energy expenditure, EA = energy availability, R = Regular, I = Irregular, AG = Amenorrhea G (AG), ANG = Amenorrhea NG.
The six runners visited the gynecologist at different times (three months prior was the latest time for one of the runners to see a gynecologist, and the longest time was two years prior). Nevertheless, no changes in menstrual cycle were seen after consulting a gynecologist. To prove that eating more can change menstrual conditions, longitudinal research is needed rather than cross-sectional research such as our study.

**Limitations**

Limitations of this study included the small number of participants, all of whom belonged to one team. This cross-sectional study showed the present condition of FAT risk. However, the study could not determine causes of menstrual dysfunction. It is necessary to build evidence through retrospective studies that examine the historical background and prospective observational studies regarding the effects of countermeasures against menstrual disorders, including the possibility that the causes of menstrual disorders may differ from person to person. It is not easy to conduct longitudinal studies of female athletes due to time, cost, and availability of practical, in-the-field data collection methods. Cross-sectional research is helpful for understanding the current menstrual disorders of competitive athletes so that preventive measures can be taken to ensure irregular menstruation does not worsen.

The comprehensive approach taken in our cross-sectional study to measure anthropometry, metabolic, and energy variables likely to be related to menstrual dysfunction only showed differences between amenorrhea G and the other groups for anthropometry characteristics (BMI, Σ8SF, LRC, somatotype score, and fat mass). Therefore, it is proposed that longitudinal studies of competitive female athletes are needed, where height, body mass, and skinfolds are measured regularly to enable evaluation of BMI, Σ8SF, and the LRS as possible indicators for menstrual dysfunction over time.

**Conclusions**

There was a high prevalence of menstrual dysfunction in female Japanese college long-distance runners. Runners with amenorrhea had lower BMI and lower skinfolds than the other groups, even after having consulted a gynecologist for medical support. All runners had an energy deficiency higher than the published threshold; therefore, this threshold did not differentiate the amenorrhea or irregular menstruating athletes from regular menstruating athletes.

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Conflict of interest

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