Energy expenditure adjusted for body composition differentiates constitutional thinness from both normal subjects and anorexia nervosa

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Departments of 1Endocrinology, 2Rheumatology, 3Nuclear Medicine, and 4Psychiatry, Centre Hospitalier Universitaire, Saint-Etienne, France; 5Department of Endocrinology, University of Medicine and Pharmacy, “Gr.T.Popa,” Romania; and 6Centre de Recherche en Nutrition Humaine de Lyon, France

Submitted 22 May 2006; accepted in final form 8 August 2006

Bossu C, Galusca B, Normand S, Germain N, Collet P, Frere D, Lang F, Laville M, Estour B. Energy expenditure adjusted for body composition differentiates constitutional thinness from both normal subjects and anorexia nervosa. Am J Physiol Endocrinol Metab 292: E132–E137, 2007. First published August 15, 2006; doi:10.1152/ajpendo.00241.2006.—Constitutional thinness (CT) is characterized by a low and stable body mass index (BMI) without any hormonal abnormality. To understand the weight steadiness, energetic balance. Fat-free mass (FFM) (kg) was similar in CT and AN (32.5 ± 2.9 vs. 34.1 ± 1.6). While RMR absolute values (kJ/day) were lower in CT (4,839 ± 209), RMR values adjusted for FFM were the highest in CT. TEE-to-FFM ratio was also higher in CT than in controls. Energetic metabolism balance maintains a stable low weight in CT. An increased energy expenditure-to-FFM ratio differentiates CT from controls and could account for the resistance to weight gain observed in CT.

energy metabolism balance; doubly labeled water

CONSTITUTIONAL THINNESS (CT), or leanness, was identified over the last decades as a nonpathological state of underweight that does not meet the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria of anorexia nervosa (AN) (4). Contrary to the later pathology, the nonpathological state of CT in adult women is supported by the presence of menstruations (43), normal thyroid (32) and cardiac functions (36), and normal insulin sensitivity (47). Moreover, CT individuals are characterized by a body weight that has always been in the lower percentiles for age, gender, and ethnicity (2, 7). Familiality and heritability of thinness were also described (23, 28, 33) or demonstrated (7).

In a previous study (48), we evaluated CT women with low body mass indexes (BMIs) similar to those of AN (i.e., BMI <17.5 kg/m²). We found nutritional markers such as free T3 and IGF-I to be within normal ranges, whereas leptin levels were mildly decreased. Growth hormone (GH), ghrelin, and cortisol profiles also presented normal values, contrary to AN (48).

The mechanism behind low-weight steadiness in CT was not elucidated. Multifactorial etiology involves a combination of genetics in addition to yet unrecognized pathophysiological factors (7). Energy metabolism is considered a key target of genetic influences (31). Recently, gene determinations of thinness were postulated, some of them related to increased insulin sensitivity (12, 24) and others to regulation of feeding behavior and metabolic rate (13, 29). Conversely, it was shown that insulin sensitivity is normal in CT (47), and only some components of energy metabolism, like resting metabolic rate and postprandial thermogenesis, were evaluated (41, 49). Considering these incomplete data, we conducted a prospective study to evaluate the energy metabolism, including simultaneous assessment of food intake energy and total energy expenditure in very-low-weight CT subjects. We compared these data with those of normal BMI controls and with those of AN patients displaying similar very low body weight.

SUBJECTS AND METHODS

This study was reviewed and approved by the ethics committee on human research of Saint Etienne, France, and all subjects gave written, informed consent.

Subjects

Three groups of Caucasian female subjects were recruited for the study: seven CT, six AN, and seven controls. The studied CT and AN were matched by BMI.

The seven CT subjects were recruited at our outpatient clinic among the patients evaluated for leanness, using the following criteria: BMI between 14.5 and 16.5 kg/m², stable throughout the postpubertal period, presence of physiological menstruations without estrogen-progestative treatment, and the desire for weight gain as the main reason for medical consultation.

All six AN subjects displayed active psychiatric disease according to the criteria of the DSM (1). Before onset of disease, the BMIs of the subjects ranged between 19.1 and 21.5 kg/m². Thereafter, BMIs

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decreased to values <16.5 kg/m², where they remained nearly stable during the last month before the study. Patients were recruited before starting any therapeutic approach. None of them used oral contraceptives, and all presented with secondary amenorrhea for at least 6 mo. Secondary amenorrhea occurred at least 12 mo after the first menses period. All patients had the restrictive form of AN. None of the subjects had a previous history of endocrine disease or psychiatric therapy at the moment of inclusion in the protocol.

The weight history from birth to 18 yr for each thin patient (including AN and CT) was retrospectively reconstructed using medical records and plotted against a reference set of BMI from the 3rd to 97th percentile (40). The family pedigree of weight was also recorded for three generations.

Seven controls (mean BMI 21.2 ± 1.1 kg/m²) were matched by age (18–26 yr) with AN and CT subjects.

In CT and controls, all data were collected during the follicular phase of cycle. None of the subjects included in this study was documented with a chronic or congenital disease or a form of addictive or abusive consumption (alcohol, smoking, drugs, or physical activity, etc.). None of them was taking any medication.

**Psychological Profile**

The food-related behavioral problems were evaluated by four psychiatric reference questionnaires in all three groups of subjects: the "silhouette" questionnaire of Jaeger et al. (22), the Dutch Eating Behavior Questionnaire validated by Van Strien et al. (51), the Eating Disorder Inventory (17), and the Eating Disorders Examination of Cooper and Fairburn (10). The psychological questionnaires measured complementary behavioral or psychiatric dimensions.

**Body Composition Measurements**

Dual-energy X-ray absorptiometry (DEXA) allowed for the quantification of the percentage of total body fat mass (FM) and fat-free mass (FFM) expressed in kilograms (LUNAR, DPX-L, <1% coefficient of variation) (16, 30).

**Blood Samples**

Fasting blood samples were immediately centrifuged, and plasma was stored at −80°C for each subject. Leptin was measured by radioimmunological technique (RIA Human Leptin kit, Linco Research). The manufacturer’s reference range for a normal BMI (18–25 kg/m²) was 3.7–11.1 μg/l. The coefficient of interassay variation was 4.5%. Free T3 was measured by radioimmunological technique (Immunotech, Marseille, France). The manufacturer’s reference range was 2.5–5.8 pmol/l. The coefficient of interassay variation was 4.9%. IGF-I was measured by immunoradiometric assay technique (Immunotech). The manufacturer’s reference range was 219–644 ng/ml within the 20- to 30-yr-old age group. Interassay coefficient of variation was ≤6.8%.

**Energy Metabolism Assessment**

**Energy intake.** The assessment of dietary intake was realized over a period of 4 days, including 2 weekdays and a weekend. The subjects reported the four 24-h dietary records using an instruction manual for codification of foods, including photographs for estimations of portion size (3). Foods were presented in three sizes, including intermediate and extreme positions, permitting seven choices of the amount. Photographs of the portion sizes were previously validated for the SU.VI.MAX study (26). A nutritionist met with subjects to explain the collection of the data and then to ensure the accuracy of the collected data.

Food quotient (FQ) was calculated using Black’s formula (5):

FQ = [(710.71)P + (1,377.06F) + (746C)]/(879.06P + (1,948.34F) + (746C)), where P, F, and C are protein, fat, and carbohydrate intakes, respectively, expressed as grams per day.

**Total energy expenditure.** Total energy expenditure (TEE) was measured in ambulatory using doubly labeled water (DLW), a gold standard method (6, 44). The DLW technique uses a mixture of water labeled with two stable isotopes (²H,¹⁸O, Eurisoprt): deuterium (²H) and oxygen-18 (¹⁸O) (6, 45). After baseline urine samples were provided, a premixed dose of 0.05 g/kg ²H₂O (99.9% ²H) and 1.05 g/kg H₂¹⁸O (10% ¹⁸O) was administered to the subjects. After dosing, two urine samples were collected at 3 and 4 h. Ten and fourteen days after dosing, the subjects returned to the laboratories, and urine samples were collected from two consecutive voids. Urine was stored at −20°C in cryogenically stable tubes until analysis by isotope ratio mass spectrometry. TEE was determined using the two-point method according to Schoeller et al. (44). The dilution spaces for ²H and ¹⁸O were calculated from the baseline and urine samples according to Coward et al. (38). The total body water (TBW) was calculated from the average of the dilution space of deuterium and oxygen-18, after correction for the isotopic exchanges, of 1.041 and 1.007, respectively. The production of CO₂ was calculated according to the equation of Schoeller and al. (44)

\[
r\text{CO}_2 (\text{mol/day}) = \frac{N}{2.078} (1.007k_o - 0.0246 \cdot N \cdot 1.05(1.007k_o - 0.041k_d))
\]

with N being the average space of dilution of ²H and of ¹⁸O calculated by the method of the “plateau” using the isotopic enrichments of the 4-h postdose sample; k₀ and k₁ represent the hillsides of elimination calculated by linear regression of the logarithm of the isotopic enrichment according to time (days).

The following formula was used to calculate TEE:

\[
\text{TEE (kJ/day)} = r\text{CO}_2 \times (1.1 + 3.9/RQ) \times 22.4 \times 4,018 \times 4,187/1,000
\]

Fasting respiratory quotient (RQ) was measured by indirect calorimetry.

**Resting metabolic rate.** Resting metabolic rate (RMR) was determined by use of the Deltatrac device for indirect calorimetry (Datex Instrumentarium, Helsinki, Finland) (15). Data were collected in the morning, after 12 h of fasting (alcohol and caffeine ingestion included) and physical activity restriction. A 1-h resting period preceded the assessment of RMR.

**Energy expenditure related to physical activity.** Physical activity was assessed with the MONICA Optional Study of Physical Activity Questionnaire (MOSPA-Q) (34), whose validity and reliability have been reported (39). MOSPA-Q quantified the average and daily energy expenditure over the last year, via professional, leisure, or sport activities.

Physical activity level (PAL = TEE/RMR) and activity energy expenditure (AEE = TEE − RMR) were also estimated.

To adjust energy expenditure for metabolic mass (FM), RMR-to-FFM and TEE-to-FFM ratios were calculated as previously reported (25, 37).

**Statistical Analysis**

The results are presented as means ± SD. ANOVA was first used to perform a three-group analysis comparing constitutionally thin women with anorexic women and controls. When ANOVA was significant, we performed post hoc ANOVA tests (Fisher’s protected least significant difference and Tukey’s-S-Kramer’s test) for comparisons within all groups. Spearmann correlation index was calculated between leptin and FM in thin patients group (including CT and AN subjects) and between FFM and RMR in overall group. P < 0.05 was considered statistically significant (Statview 4.5 software).
RESULTS

The psychological profile of CT women indicated that they wanted to gain weight. Typical psychological profile including food limitation, emotionality, and external excitement was evidence in AN patients but not in CT (data not shown).

In the CT group, the mean BMI was very low, approximately the 3rd percentile, throughout the growth period until the age of 18 (Fig. 1). Conversely, the mean BMI in AN subjects was normal (20.7 ± 1.0 kg/m²) before the onset of anorexic tendencies.

After excluding the index cases, we found an average of 2.5 thin subjects per family within the CT group. After a similar exclusion, the average of thin subjects in AN families was ~0.5 per family.

The results of energy balance in every group of study are shown in Table 1. The self-reported caloric intake for the CT subjects (7,565 ± 908 kJ/day) was similar to that of controls (7,961 ± 1,452 kJ/day) and significantly higher than that of the AN subjects (4,894 ± 703 kJ/day, P < 0.05). Similar percentages of nutrient consumed were found in CT and control groups. Compared with both controls and CT groups, the percentage of lipids consumed in AN patients was significantly lower.

TEE measured by DLW (kJ/day) was similar in all three groups. Compared with both controls and CT groups, the mean calculated difference between energetic expenditure and caloric intake was similar in CT (816 kJ/day) and controls (832 kJ/day) and significantly higher in AN (3,161 kJ/day, P < 0.05) (Fig. 2).

By use of indirect calorimetry, the RMR of CT (4,839 ± 473 kJ/day) was found to be lower than in controls (5,576 ± 209 kJ/day) and higher than AN (3,810 ± 937 kJ/day, P < 0.05). The CT energy expenditure due to physical activity, evaluated by MOSPA-Q (1,430 ± 284 kJ/day), was not significantly different from that of controls (2,051 ± 1,314 kJ/day) and AN subjects (1,511 ± 962 kJ/day). PAL and AEE of CT subjects were similar to those of controls, whereas in AN, they tend to be higher (Table 1).

The results of body composition measurements are shown in Table 2. The mean BMI (kg/m²) does not differ between the CT and AN groups (16.1 ± 0.6 vs. 15.8 ± 0.8, P = 0.35). The FFM (kg) was significantly lower in CT compared with controls (32.5 ± 2.9 vs. 37.8 ± 1.6, P = 0.026) and similar to that of AN (34.1 ± 1.9, P = 0.31). The FM (%) was significantly different among the three groups (CT, 18.3 ± 2.9; AN, 9.4 ± 5.4; controls, 26.2 ± 4.1, P < 0.05). RMR displayed a strong linear relationship with FFM (r = 0.522, P < 0.05) within the overall group (Fig. 3).

When adjusted for FFM, RMR and TEE values (kJ/kg FFM) were significantly higher in CT than in controls (148.6 ± 5.4 vs. 131.8 ± 10.4, P = 0.009; 259.5 ± 40.6 vs. 208.0 ± 29.5, P = 0.03, respectively) (Table 1).

Similar FQ values were found in all groups. RQ was not found significantly different in CT and controls and was higher in AN.

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Table 1. Energy balance of constitutional thinness, anorexia nervosa, and control subjects of the study

<table>
<thead>
<tr>
<th></th>
<th>CT (n = 7)</th>
<th>C (n = 7)</th>
<th>AN (n = 6)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Self-reported food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intake, kJ/day</td>
<td>7,565±908</td>
<td>7,961±1,452</td>
<td>4,894±703</td>
<td>a, c</td>
</tr>
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<td>Protein intake, %</td>
<td>13.0±2.2</td>
<td>14.5±1.9</td>
<td>18.8±5.4</td>
<td>a, c</td>
</tr>
<tr>
<td>Carbohydrate intake, %</td>
<td>50.7±7.5</td>
<td>46.4±5.1</td>
<td>50.6±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Lipids intake, %</td>
<td>36.2±6.7</td>
<td>39.0±5.6</td>
<td>30.5±8.1</td>
<td>a</td>
</tr>
<tr>
<td>TEE, kJ/day</td>
<td>8,382±988</td>
<td>8,793±845</td>
<td>8,001±215</td>
<td>NS</td>
</tr>
<tr>
<td>RMR, kJ/day</td>
<td>4,839±473</td>
<td>5,576±209</td>
<td>3,810±937</td>
<td>a, c</td>
</tr>
<tr>
<td>RQ</td>
<td>0.82±0.01</td>
<td>0.83±0.01</td>
<td>0.89±0.02</td>
<td>a, c</td>
</tr>
<tr>
<td>FQ</td>
<td>0.85±0.01</td>
<td>0.84±0.01</td>
<td>0.86±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>AEE, kJ/day</td>
<td>5,542±464</td>
<td>3,207±410</td>
<td>4,191±967</td>
<td>NS</td>
</tr>
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<td>PAL</td>
<td>1.75±0.12</td>
<td>1.57±0.07</td>
<td>2.14±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>TEE/FFM ratio</td>
<td>259.5±40.6</td>
<td>208.0±29.5</td>
<td>234.4±69.5</td>
<td>b</td>
</tr>
<tr>
<td>RMR/FFM ratio</td>
<td>148.6±5.4</td>
<td>131.8±10.4</td>
<td>111.3±25.0</td>
<td>b, c</td>
</tr>
</tbody>
</table>

Values are means ± SD. CT, constitutional thinness; AN, anorexia nervosa; C, control; TEE, total energy expenditure; RMR, resting metabolic rate; RQ, respiratory quotient; FQ, food quotient; AEE (TEE – RMR), activity energy expenditure; PAL (TEE/RMR), physical activity level. *P < 0.05 between C and AN; †P < 0.05 between C and CT; ‡P < 0.05 between AN and CT; NS, nonsignificant.

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Fig. 1. Mean body mass index (BMI) growth curve of constitutionally thin (CT) subjects (n = 7). The BMI history from birth to 18 yr was reconstituted retrospectively (using medical records) for each thin patient; mean values were calculated and plotted against a reference set of BMI from 3rd to 97th percentile (40).

Fig. 2. Energy metabolism in CT, young women controls (C), and anorexia nervosa (AN). Shown are mean values of self-reported food intake energy, total energy expenditure (TEE), resting metabolic rate (RMR), and physical activity (PA).
Among the three groups, free T3, IGF-I, and leptin were significantly lower in AN subjects and showed no significant difference between CT and controls (Table 2).

In observing very thin patients (consisting of both AN and CT subjects), we found a strong correlation between leptin and BMI.

Among severely underweight subjects (including AN and CT subjects), a strong correlation between leptin and FM was measured by DEXA was found. In AN patients, serum leptin is extremely low (<3 ng/ml), whereas in CT subjects, leptin levels were in the normal range (8–12 ng/ml). Similarly, FM is diminished in AN subjects but in CT subjects is present with normal percentages. Therefore, very thin patients with nearly normal levels of leptin and FM should not be confused and diagnosed as AN. This evidence proposes leptin alongside other clinical features in differentiating CT from AN.

To date, no studies have been published on the complete evaluation of energy metabolism observed in CT. Two major aspects of this entity, the steadiness and the etiology of their very low body weight, remain unexplained. To observe whether a possible relationship exists with these aspects, we compared CT energetic profile with those of AN and controls.

First, we found a negative energy balance in restrictive AN. To our knowledge, this is the first study to perform a complete evaluation of energy metabolism (considering both energy intake and energy expenditure) in AN. Although food intake evaluation is questionable in AN, our AN subjects displayed severe-to-very severe reduction of caloric intake (around 4,500 kcal/day), similar to results found in previous studies (18, 20). AN energy expenditure profiles including TEE (8,001 ± 2,152 kcal/day) and RMR (3,810 ± 937 kcal/day) were also found to be similar to those in other studies (35, 52). These collaborative data lead us to say that negative balance of AN is not questionable. The real question is the discrepancy between this negative energy balance and the apparent steady weight in AN (weight loss slows when very low values of BMI are reached). However, the study design was not focused on this pathology and thus did not allow for the clarification of this problem.

Next, in our study, self-reported caloric intake of CT subjects was not found significantly different from that of controls but was higher than in AN. Moreover, the percentages of each nutrient consumed in CT subjects were also found to be similar to those of controls. As already mentioned above, AN subjects showed a lower caloric and lipid intake and a higher percentage of protein intake (18, 19). Using DLW assessment, we observed that TEE in the CT group was not significantly different from that of controls and AN. In the CT group, the difference between the energy expenditure and the energy intake (816 kcal/day) was similar to that of controls (832 kcal/day), values that, according to literature, are insignificant (42). This demonstrates for the first time that energy metabolism profile explains the steady weight in this phenotype of thinness.

TEE is accounted for by three main components: RMR (resting energy expenditure − ~60% of TEE), physical activity (nonresting energy expenditure − ~30% of TEE), and the thermic effect of feeding (~10% of TEE) (25, 37). RMR was found to be lower in the CT group than in controls but higher than in AN. Similar results have been found in constitutionally lean children (49). Physical activity evaluated by MOSPA-Q...
displayed no difference between CT and controls. Because TEE found in CT was equivalent to that of controls, composed of similar physical activity but lower RMRs, we deduced an increase in nonexercise activity thermogenesis in CT that could explain their resistance to weight gain (27).

RMR is essentially connected to active cellular mass mainly represented by muscles and compartments of FFM (8, 50), and resistance to diet-induced obesity (11). When adjustments for the differences in metabolic mass were made, RMR and TEE values were found significantly higher in CT than in controls. This relative increase in energy expenditure could also account for the “inability” of CT subjects to gain weight when desired. Interestingly, a similar lean phenotype was described in RIβ knockout mice including normal food intake, increased metabolic rate and body temperature, and resistance to diet-induced obesity (11).

Because a stable weight in CT denotes equilibrium in energy metabolism, the etiology of this phenotype remains to be explored. Candidate genes, controlling body composition and resistance to the development of obesity (7), should be considered in further research. Two major traits sustaining this hypothesis, the consistency of a low BMI throughout life and familiality, were confirmed in our study.

ACKNOWLEDGMENTS

We thank Ms. Iochebie Jolie Pun from the Michael G. DeGroote School of Medicine, Hamilton, ON, Canada, for correcting the English of this paper.

GRANTS

This work was supported by a grant from the Direction Régionale de la Recherche Clinique Saint Etienne, France.

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