**CLINICAL STUDY**

**L-Tri-iodothyronine is a major determinant of resting energy expenditure in underweight patients with anorexia nervosa and during weight gain**

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**Abstract**

*Objective:* We aimed to define the effect of L-3,5,3'-tri-iodothyronine (T3) on metabolic adaptation in underweight patients with anorexia nervosa (AN) as well as during weight gain.

*Methods:* This involved clinical investigation of 28 underweight patients with AN, who were compared with 49 normal-weight controls. A subgroup of 17 patients was followed during weight gain. Resting energy expenditure was measured by indirect calorimetry. Body composition was measured by anthropometry as well as bioelectrical impedance analysis. Energy intake (EI) was assessed by a 3-day dietary record. Plasma concentrations of thyroid hormones (thyroxine (T4), T3 and thyrotropin (TSH)) were analyzed by enzyme immunoassays.

*Results:* When compared with normal-weight women, underweight patients with AN had reduced fat mass (FM) (-27.1%), fat-free mass (FFM) (-13.1%), resting energy expenditure (REE) (-21.8%), T3 (-33.4%) and T4 concentrations (-19.8%) at unchanged TSH. REE remained reduced after adjustment for FFM (-24.6%). T3 showed a close association with REE. This association remained after adjustment of REE for FFM. Treatment of underweight AN patients resulted in a mean weight gain of 8.3 kg. This was mainly explained by an increase in FM with small or no changes in FFM. REE and T3 also increased (+9.3% and +33.3% respectively) at unchanged TSH and T4. There was a highly significant association between weight gain-induced changes in T3 and changes in adjusted REE (r = 0.78, P < 0.001, based on Pearson’s correlation). An increase in plasma T3 concentrations of 1.8 pmol/l could explain an increase in REE of 0.6 MJ/day (that is, a 32% increase in T3 was associated with a 13% increase in REE).

*Conclusions:* Our data provide evidence that the low T3 concentrations add to metabolic adaptation in underweight patients with AN. During weight gain, increases in T3 are associated with increases in REE, which is independent of FFM. Both results are evidence for a physiologic role of T3 in modulation of energy expenditure in humans.

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**Introduction**

Thyroid status is a major determinant of resting energy expenditure (REE). Hyperthyroidism increases while hypothyroidism decreases REE (see reviews (1, 2)). In addition, physiologic variations in plasma L-3,5,3'-tri-iodothyronine (T3) concentrations are also associated with between- and within-subject variations in REE (3–6). There is some experimental and also human evidence that thyroid hormones modulate energy expenditure in response to changes in energy and macronutrient intake (7–10). A low T3 status is observed in starvation and in underweight subjects, suggesting a role for T3 in metabolic adaptation to these situations (8, 9).

Compared with normal-weight subjects, underweight patients with anorexia nervosa (AN) have low serum T3 concentrations, reduced fat-free mass (FFM) and reduced REE (11–16). However, since FFM is the major determinant of REE (17), the individual contributions of both factors (FFM and T3) to the adaptation of REE to underweight are unclear (10, 18–20). The present study investigated the association between REE, FFM and T3 in a group of underweight patients with AN who were compared with a normal-weight control group (cross-sectional study). In addition, changes in REE were compared with changes in FFM and T3 in a subgroup of AN patients during weight gain (longitudinal study). The results provide evidence for a mass-independent effect of T3 on REE.
Materials and methods

Study population and design
A total of 28 women (mean age 25±7 years) meeting criteria for AN based on the Diagnostic and Statistical Manual of Mental Disorders – IV (21) were recruited before the start of in-patient treatment in the Psychosomatic Clinic Bad Bramstedt, Germany. During the study period, all patients were enrolled in a multidisciplinary psychotherapy and nutritional treatment program, and encouraged to gain an average of 700 g body weight weekly. No patient had a medical condition (other than AN) or received hormonal or other medication known to affect body composition and/or energy expenditure. Body composition, energy intake and resting energy expenditure were assessed, and serum hormone concentrations were analyzed within the first days after referral in all patients (time (T) 0). Delta (T0-T1) was 43±17 patients were re-investigated during weight gain (that is, at T1 and T2). Delta (T0-T2) was 84±11 days. A control group (C) comprised 49 age-matched, normal-weight, healthy women (mean age 25±3 years), who were examined at the Institute of Human Nutrition, University of Kiel, Germany. Control subjects were recruited by advertisement from students of nutritional sciences.

Body composition
Standard procedures were used to assess body height and weight (SECA, Model 2200; Vogel & Halke, Hamburg, Germany). Skinfold thickness was determined with a skinfold caliper (Model 01 127; Lafayette Instrument Co., Lafayette, IN, USA.) at four sites (biceps, triceps, subscapular and supra-iliacal). Body fat was calculated by the equation of Durnin and Womersley (22). Bioelectrical impedance analysis (BIA) was carried out (Body Composition Analyser, Model TVI-10, Danninger Medical Technology, Detroit, MI, USA), and fat mass (FM) and fat free mass (FFM) were calculated with a software package (Bodycomp; Danninger Medical), as described previously (23). FM as assessed by BIA, and skinfold measurements showed a strong correlation (r = 0.89 for all AN + C; r = 0.76 for AN alone).

Energy expenditure and energy intake
REE was measured by indirect calorimetry (Deltatrac TM; Datex Instrumentarium, Helsinki, Finland), as described previously (23), using Weir’s equation (24). REE was also predicted by Harris and Benedict’s equation (25). Energy and macronutrient intake was assessed by a 3-day dietary record, using a software program for data analysis (PRODI 4.5. LE 2001 Expert; Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany). The diet consumed by the patients during weight gain was provided as normal food. All subjects received detailed instructions for completing the 3-day food records. Dietary intake data was obtained from 96.4% (27/28) of patients at T0, 94.1% (16/17) at T1 and 100% (6/6) at T2 respectively. Food records were tested for plausibility by using REE x 1.25 as the cutoff for EI. None of the AN patients were below the cutoff.

Blood samples
Blood samples were obtained between 0700 and 0800 h after overnight fast. Plasma was immediately frozen at −80 °C until analysis. Plasma concentration of T3, T4 and TSH were analyzed by Electro Chemiluminescence Immuno Assay (ECLIA, Elecsys; Roche Diagnostics, Mannheim, Germany). The normal ranges were 2.8–7.1 pmol/l, 12.0–22.0 pmol/l and 0.27–4.2 mU/l for T3, T4 and TSH respectively.

Ethics
The study protocol was approved by the ethics committee (Medical Faculty of the Christian-Albrechts University of Kiel, Germany). After being informed, patients gave written consent.

Statistics
Statistical analyses were performed by SPSS for Windows (Statistical Package for Social Science 8.0; SPSS, Chicago, IL, USA). Data are presented as means±S.D. Pearson’s correlation or Spearman’s correlation coefficients (for noncontinuous variables) were calculated to test for relationships among different parameters. The Mann–Whitney U-test was used for comparisons between groups, and the Wilcoxon test for intraindividual, longitudinal changes of one parameter. P values of <0.05 were considered statistically significant. Resting energy expenditure was adjusted (adj) for FFM by the following equation: $REE_{adj} = \frac{REE_{meas} - \text{FFM}_{BIA} \cdot \text{group mean} - \text{FFM}_{BIA} \cdot \text{individual}}{\text{kg}} \times a$, where $d$ = day, $REE_{meas}$ = REE measured by indirect calorimetry, and $a$ = slope of regression line FFM vs $REE_{meas}$ for patients and controls. A stepwise multiple regression analysis was performed with REE as dependent variable and FFM and T3 as determinants.

Results
Cross-sectional data (Table 1 and Fig. 1a–c)
Compared with normal-weight women, underweight patients with AN (AN T0) had reduced FM ($-71.3\%$), FFM ($-13.1\%$), REE ($-21.8\%$), $T_3$ ($-33.4\%$) and $T_4$ concentrations ($-19.8\%$) at unchanged TSH (Table 1). REE remained reduced after adjustment for FFM ($-24.6\%$). There was a close association between REE and FFM in controls as well as in underweight patients with AN (Fig. 1a).
Table 1 Nutritional status, resting energy expenditure (REE) and serum concentrations of thyrotropin (TSH), free 3,5,3’-tri-iodothyronine (fT3) and free thyroxine (fT4) of patients with anorexia nervosa (AN) studied at admission (AN T0), after 6 weeks (AN T1) and after 12 weeks (AN T2) of clinical treatment (longitudinal study). Data were compared with healthy controls and are presented as means±S.D.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 49)</th>
<th>AN T0 (n = 28)</th>
<th>AN T1 (n = 17)</th>
<th>AN T2 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>63.1±7.8</td>
<td>42.9±4.5*</td>
<td>49.6±4.2*</td>
<td>49.1±2.1*</td>
</tr>
<tr>
<td>BMIs (kg/m²)</td>
<td>22.3±2.3</td>
<td>15.1±1.5*</td>
<td>17.5±1.7*</td>
<td>17.2±1.1*</td>
</tr>
<tr>
<td>FMa (%)</td>
<td>36.5±3.4</td>
<td>31.4±1.2*</td>
<td>39.7±2.1*</td>
<td>41.0±3.5*</td>
</tr>
<tr>
<td>FFMa (kg)</td>
<td>42.2±3.4</td>
<td>36.7±3.6*</td>
<td>39.0±3.1</td>
<td>36.9±2.0</td>
</tr>
<tr>
<td>∑Skinfolds (mm)</td>
<td>51.9±12.9</td>
<td>22.4±8.7*</td>
<td>38.9±9.7*</td>
<td>41.0±6.5*</td>
</tr>
<tr>
<td>REEa (MJ/day)</td>
<td>5.5±0.7</td>
<td>4.3±0.5*</td>
<td>5.1±0.6*</td>
<td>4.7±0.6</td>
</tr>
<tr>
<td>REEq (MJ/day)</td>
<td>5.7±0.5</td>
<td>4.3±0.4*</td>
<td>5.1±0.6*</td>
<td>4.7±0.6</td>
</tr>
<tr>
<td>REE–REEp (MJ/day)</td>
<td>−0.5±1.0</td>
<td>−1.0±0.4*</td>
<td>−0.5±0.6*</td>
<td>−0.9±0.6</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.4±0.9</td>
<td>1.7±1.1</td>
<td>1.6±0.8</td>
<td>2.2±1.2</td>
</tr>
<tr>
<td>fT3 (pmol/l)</td>
<td>16.7±2.7</td>
<td>13.4±1.4*</td>
<td>13.8±2.4</td>
<td>15.3±2.5</td>
</tr>
<tr>
<td>fT4 (pmol/l)</td>
<td>5.4±1.1</td>
<td>3.6±0.9*</td>
<td>4.7±1.0*</td>
<td>4.5±1.1</td>
</tr>
</tbody>
</table>

*Significant differences between AN and C (Mann–Whitney–U test); P < 0.001.
1,2,3Significant longitudinal changes for AN T1 vs AN T0 and AN T2 vs AN T0 (Wilcoxon test): 1P < 0.05; 2P < 0.01; 3P < 0.001.
4Significant longitudinal changes for AN T2 vs AN T1 (Wilcoxon test): P < 0.05; 4P < 0.01.

AN patients had lower REE values at any given FFM. The two regression lines differ with respect to slope (lower in patients), the y-intercept (both different from zero but higher in AN patients) and predictive value (lower in AN patients). T1 also showed an association with REE (Fig. 1b) (r = 0.49 and 0.56 in AN patients and controls alone respectively; P < 0.01). This association remained after adjustment of REE for FFM (Fig. 1c).

Longitudinal data (Table 1 and Fig. 2)

Treatment of underweight AN patients resulted in a considerable weight gain (5.9±2.2 and 8.2±2.3 kg at T1 and T2 respectively; P < 0.0001) (Table 1). This was mainly explained by an increase in FM (4.5±1.7 and 7.4±2.2 kg at T1 and T2 respectively; P < 0.05) at small or no changes in FFM (n.s.) (Table 1). Concomitantly, REE (18.6% at T1 and 14.0% at T2 respectively; P < 0.05) and free T3 (30.5% at T1 and 47% at T2 respectively; P < 0.01) also increased, while TSH and T4 remained unchanged (Table 1). There was no significant association between changes in FFM and changes in REE. In contrast, there was a highly significant association between changes in T3 and changes in adjusted REE (Fig. 2). Patients with a high (>1.7 pmol/l) median of response, ΔT3/T3 and those with a low response (<1.7 pmol/l) did not differ in changes in body weight, FM and FFM. However, there were significant between-group differences in REE (0.1±0.3 vs 1.1±0.8 MJ/day in low and high T3 responders respectively; P < 0.05) and REEadj for FM (0.8±0.2 vs 1.0±0.8 MJ/day respectively; P < 0.05).

At T0, T1 and T2, there were no differences in dietary intake (EI 181 ± 12 kcal/day, including 33.0% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05). At T0, T1 and T2, there were no differences in dietary intake (EI 1821 ± 92 kcal/day, including 30.3% fat (F), 55.2% carbohydrates (CHO) and 14.5% protein (P) (P < 0.05).

Figure 1 (a–c) Regression between fat-free mass (FFM) and resting energy expenditure (REE) in 49 healthy, normal-weight controls (C) and in 28 patients with anorexia nervosa (AN) before treatment (that is, at time T0).
Discussion

Thyroid hormones increase REE. The present human data support the idea that physiologic changes in plasma T₃ contribute to REE in underweight patients with AN and also during weight gain. The effect of T₃ on REE was independent of FFM, which is considered to be the strongest determinant of REE.

When compared with normal weight controls, REE was reduced in underweight patients with AN (Table 1). In the relationship between REE and FFM in controls and patients with AN, the between-group difference was 0.7 MJ/day at a given FFM of 40 kg. When we take into account the between-group differences in plasma T₃ concentrations (that is, 1.8 pmol/l) (Table 1) together with the association between plasma T₃ concentrations and REE adjusted for FFM (Fig. 1c), the increase in T₃ could explain an energy expenditure of about 0.6 MJ/day. We take the close agreement between the data (that is, 0.6 vs. 0.7 MJ/day) as in vivo evidence for a thermic effect of T₃ in humans. The idea is further supported by the observation that during weight gain, REE and plasma T₃ both increased at nearly unchanged FFM (Table 1). Again changes in plasma T₃ concentrations were closely associated with changes in REE (Fig. 2). The calculation revealed that an increase in plasma T₃ of 1.8 pmol/l increased the adjusted REE by about 0.5 MJ/day. This number is again close to the calculation based on our cross-sectional data (see above).

The present data support previous results from animal experiments (7, 8). They are also in line with one previous study on overfeeding (plus 10% energy intake above usual) in obese subjects (10). In this situation, body-mass index (BMI) increased from 34.6 to 38.0 kg/m². Concomitantly, FM and FFM showed mean increases of 5.6 and 3.9 kg respectively, while T₃ increased by only 0.2 pmol/l together with small changes in REE. The authors also found a positive association between changes in plasma T₃ concentrations and changes in REE. By their regression algorithm, a 40% change in plasma T₃ concentrations would result in a 2% increase in REE only. This is less than the 12% increase in REE seen in our study. However, in the obese subjects studied by Rosenbaum et al. (34), a weight gain of about 8–10 kg, which is close to the weight gain observed in our study (Table 1), showed only minor effects on both plasma T₃ concentrations and REE. Our data would therefore suggest that when compared with the effects of over-feeding seen in obese subjects, weight gain in underweight patients with AN resulted in a greater increase in plasma T₃ concentrations as well as a more pronounced effect of T₃ on REE. It is tempting to speculate that the low T₃ status seen before weight gain adds to energy sparing and thus to metabolic economy during starvation. From a teleologic point of view, one may further speculate that increases in plasma T₃ concentrations and T₃ sensitivity associated with weight gain will enhance energy expenditure and thus inhibit further weight gain in anorectic patients. Thus, the ‘physiologic’ T₃ signal seems to be intended to maintain body weight by regaining a zero energy balance. This idea is contrary to the situation in hyperthyroid patients, where hypermetabolism leads to a negative energy balance and thus weight loss. This is evidence for the difference between the physiologic and supraphysiologic thermic effect of thyroid hormones.

There is an apparent controversy on energetics in undernutrition (see review (26)). Increased, unchanged and decreased REEs were observed in heterogeneous groups of undernourished subjects (including patients with malignant or other wasting diseases). Besides disease-dependent factors, the variance in metabolic adaptations to underweight are explained by reductions in body mass as well as by variations of body composition (see reviews (26, 27)). In undernourished subjects, weight loss is explained by losses in FM as well as FFM. Losses in FFM are mainly due to a reduction in muscle mass, which has a low organ metabolic rate. In contrast, the losses in visceral organ masses, which have a high metabolic rate, are moderate. Thus, their relative contribution to REE may increase in some underweight subjects (26). We have recently shown that, when compared with normal-weight controls, REE (adjusted and unadjusted for FFM), muscle mass (MM) and organ mass (OM = sum of visceral organs) were all reduced in a group of constitutionally thin women (28). In addition, there was a significant between-group difference in the ratio of OM to MM (0.16 and 0.18 in underweight and normal-weight women respectively; \( P < 0.005 \)), suggesting that the OM/MM ratio is reduced rather
than increased in underweight subjects. If this also occurred in our anorectic patients, a lower OM/MM ratio might add to a lower REE. In the present study, underweight subjects with AN had reduced REE (either unadjusted or adjusted for FFM) (Table 1 and Fig. 1). We take this as evidence of effective metabolic adaptation. Our finding is contrary to some other studies in which the difference in REE between underweight AN patients and normal-weight controls disappeared after adjustment of REE for FFM (see review (29)). The latter finding suggested that REE in AN patients was reduced only to the extent of reduced FFM. However, other authors showed that in AN patients the metabolic activity of FFM was reduced in addition to the loss of FFM (14). This is in line with our data, supporting the idea that in underweight AN patients, metabolic adaptation is explained by the loss of FFM plus the fall in plasma concentrations of the major thermic hormone, T₃. Our data do not exclude the possibility that changes in FFM composition also add to metabolic adaptation. However, since most of the observed decrease in the metabolic activity of FFM could be explained by T₃ (Figs 1 and 2, results), the unexplained variance left is small.

In AN patients, weight gain is mainly explained by increases in FM while FFM remains nearly unchanged (Table 1). This finding reflects the ‘catch-up fat’ phenomenon (30) and supports previous data on body composition changes in patients with AN (31, 32). The increase in FM is associated with an increase in REE. Since fat cells, per se, have a very low metabolic rate and fat is metabolically inert, the association between FM and REE may reflect FM-associated metabolic and/or hormonal changes (such as increases in sympathetic nervous system activity) that add to the increase in REE. There were significant correlations between both FM and REE, and plasma T₃ concentrations. Besides its role in energy storage and thermal insulation, adipose tissue has an endocrine and secretory function (see review (33)). Leptin is one of a number of secretory proteins that increase with increasing adipose tissue. Although a direct thermic effect of leptin is unlikely in humans, the replacement of physiologic amounts of exogenous leptin normalized the low T₃ concentrations seen in weight-reduced subjects (34). Leptin also prevented fasting-induced suppression of prohypothalamic releasing hormone messenger RNA in hypothalamic neurons (35, 36) as well as stimulating TSH secretion in vivo (37). Thus, FM, leptin and T₃ may have a modulating effect on energy expenditure in underweight subjects and also during weight gain.

Taken together, our data provide evidence that low T₃ concentrations add to metabolic adaptation in underweight patients with AN. During weight gain, increases in T₃ are closely associated with increases in REE. Both results are independent of body mass and thus are evidence of a physiologic role of T₃ in the modulation of energy expenditure in humans.

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