Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited1,2

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ABSTRACT

Background: Adaptive thermogenesis (AT) is the fat-free mass (FFM)-independent reduction of resting energy expenditure (REE) to caloric restriction (CR). AT attenuates weight loss and favors weight regain. Its variance, dynamics, and control remain obscure.

Objectives: Our aims were to address the variance and kinetics of AT, its associations with body composition in the context of endocrine determinants, and its effect on weight regain.

Design: Thirty-two nonobese men underwent sequential overfeeding (1 wk at +50% of energy needs), CR (3 wk at −50% of energy needs), and refeeding (2 wk at +50% of energy needs). AT and its determinants were measured together with body composition as assessed with the use of quantitative magnetic resonance, whole-body MRI, isotopedia dilution, and nitrogen and fluid balances.

Results: Changes in body weight were +1.8 kg (overfeeding), −6.0 kg (CR), and +3.5 kg (refeeding). CR reduced fat mass and FFM by 114 and 159 g/d, respectively. Within FFM, skeletal muscle (−5%), liver (−13%), and kidneys (−8%) decreased. CR also led to reductions in REE (−266 kcal/d), respiratory quotient (−15%), heart rate (−14%), blood pressure (−7%), creatinine clearance (−12%), energy cost of walking (−22%), activity of the sympathetic nervous system (SNS) (−38%), and plasma leptin (−44%), insulin (−54%), adiponectin (−49%), 3,5,3′-tri-iodo-thyronine (T3) (−39%), and testosterone (−11%). AT was 108 kcal/d or 48% of the decrease in REE. Changes in FFM composition explained 36 kcal, which left 72 kcal/d for true AT. The decrease in AT became significant at ≤3 d of CR and was related to decreases in insulin secretion (r = 0.92, P < 0.001), heart rate (r = 0.60, P < 0.05), creatinine clearance (r = 0.79, P < 0.05), negative fluid balance (r = 0.51, P < 0.01), and the free water clearance rate (r = −0.90, P < 0.002). SNS activity and plasma leptin, ghrelin, and T3 and their changes with CR were not related to AT.

Conclusion: During early weight loss, AT is associated with a fall in insulin secretion and body fluid balance. This trial was registered at clinicaltrials.gov as NCT01737034. Am J Clin Nutr 2015;102:807–19.

Keywords: body composition, energy balance, energy expenditure, weight change, metabolic adaptation, weight loss, starvation, refeeding, MRI

INTRODUCTION

Caloric restriction (CR)7 and weight loss are associated with decreases in resting energy expenditure (REE) and adaptive thermogenesis (AT). AT refers to the decrease in REE beyond those decreases accounted for by changed fat-free mass (FFM) and fat mass (FM). This effect is seen with diet, exercise, diet-and-exercise, pharmacologic, and surgical interventions (1–3). The extent of AT relates to the degree of energy deficit (4), and it reduces the magnitude of the negative energy balance. In obese patients, AT might persist beyond weight loss (5, 6). It has been hypothesized that AT favors weight instability and regain (1, 5–8). By contrast, AT may be beneficial in patients with anorexia nervosa in whom the metabolic adaptation favors weight gain during refeeding (9). The quantification of AT is important when considering the thrifty gene hypothesis (10, 11), the modeling of weight change in response to changes in energy intake (12, 13), weight loss, and a disproportional regain of FM in patients with

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2Supplemental Tables 1–4 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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7Abbreviations used: AEE, activity energy expenditure; AT, adaptive thermogenesis; CAU, Christian-Albrechts University; CR, caloric restriction; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; FWCR, free water clearance rate; GIT, glucose-induced thermogenesis; HF, high frequency; HFF, hepatic fat fraction; ICW, intracellular water; IUV, isosmotic urine volume; LF, low frequency; MM, muscle mass; Psum, plasma osmolality; p-ratio, percentage of protein gained or lost of body weight gained or lost; REE, resting energy expenditure; REEmeasured, resting energy expenditure measured with the use of indirect calorimetry; REEpredicted, resting energy expenditure calculated from individual organ masses times their specific metabolic rate; RJ, respiratory quotient; SNS, sympathetic nervous system; TBW, total body water; TE, time to echo; TR, time to repeat; T3, 3,5,3′-tri-iodo-thyronine; Usum, urine osmolality; VAT, visceral adipose tissue.

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anorexia nervosa and weight-reduced obese patients (9, 14, 15) and modern concepts of bioenergetics as target for obesity treatment (16).

AT could be a regulated or a forced change. Most authors have assumed that AT is an autoregulatory response explained by the reduced activity of the sympathetic nervous system (SNS) and low plasma concentrations of 3,5,3′-tri-iodo-thyronine (T3) and leptin (6, 17, 18). Keys et al. (19) were first to quantitatively describe AT. In the Minnesota Starvation Experiment (19), REE declined by 39% or ~600 kcal/d. Approximately 35% of the starvation-induced fall in REE (i.e., ~200 kcal/d) was independent of losses in FFM (19). In a subsequent study on 3 wk of semistarvation, AT reached 73% of the fall in REE (20). With weight recovery, REE increased and surpassed that in the prestarvation state (19). In another seminal study, Leibel et al. (21) showed that a 10% weight reduction and subsequent stabilization yielded an AT between 54 and 137 kcal/d. Although AT seems to be established, it is still causally enigmatic to physicians. Some pertinent questions remain to be answered.

The current study followed a subsequent CR-refeeding protocol. Our primary hypotheses were that AT 1) is partly explained by changes in the composition of FFM, 2) is linked to early rather than prolonged starvation, and 3) has no effect on short-term weight regain.

METHODS

The investigation of AT was the primary aim of our 6-wk subsequent overfeeding-CR-refeeding study. Specific aspects of glucose metabolism were secondary aims and were already published (22). Data on energy expenditure, energy balance, and detailed body composition were not part of the previous study.

Subjects

Thirty-two healthy young men were recruited from the campus of the Christian Albrechts University (CAU) between February 2010 and September 2012. Participants were kept in residence during the day from 0800 to 1800 at our institute, which is part of the CAU. Participants were students outside the area of nutritional and medical science. They were monitored for compliance with the use of 24-h individual glucose monitoring and pedometers. Exclusion criteria were smoking, obesity, chronic diseases, regular use of medications, recent weight changes, vegetarianism, heavy exercise, and food allergies. Subjects had a normal insulin sensitivity that was verified with the use of a hyperinsulinemic euglycemic clamp (1 mU insulin · kg body weight⁻¹ · min⁻¹). The study protocol followed the Declaration of Helsinki, and all procedures were approved by the Ethics Committee of the Medical Faculty of the CAU. Given the 1) normal weight of our volunteers and 2) the expected weight loss in response to a 3-wk period of CR (i.e., ~6 kg body weight), the ethical committee of the CAU asked us to precede CR by a 1-wk overfeeding period (at +50% of energy needs). All subjects provided written informed consent before participation. Participants received an honorarium of €1200 (US$1316) for participation.

Study protocol

We performed 2 studies. Study 1 followed the original 6-wk intervention protocol. On the basis of the results of study 1, a short-term study 2 was performed 1.5 y later on early metabolic adaptation.

Study 1 was performed in 32 subjects aged 20–37 y [BMI (in kg/m²) ranged between 20.7 and 29.3; mean ± SD FM: 17.9 ± 5.7%]. Details of the protocol have been previously described (22). During a 10-wk baseline period in residence, habitual food intake (with the use of dietitian-guided dietary records), REE (with the use of indirect calorimetry), and physical activity [with the use of 24-h heart rate and accelerometry] were assessed 3 times to calculate individual energy needs. A physical activity level of 1.4 was taken to resemble a sedentary lifestyle. Dietary interventions comprised 1 wk of overfeeding (at +50% of energy requirements; 4059 ± 5.2 kcal/d) followed by 3 wk of CR (at −50% of energy requirements; 1353 ± 154 kcal/d) and a subsequent 2 wk of refeeding (at +50% of energy requirements; 4059 ± 452 kcal/d). Protein intake was 97 ± 11 g/d (baseline), 146 ± 17 g/d (overfeeding), 49 ± 6 g/d (CR), and 146 ± 17 g/d (reef- feeding), respectively. Body weight, body composition (with the use of quantitative magnetic resonance), the fluid balance, and urinary nitrogen excretion were measured daily. Detailed body composition was assessed with the use of whole-body MRI, air-displacement plethysmography, and dilution techniques together with measurements of energy expenditure and plasma concentrations of hormones and substrates.

Study 2 was performed in a subgroup of 8 subjects who had already participated in study 1 to analyze the short-term effects of CR on AT. Contrary to study 1, daily measurements were done for 1 wk of overfeeding followed by 1 wk of CR. In addition, study 2 gave us the opportunity to test the reproducibility of metabolic adaptation to starvation.

In both studies, all foods and drinks were provided. The preparation and consumption of foods and beverages were supervised by skilled nutritionists. During the initial overfeeding period, all participants received a normal mixed diet (15% protein, 50 or 65% carbohydrate, and 35 or 20% fat). To standardize dietary intake during CR and refeeding, 50% of the energy intake was given as a liquid-formula diet (InsuLean; D Pape, Essen, Germany). The remaining 50% of energy was provided as high–glycemic index and low–glycemic index mixed meals and snacks. For compliance, continuous 24-h glucose monitoring was performed with the use of the FreeStyle Navigator (continuous glucose monitoring) device (Abbott Diabetes Care). In addition, dietary records and pedometers were used each day throughout the study. The energy content of selected duplicate meals was analyzed with the use of bomb calorimetry to check for the calculated energy intake given in the meal plan. Any food left uneaten was included in the final calculations of individual energy balances. Throughout the whole study protocol, ad libitum intake of water as well as of alcohol- and caffeine-free beverages was allowed.

Energy expenditure

Oxygen uptake and carbon dioxide uptake were measured with the use of open circuit indirect calorimetry after an overnight fast. Four ventilated hood systems were used (3 Vmax Spectra 29n devices [SensorMedics, ViasysHealthcare] and one Quark RMR device (COSMED)) with a precision of 4.4–6.5%. Each subject was familiarized with the equipment and was always measured with the same device. Alcohol burning tests were performed as a post- calorimetric test, and any deviation in oxygen uptake and carbon dioxide uptake from the theoretical value was used for device-specific corrections. Mean corrections for REE were −0.60% (Vmax 1 device), 4.23% (Vmax 2 device), −2.25% (Vmax 3 device), and 1.01% (Quark RMR device), respectively. It was assumed that these values were representative for each indirect
calorimeter condition throughout the tests. Because our measurements were done for 1 h and under steady state conditions, this assumption was true (23). Glucose-induced thermogenesis (GIT) was calculated from the AUC of the 2-h increase in energy expenditure after a 75-g oral glucose load. Accelerometry (SenseWear Armband Systems; Body-Media Inc.) combined with synchronized heart rate monitoring (Actiheart; CamNtech Ltd.) was used to assess activity energy expenditure (AEE). Total or 24-h energy expenditure was the sum of

\[ \text{REE} + (\text{GIT} \times 3) + \text{AEE} \]  

\[ (I) \]

where 3 stand for 3 meals/d. The flex heart rate was defined from the individual minute-to-minute relation between the heart rate and submaximal oxygen uptake during an incremental treadmill exercise protocol as the lowest heart rate at light exercise (24). The use of heart rate–synchronized accelerometry to measure free-living physical AEE has been validated compared with the use of doubly labeled water in lean young men with various fitness levels (25).

For heart rate variability, low frequency (LF) (ms²) was in the range of 0.04–0.15 Hz, and high frequency (HF) (ms²) was between 0.15 and 0.4 Hz. The LF:HF ratio was taken as a measure of SNS activity. The incremental exercise tolerance test was performed on an electronically braked treadmill ergometer (QuarkRMR, 170D; COSMED). Skeletal muscle work efficiency was assessed on the same treadmill ergometer at different low work loads of 1, 2.5, and 5 km/h. Data are expressed in kcal/min above REE.

**Body composition**

Analyses of body composition have been described previously. Daily measurements of FM were performed with the use of quantitative magnetic resonance (ECHOMRI-AH; Echo Medical Systems) (26). Whole-body MRI was used to assess the volumes of adipose tissue, skeletal muscle mass (MM), and internal organs (Magnetom Avanto 1.5 T; Siemens Medical Systems) (27, 28). Transversal images were obtained from the wrist to ankle with the use of a continuous axial T1-weighted gradient-echo sequence [time to repeat (TR): 1.57 ms; time to echo (TE): 4 ms]. The protocol for the brain comprised continuous 4-mm slices with 1-mm interslice gaps (TR: 313 ms; TE: 14 ms). The rest of the body images were obtained with an 8-mm slice thickness and 2-mm interslice gaps. Image acquisition for the volumetric assessment of the thoracic and abdominal region was obtained in breath hold, and heart mass was assessed with the use of a breath-navigated and pulse-triggered T2-weighted half Fourier acquisition single-shot turbo spin-echo sequence (TR: 700 ms; TE: 24 ms) (27, 28). The volume of visceral adipose tissue (VAT) was acquired from the top of the liver or the base of the lungs to the femur heads. All images were segmented manually (Slice-O-Matic 4.3 software; TomoVision) by the same investigator (MP). The intra-observer variance for repeated measurements was <2% (e.g., 0.9% for subcutaneous adipose tissue, 1.0% for VAT, and 1.8% for skeletal MM). Total organ and tissue volumes were calculated from the sum of all areas (cm²) multiplied by the slice thickness and interslice gap. Volume data were transformed into masses with the use of the following organ and tissue densities: 1.036 g/cm³ for the brain, 1.06 g/cm³ for the heart and liver, 1.05 g/cm³ for the kidneys, 1.04 g/cm² for skeletal MM, and 0.92 g/cm² for VAT and subcutaneous adipose tissue (compare references 27–29).

Liver fat was determined with the use of the 2-point Dixon method with a volume interpolated breath-hold examination as previously described (30). Briefly, a T1-weighted gradient-echo sequence with in-phase and out-of-phase imaging was performed with the use of the following variables: TR, 10.4 ms; TE, 4.76 ms (in phase), and 7.14 ms (opposed phase); flip angle, 10° matrix, 80 × 128; and field of view, 440 mm. Fat-only and water-only images were calculated from in-phase and opposed-phase images as follows:

\[ \text{Water only} = 1/2 \times (\text{in phase} + \text{opposed phase}) \]  

\[ (2) \]

\[ \text{Fat only} = 1/2 \times (\text{in phase} – \text{opposed phase}) \]  

\[ (3) \]

Forty adjacent slices were acquired within a 19-s breath hold to cover the liver with a slice thickness of 5 mm and a 1-mm inter-slice gap. Images were analyzed and processed with the use of ImageJ software (version 1.50, 2012; NIH) to calculate hepatic fat fraction (HFF) images from fat-only and water-only images. A single continuous region of interest was defined (20.62 × 20.62) in each of 5 adjacent HFF images and was placed in the liver parenchyma with the avoidance of major blood vessels. The region of interest was placed in the same area for all repeated measurements. The quantity of liver fat was determined as the percentage of the total liver core and was averaged for the 5 HFF images. The intra-organ fat percentage was evaluated from 2 liver regions of interest as defined and averaged by one observer (AB-W).

In study 2, total body water (TBW) and extracellular water (ECW) were measured in 8 subjects with the use of deuterium and sodium bromide dilution (see reference 31 for additional details). After 40-mL venous blood samples were obtained, each subject drank an oral dose of 0.4 g deuterium oxide/kg body weight with an amount of 100 mL tap water. Four hours later, a second blood sample was taken. The concentration of deuterium oxide was measured in the ultrafiltrate as 3H/H enrichment of the serum samples with the use of isotope ratio mass spectrometry. The intra-individual CV for plasma deuterium atom percent excess was 0.18 ± 0.09%. To assess ECW, an oral dose of sodium bromide that provided 50 mg bromide/kg body weight was administered simultaneously with deuterium-enriched water. Bromide was quantified in plasma samples with the use of a nondestructive liquid X-ray fluorescence technique with a reproducibility of ± 0.8%. Corrected bromide space was used as the proxy for ECW and calculated with the use of the following formula:

\[ \text{ECW} = \text{bromide dose} \times \text{bromide elevation in plasma} \times 0.90 \times 0.95 \times 0.94 \]  

\[ (4) \]

where 0.90 is the correction factor for nonextracellular distribution, 0.95 is the Donnan equilibrium factor, and 0.94 is the correction for water content in plasma (32). The following equation was used to calculate TBW:

\[ \text{TBW}(kg) = \left[ (\text{dose} \times 99.9) / 20 \right] \times (18.02 + \text{atom percent excess}) \times 10^9 + 1.04 \]  

\[ (5) \]

The dose is the dose of ²H₂O expressed in g, 99.9 is the AP (abundance over natural occurrence) of ²H₂O, 20 is the molecular
The difference between REE predicted and resting energy expenditure calculated from body composition data with the assumption of energy densities of 1100 kcal/kg FFM and 9300 kcal/kg FM (8000 kcal/kg adipose tissue), respectively (34). The percentage of protein gained or lost (p-ratio; i.e., protein energy mobilized/total energy mobilized) was calculated according to Dulloo et al. (35). Body protein was assessed from the cumulative changes in nitrogen balance during overfeeding, CR, and refeeding.

Fluid and sodium balances were calculated from fluid and sodium intake plus the amount of water produced by macronutrient oxidation. Urea, creatinine, and plasma indexes were used to assess the whole-body water balance (37). Obligatory urine volume (or iso-osmotic urine volume (UIV)) is defined as the volume of water necessary to excrete the extracellular osmotic load in urine that has the same concentration as serum. IUV is calculated as

\[
\text{IUV} = \frac{\text{24-h urine osmolar clearance} \times \text{plasma osmolality}}{24-h \text{ urine volume}}
\]

where 24-h urine osmolar clearance is calculated from 24-h urine osmolality \(\mu\text{osm}\), and the 24-h urine volume is calculated as

\[
\text{24-h urine osmolar clearance} = \frac{\text{urine osmolar clearance}}{\text{urine volume}}
\]

The free water clearance rate (FWCR) (in mL/24h) is the difference between the total 24-h urine volume and the IUV. A negative value represents the retention of excess water by the kidneys. By contrast, a positive FWCR reflects water removal from plasma in excess of solutes. In addition, the ratio of \(U_{\text{osm}}\) to plasma osmolality ratio \(P_{\text{osm}}\) was used as a hydration biomarker with \(U_{\text{osm}}/P_{\text{osm}} < 1.0\) reflecting a relative water excess, whereas \(U_{\text{osm}}/P_{\text{osm}} > 1.0\) reflects a relative water deficiency. Therefore, the IUV can also be calculated as

\[
\text{IUV} = \frac{U_{\text{osm}} + P_{\text{osm}} \times \text{UV}}{\text{urine volume}}
\]

The insulin:glucagon molar ratio was calculated from measured plasma concentrations as follows:

\[
\text{Insulin:glucagon ratio} = \frac{\text{insulin (in \(\mu\text{U/mL}\))}}{\text{glucagon (in pg/mL) \times 23.3}}
\]

Statistical analysis

Results are expressed as means ± SDs. All statistical analyses were conducted with the use of SPSS 21.0 statistical software (IBM). The prediction of weight loss was determined with the use of nonlinear regression derived from semilog plots of body weight vs. the day of CR. Data were described with the use of a 2-compartment model developed with WinNonlin software (version 6.3; Pharsight) with an early rapid phase (decay constant \(k_1\)) and a later slow phase (decay constant \(k_2\)). REE was adjusted for 1) FFM and 2) FFM plus FM (38). AT was the primary endpoint of our study. To identify possible determinants, associations between AT and 1) body composition and 2) hormones [leptin, 3,5,3'-triiodothyranine (T3), insulin, and SNS activity] were tested with the use of the Pearson correlation coefficient. In study 2, the specific focus was on the kinetics of AT. Throughout days 1–7 we repeatedly tested REE and compared the results with the previous day’s measurements. Associations between AT and its determinants were tested at day 3 of CR (end of the initial more-rapid phase of
### TABLE 1

Body-composition data of male subjects during sequential overfeeding, underfeeding, and refeeding (*n* = 32; study 1)

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Basal</th>
<th>7 d of overfeeding</th>
<th>21 d of caloric restriction</th>
<th>14 d of refeeding</th>
<th>7 d of overfeeding — basal</th>
<th>21 d of caloric restriction — basal</th>
<th>21 d of caloric restriction — 7 d of overfeeding</th>
<th>14 d of refeeding — 21 d of caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>77.68 ± 7.637</td>
<td>79.45 ± 7.808</td>
<td>73.45 ± 7.398</td>
<td>76.94 ± 7.911</td>
<td>1.76 ± 0.676***</td>
<td>-4.22 ± 0.873***</td>
<td>-6.00 ± 0.832***</td>
<td>3.48 ± 1.155***</td>
</tr>
<tr>
<td><strong>Fat mass, kg</strong></td>
<td>13.83 ± 5.119</td>
<td>14.63 ± 5.255</td>
<td>12.00 ± 4.988</td>
<td>13.20 ± 4.967</td>
<td>0.80 ± 0.601***</td>
<td>-1.83 ± 0.490***</td>
<td>-2.63 ± 0.536***</td>
<td>1.20 ± 0.639***</td>
</tr>
<tr>
<td><strong>FM index</strong></td>
<td>4.23 ± 1.692</td>
<td>4.47 ± 1.734</td>
<td>3.67 ± 1.637</td>
<td>4.03 ± 1.639</td>
<td>0.24 ± 0.178***</td>
<td>-0.56 ± 0.153***</td>
<td>-0.80 ± 0.162***</td>
<td>0.36 ± 0.191***</td>
</tr>
<tr>
<td><strong>FFM, kg</strong></td>
<td>63.84 ± 7.493</td>
<td>64.82 ± 7.377</td>
<td>61.46 ± 7.070</td>
<td>63.72 ± 7.300</td>
<td>0.98 ± 0.654***</td>
<td>-2.38 ± 0.883***</td>
<td>-3.36 ± 0.787***</td>
<td>2.26 ± 0.895***</td>
</tr>
<tr>
<td><strong>Cumulative N balance, g/d</strong></td>
<td>6.48 ± 5.671</td>
<td>33.54 ± 31.536</td>
<td>-78.98 ± 33.569</td>
<td>140.23 ± 67.454</td>
<td>27.06 ± 28.201***</td>
<td>-85.46 ± 31.636***</td>
<td>-112.53 ± 32.468***</td>
<td>219.22 ± 64.758***</td>
</tr>
<tr>
<td><strong>N retention, %</strong></td>
<td>0.58 ± 26.366</td>
<td>15.00 ± 61.368</td>
<td>-2.19 ± 9.488</td>
<td>23.68 ± 73.031</td>
<td>15.58 ± 66.022</td>
<td>-1.61 ± 27.811</td>
<td>-17.19 ± 61.869</td>
<td>25.88 ± 74.051</td>
</tr>
<tr>
<td><strong>Protein, kg</strong></td>
<td>12.39 ± 1.454</td>
<td>12.60 ± 1.517</td>
<td>12.10 ± 1.518</td>
<td>12.96 ± 1.705</td>
<td>0.21 ± 0.197***</td>
<td>-0.28 ± 0.353***</td>
<td>-0.49 ± 0.209***</td>
<td>0.86 ± 0.430**</td>
</tr>
<tr>
<td><strong>p-ratio</strong></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.14 ± 0.154</td>
<td>—</td>
<td>—</td>
<td>0.10 ± 0.044**</td>
</tr>
</tbody>
</table>

**Adipose tissue**

| **Whole body, kg** | 15.22 ± 4.931 | — | 13.28 ± 4.260 | 13.92 ± 4.059 | — | -1.94 ± 1.269*** | — | 0.63 ± 2.128 |
| **VAT, kg** | 1.22 ± 0.846 | — | 1.03 ± 0.768 | 1.16 ± 0.816 | — | -0.23 ± 1.368 | — | 0.13 ± 0.140 |
| **SAT, kg** | 12.83 ± 3.836 | — | 12.25 ± 3.686 | 12.76 ± 3.301 | — | -0.72 ± 3.351 | — | 0.50 ± 2.199 |
| **Arms** | 1.67 ± 0.440 | — | 1.66 ± 0.566 | 1.74 ± 0.431 | — | -0.03 ± 0.834 | — | 0.08 ± 0.487 |
| **Legs** | 6.09 ± 1.600 | — | 5.79 ± 1.500 | 6.06 ± 1.340 | — | -0.36 ± 2.484 | — | 0.27 ± 0.890 |
| **Trunk** | 5.07 ± 1.903 | — | 4.80 ± 1.752 | 4.95 ± 1.649 | — | -0.33 ± 3.194 | — | 0.15 ± 0.793 |
| **VAT:SAT** | 0.09 ± 0.047 | — | 0.08 ± 0.040 | 0.08 ± 0.036 | — | -0.01 ± 0.065 | — | 0.01 ± 0.013 |

**Skeletal muscle**

| **Whole body** | 30.83 ± 3.186 | — | 29.37 ± 3.200 | 30.88 ± 3.502 | — | -1.45 ± 0.764*** | — | 1.51 ± 0.953*** |
| **Arms** | 46.1 ± 0.708 | — | 4.55 ± 0.832 | 4.73 ± 0.660 | — | -0.07 ± 0.695 | — | 0.18 ± 0.520 |
| **Legs** | 15.17 ± 1.413 | — | 14.60 ± 1.582 | 15.09 ± 1.662 | — | -0.57 ± 0.593*** | — | 0.49 ± 0.512*** |
| **Trunk** | 11.04 ± 1.277 | — | 10.22 ± 1.423 | 11.07 ± 1.359 | — | -0.82 ± 0.417*** | — | 0.85 ± 0.940*** |

**Organ mass**

| **Sum of organ masses, kg** | 3.74 ± 0.462 | — | 3.45 ± 0.410 | 3.79 ± 0.405 | — | -0.29 ± 0.173*** | — | 0.34 ± 0.157*** |
| **Heart, kg** | 0.25 ± 0.048 | — | 0.25 ± 0.047 | 0.23 ± 0.032 | — | -0.00 ± 0.040 | — | -0.02 ± 0.033 |
| **Liver, kg** | 1.68 ± 0.399 | — | 1.47 ± 0.311 | 1.83 ± 0.329 | — | -0.21 ± 0.203*** | — | 0.36 ± 0.145 |
| **Liver fat, %** | 6.2 ± 3.11 | — | 5.8 ± 3.10 | 6.9 ± 2.78 | — | -1.1 ± 1.33** | — | 2.2 ± 1.63*** |
| **Kidneys, kg** | 0.24 ± 0.040 | — | 0.22 ± 0.042 | 0.25 ± 0.050 | — | -0.02 ± 0.019** | — | 0.02 ± 0.030* |
| **Brain, kg** | 1.55 ± 0.131 | — | 1.50 ± 0.115 | 1.48 ± 0.111 | — | -0.05 ± 0.051** | — | -0.02 ± 0.047 |
| **Residual mass, kg** | 29.56 ± 3.570 | — | 29.06 ± 3.528 | 30.55 ± 4.162 | — | -0.50 ± 1.399 | — | 1.50 ± 2.157* |

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1. **Significant differences from preceding periods (repeated-measures ANOVA): *P < 0.05, **P < 0.01, ***P < 0.001. FFM, fat-free mass; FM, fat mass; N, nitrogen; p-ratio, percentage of protein gained or loss of body weight gained or lost; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.**

2. **FM (in kg) divided by height (in m²).**

3. **Determined with the use of quantitative magnetic resonance.**

4. **Calculated from nitrogen-balance data.**
weight loss). Data normality was tested with the use of the Kolmogorov-Smirnov test. A 1-factor ANOVA was conducted to test for differences in outcome variables after CR and refeeding. A repeated-measures ANOVA was used to observe differences across intervention periods followed by Bonferroni post hoc tests. The α level of significance was set at 0.05. Bonferroni post hoc tests were performed to examine differences between AT-positive and AT-negative subgroups.

RESULTS

Body weight and body composition

In study 1, subjects gained 2.3% of body weight with overfeeding, whereas CR resulted in a weight reduction of 7.5% with a regain of 4.5% during refeeding (Table 1, Figure 1A). During CR and refeeding, day-to-day variances in body weight were 2.1% and 1.5%, respectively. Total weight loss correlated with body weight before CR ($r = -0.73$, $P < 0.01$). The higher the body weight was before CR, the higher the weight loss was. During refeeding, weight gain correlated with body weight at the end of CR ($r = 0.61$, $P < 0.05$). Average percentage changes in FM were +5.8% (overfeeding), −17.8% (CR), and +10.0% (refeeding), respectively (Table 1, Figure 1A) with day-to-day variances of 6.6% (CR) and 5.1% (refeeding). As average percentage values, total body protein increased with overfeeding (by +1.4%), decreased with CR (by −2.6%), and increased again with subsequent refeeding (+5.6%).

Weight loss was associated with nonlinear changes in body composition. FFM decreased by 313 ± 40 g/d (week 1), 90 ± 83 g/d (week 2), and 66 ± 60 g/d (week 3), respectively. Corresponding losses of FM were 168 ± 43 g/d (week 1), 109 ± 50 g/d (week 2), and 142 ± 42 g/d (week 3). With CR, weight loss was correlated with the loss of FFM ($r = 0.78$, $P < 0.01$) as well as off FM ($r = 0.42$, $P < 0.05$). Vice versa, weight gain was correlated with gains in FFM ($r = 0.85$, $P < 0.01$) and in FM ($r = 0.65$, $P < 0.01$). Whole-body MRI data showed that the loss of FFM was explained by losses in skeletal MM and organ masses (significant for the liver and kidneys) and a nonsignificant decrease in residual mass (24%) (Table 1). A total of 72% of the loss in organ masses were explained by decreases in liver mass and liver fat (−30 g/3 wk). In study 2, liver mass decreased by −0.15 ± 0.11 kg ($P < 0.01$) within the first week of CR. With refeeding, organ and tissue masses regained to baseline amounts (Supplemental Table 1).

Urinary nitrogen excretion rates were 17.15 ± 4.716 g/d (overfeeding: $P < 0.01$ compared with at basal), 11.08 ± 3.529 g/d (CR: 812 MU LLER ET AL. Downloaded from https://academic.oup.com/ajcn/article-abstract/102/4/807/4564599 by guest on 11 June 2018
$P < 0.05$ compared with at basal), and $19.47 \pm 4.811\text{ g/d (refeeding; not significantly different from at basal)}$ (Table 1). Throughout CR, the $r$-ratio remained constant (Figure 1B).

In study 2, the urinary excretion of creatinine was $177.14 \pm 25.247\text{ mM/d (overfeeding), 169.50 \pm 32.799\text{ mM/d (3 d of CR), and 130.45 \pm 37.553\text{ mM/d (7 d of CR; overfeeding compared with 7 d of CR, } P < 0.05)$ (Supplemental Table 2). Plasma creatinine concentrations were $78.00 \pm 8.194\text{ mg/L (overfeeding), 84.63 \pm 9.899\text{ mg/L (3 d of CR), and 91.13 \pm 15.487\text{ mg/L (7 d of CR), respectively (overfeeding compared with either 3 or 7 d of CR, both } P < 0.05$ (Supplemental Table 2). Sodium excretion decreased during CR. Concomitantly, the FWCR became positive and was correlated with changes in ECW ($r = -0.73, P < 0.05$) as well as the ICW:ECW ratio ($r = -0.76, P < 0.05$) (Supplemental Table 2).

As regards the dynamics of weight loss, curve fitting revealed 2 different functions. The first function was early and rapid weight loss within the first 5 d with a corresponding decay constant $(k_1)$ of $-0.78 \pm 0.19\text{ kg/d}$. The second function was a curve-linear weight loss with a decay constant $(k_2)$ of $-0.19 \pm 0.03\text{ kg/d}$. With refeeding, a rapid weight gain (gain constant $k_3: 0.63 \pm 0.07\text{ kg/d}$) was seen within the first 4 d followed by a curve-linear increase until the end of the refeeding period (gain constant $k_4: 0.15 \pm 0.07\text{ kg/d}$). Contrary to biphasic changes in body weight, changes in FM were curve linear with either CR (decay constant: $-0.12 \pm 0.02\text{ kg/d}$) or refeeding (gain constant: $0.11 \pm 0.05\text{ kg/d}$).

Energy expenditure and energy balance

During CR, REE, 24-h energy expenditure, the respiratory quotient (RQ), heart rate, and blood pressure all decreased in both studies (Figure 1B, Figure 2, Table 2, Supplemental Table 3). Day-to-day variances in REE and REEadj,FM+FM were 5.8 and 5.2%, respectively. The interindividual variance in decreases in REE with CR was 22.9%.

In study 2, changes in REE, AT ($P < 0.05$), and the RQ became significant at day 3 and are shown in Figure 3. There was a close association between decreases in REE and the RQ and their subsequent decreases with refeeding ($r = -0.59, P < 0.01$). The higher REE and the RQ were before CR, the higher were their decreases with CR ($r = -0.64, P < 0.01; r = 0.66, P < 0.05$, respectively). For the comparison of REEpredicted (calculated from individual organ masses times their specific metabolic rates) with REEmeasured, at baseline, the difference was $+10 \pm 86\text{ kcal/d}$. During CR, REEpredicted underestimated REEmeasured by $72 \pm 115\text{ kcal/d}$ with a corresponding value of $+35 \pm 99\text{ kcal/d}$ after refeeding (Table 2). Cumulative energy balances that were based on changes in body composition were $8509 \pm 5342\text{ kcal/1 wk of overfeeding, -28,181 \pm 4852\text{ kcal/3 wk of CR, and 13,654 \pm 6174\text{ kcal/2 wk of refeeding, respectively.}$

Compared with at baseline, metabolic costs of walking at a low speed decreased with CR and increased again with refeeding (Table 2). Part of the effect persisted during 2 wk of refeeding. With refeeding, all other variables normalized. Over the whole study, submaximal oxygen uptake remained unchanged.

Substrates and hormones

In study 1, CR reduced SNS activity and plasma T3, leptin, adiponectin, testosterone, and insulin concentrations (Table 3). With CR, plasma concentrations of free fatty acids (study 2) and ghrelin (study 1) increased (Table 3, Supplemental Table 4).

In study 1, there were no statistically significant associations between the energy balance or changes in FM and 1) baseline plasma concentrations of either leptin or insulin and 2) their changes with CR or refeeding. Plasma concentrations of insulin and leptin were correlated with each other (CR: $r = 0.43, P < 0.02$; refeeding: $r = 0.48, P < 0.01$). Euglycemic hyperinsulinemic clamp data suggested that insulin sensitivity was normal and remained unchanged over the 6-wk weight cycle (Table 3).

Correlates of AT

In study 2, the decrease in REE correlated with the extent of weight and FFM losses ($r = 0.43$ and 0.45, respectively, $P < 0.05$). AT had no associations with changes in body fat, VAT, liver fat, organ masses, ratios of organ masses:FFM, and nitrogen and sodium balances. During the first week of CR weight loss ($r = 0.58, P < 0.01$), the decrease in FFM ($r = 0.53, P < 0.001$), the negative fluid balance ($r = 0.51, P < 0.05$), and the fall in the basal heart rate ($r = 0.60, P < 0.05$) were all significantly correlated with the extent of AT.

As regards hormonal determinants, there were no correlations between AT and changes in either T3 ($r = 0.20, NS$), insulin ($r = -0.10, NS$), leptin ($r = -0.03, NS$), the leptin:FM ratio ($r = -0.03, NS$), ghrelin ($r = 0.09, NS$) or variables of SNS activity (i.e., urinary noradrenaline excretion: $r = 0.04, NS$; SD of all normal-to-normal-intervals: $r = 0.13, NS$; root mean square successive difference: $r = -0.07, NS$; LF:HF ratio: $r = 0.20, NS$) in study 1. In study 2, the extent of AT was correlated with reductions in insulin secretion assessed by the excretion of C-peptide ($r = 0.92, P < 0.001$) (Figure 4). There was a positive association between AT and the plasma glucagon concentration ($r = 0.81, P < 0.01$). At the third day of CR, there were significant correlations between AT and either the $U_{\text{osm}}$/$P_{\text{osm}}$ ratio ($r = -0.80, P < 0.001$) or the FWCR (Figure 4). The more water that was removed in excess of solutes resulted in lower AT. Both 24-h urinary C-peptide secretion and plasma leptin concentrations were associated with the $U_{\text{osm}}$/$P_{\text{osm}}$ ratio ($r = -0.76, P < 0.001$; $r = -0.71, P < 0.05$, respectively) and FWCR (for C-peptide secretion, see Figure 4; for leptin: $r = -0.71, P < 0.05$). AT was also related to the ICW:ECW ratio ($r = 0.66, P < 0.05$). AT was most pronounced at a low FWCR and a high ICW:ECW ratio. No associations were shown between AT and 1) changes in 24-h urinary sodium and aldosterone excretion and 2) plasma brain natriuretic peptide concentrations. In study 1, AT had no associations with regains of weight, FM, or nitrogen balance.

Reproducibility of AT

Baseline values of 8 volunteers obtained before the first and the second studies showed minor differences in body weight (2.0%) and in resting energy expenditure adjusted for FFM plus FM (0.3%), respectively. Individual data were highly correlated (data not shown). CR resulted in similar decreases in body weight ($-3.48 \pm 0.1155\text{ kg in study 1 compared with }-3.21 \pm 0.709\text{ kg in study 2}$). REE ($-226 \pm 138\text{ kcal/d in study 1 compared with }-208 \pm 144\text{ kcal/d in study 2}$), and the RQ ($-0.14 \pm 0.047$ in study 1 compared with $-0.11 \pm 0.047$ in study 2).
DISCUSSION

We showed that AT is an immediate phenomenon that occurs during early starvation (Figures 2 and 3) and is maintained throughout CR (Table 2, Figures 2 and 3). There was a considerable between-subject variance in metabolic adaptation and weight-loss-associated changes in body composition (Figure 1A, Tables 1 and 2, Supplemental Tables 1–3). Because AT was reproducible, and the p-ratio (i.e., protein energy mobilized/total energy mobilized) was kept nearly constant throughout the whole semistarvation period (Figure 1B), there was evidence of biological regulation.

There were considerable between-study differences in AT. With the use of a 2-compartment model, the mean AT was 104 kcal/d in normal-weight subjects (Figures 2 and 3, Table 2). By contrast, AT reached 504 kcal/d in obese patients after weight loss (39, 40). The between-study difference in AT was partly explained by the extent of weight loss (i.e., 6 kg in our study compared with ≤40 kg in obese patients; see Table 1 and references 39 and 40). Furthermore, in these clinical studies, the authors adjusted REE with the use of the REE-FFM relation before weight loss (39, 40). This method did not take into account the impact of obesity on the organ mass–FFM relation (41). The loss if FM increased the brain:FFM ratio, whereas liver:FFM, heart:FFM, and kidneys:FFM ratios decreased (41). These changes affected the REE-FFM relation and the calculation of AT.

CR decreased REE as well as non-REE (Table 2). It is already known that CR decreases metabolic costs of movement (17, 19). This effect was not fully compensated within 2 wk of refeeding (Table 2). CR had no significant effect on GIT (Table 2).

Impact of endocrine determinants on AT

CR-associated decreases in plasma concentrations of T3 and leptin as well as in SNS activity are considered as major determinants of AT (7, 17, 18). Although we reproduced the endocrine changes with weight loss (Table 3, Supplemental Table 4), these hormones had no associations with AT. In addition, there was no association between AT and FM. These results are in line with data on obese patients after weight loss that showed no associations between AT and decreases in absolute plasma concentrations of either T3 or leptin (39, 40). Our results do not argue against the findings that a low-dose administration of either T3 (42) or leptin (17, 43) and catecholamine replacement (44) partly reversed AT, which suggested the thermic effects of these hormones. However, these effects may not resemble regulation during CR. This idea is supported by the finding that the inhibition of the conversion of 3,5,3′,5′-tetra-iodo-thyronine to T3 was without effect on REE (45). Vice versa, refeeding increased plasma T3 and SNS activity but not REE (46). These findings question a regulatory role of T3 and SNS activity in AT.

Following the associations between plasma leptin concentrations and REE in anorectic patients compared with in normal-weight and overweight subjects, there was a steep relation at very low leptin concentrations, whereas no association was seen in normal-weight and overweight subjects (47). These data suggest a thermic effect in underweight patients only, which fits the idea of an asymmetric metabolic control or a so-called threshold effect of leptin (48). Our data do not exclude an effect of leptin on the nonresting compartment of energy expenditure.

Because AT was related to early starvation, its control should be explained in that context (49). At day 3 of CR, AT was closely correlated with the fall in insulin secretion (Figure 4), which was associated with changes in whole-body glucose and lipid oxidation (see the RQ in Figures 2 and 3) as well as the fluid balance and FWCR (Figure 4). The lower insulin was, the lower the free water removal was. The removal of water in excess of solutes was negatively associated with the extent of resting AT (i.e., water retention adds to AT).

Impact of detailed changes in the composition of FFM and FM on AT

Weight loss was associated with changes in the major 2 body components (i.e., FM and FFM) (Table 1, Supplemental Table 1). In the Minnesota Experiment, body fat (by underwater weighing),
TABLE 2
EE, heart rate, body temperature, energetic efficiency at low-intensity exercise, and aerobic fitness (\( \dot{V}O_2 \) submax) during sequential overfeeding, underfeeding, and refeeding (n = 32; study 1)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>7 d of overfeeding</th>
<th>21 d of caloric restriction</th>
<th>14 d of refeeding</th>
<th>7 d of overfeeding – basal</th>
<th>21 d of caloric restriction – basal</th>
<th>21 d of caloric restriction – 7 d of overfeeding</th>
<th>14 d of refeeding – 21 d of caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE, kcal/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE(_{measured})</td>
<td>1893 ± 210</td>
<td>1946 ± 183</td>
<td>1720 ± 194</td>
<td>1914 ± 210</td>
<td>53 ± 144</td>
<td>-173 ± 107***</td>
<td>-226 ± 138***</td>
<td>194 ± 110***</td>
</tr>
<tr>
<td>REE(_{adj}FM2 + FM3)</td>
<td>1846 ± 101</td>
<td>1864 ± 108</td>
<td>1757 ± 104</td>
<td>1879 ± 95</td>
<td>28 ± 135</td>
<td>-89 ± 104***</td>
<td>-166 ± 124***</td>
<td>122 ± 108***</td>
</tr>
<tr>
<td>REE(_{predicted from organ masses})</td>
<td>1911 ± 200</td>
<td>—</td>
<td>1807 ± 188</td>
<td>1926 ± 192</td>
<td>—</td>
<td>-104 ± 38***</td>
<td>—</td>
<td>119 ± 37</td>
</tr>
<tr>
<td>REE(<em>{me} – REE(</em>{p})</td>
<td>10 ± 86</td>
<td>—</td>
<td>-72 ± 115</td>
<td>35 ± 99</td>
<td>—</td>
<td>-82 ± 97**</td>
<td>—</td>
<td>-107 ± 111</td>
</tr>
</tbody>
</table>

Nonresting EE and total EE (REE)

|                       |       |                   |                             |                  |                          |                                   |                                             |                                             |
|-----------------------|-------|-------------------|-----------------------------|                  |                          |                                   |                                             |                                             |
| 24-h EE\(_{24-h heart rate monitor}\), kcal/d | 2449 ± 432 | 2527 ± 371 | 2188 ± 345 | 2548 ± 424 | 78 ± 298 | -258 ± 242*** | -349 ± 250*** | 356 ± 360*** |
| AEE, kcal/d           | 555 ± 328 | 580 ± 304 | 472 ± 213 | 634 ± 327 | 25 ± 278 | -94 ± 254 | -117 ± 231 | 100 ± 302 |
| GIT, kcal/2 h         | 21 ± 14 | — | 11 ± 10 | 24 ± 13 | — | -10 ± 15 | — | 13 ± 15** |
| RQ                    | 0.85 ± 0.052 | 0.92 ± 0.054 | 0.78 ± 0.054 | 0.90 ± 0.063 | 0.07 ± 0.072*** | -0.07 ± 0.074*** | -0.14 ± 0.047*** | 0.11 ± 0.051*** |

Physical activity, heart rate, and blood pressure

|                       |       |                   |                             |                  |                          |                                   |                                             |                                             |
|-----------------------|-------|-------------------|-----------------------------|                  |                          |                                   |                                             |                                             |
| Steps/d               | 4785 ± 1417 | 4865 ± 1896 | 5210 ± 2521 | 5456 ± 2363 | 80 ± 1453 | 426 ± 1934 | 345 ± 1788 | 245 ± 3043 |
| Basal heart rate, bpm | 65 ± 9 | 68 ± 8 | 59 ± 8 | 69 ± 8 | 3 ± 7 | -6 ± 8** | -9 ± 5** | 10 ± 6** |
| Flex heart rate, bpm (n = 6) | 96 ± 10 | — | — | 88 ± 9 | — | — | — | — |
| Temperature, °C       | 36.40 ± 0.447 | 36.79 ± 0.299 | 36.52 ± 0.500 | 36.41 ± 0.408 | 0.56 ± 0.500* | 0.39 ± 0.551 | -0.27 ± 0.482 | -0.09 ± 0.542 |
| BP, mm Hg             |       |                   |                             |                  |                          |                                   |                                             |                                             |
| Systolic              | 120 ± 9 | 120 ± 7 | 112 ± 7 | 117 ± 9 | -0.3 ± 10 | -9 ± 10*** | -8 ± 8*** | 9 ± 9 |
| Diastolic              | 80 ± 7 | 81 ± 6 | 76 ± 6 | 80 ± 7 | 0.7 ± 9 | -4 ± 8* | -5 ± 8* | 4 ± 10 |

EE at low-intensity exercise, kcal/min (n = 16)

|                      |       |                   |                             |                  |                          |                                   |                                             |                                             |
|----------------------|-------|-------------------|-----------------------------|                  |                          |                                   |                                             |                                             |
| 1 km/h               | 2.58 ± 0.406 | — | 2.02 ± 0.402 | 2.35 ± 0.399 | — | -0.55 ± 0.261*** | — | 0.33 ± 0.235*** |
| 2.5 km/h             | 3.34 ± 0.513 | — | 2.72 ± 0.435 | 3.05 ± 0.335 | — | -0.62 ± 0.256*** | — | 0.33 ± 0.295*** |
| 5 km/h               | 4.81 ± 0.660 | — | 4.31 ± 0.651 | 4.75 ± 0.534 | — | -0.50 ± 0.366*** | — | 0.44 ± 0.462** |

Aerobic fitness

|                      |       |                   |                             |                  |                          |                                   |                                             |                                             |
|----------------------|-------|-------------------|-----------------------------|                  |                          |                                   |                                             |                                             |
| \( \dot{V}O_2 \) submax, L/min | 2.50 ± 0.501 | — | 2.46 ± 0.461 | — | — | — | — | — |

\(^1\)***Significant differences from preceding periods (repeated-measures ANOVA); *P < 0.05, **P < 0.01, ***P < 0.001. AEE, activity-related energy expenditure; BP, blood pressure; bpm, beats per minute; EE, energy expenditure; FM, fat-free mass; FM, fat mass; GIT, glucose-induced thermogenesis; REE, resting energy expenditure; RQ, respiratory ratio; \( \dot{V}O_2 \) submax, submaximal oxygen uptake.

\(^2\)Measured with the use of a standard oral glucose tolerance test over 2 h.
extracellular fluid (thiocyanate space), blood volume (blue dye),
and bone mass (calculated from the X-ray density) were assessed
(19). The active tissue was calculated from the difference between
body weight and the sum of weights of FM, bone mineral, and
extracellular fluid (including plasma). Although Grande et al. (20)
could not go beyond a molecular and cellular model of body
composition, they had already speculated that starvation-induced
losses in organs and tissues add to the variance in AT. This idea
is supported by our current data. Whole-body MRI data showed
that, within 3 wk, CR yielded considerable decreases in skeletal
MM and liver and kidney masses (Table 1, Supplemental Table 1).
This effect has been overlooked in other studies on AT where a
2-compartment rather than a model at the organ and tissue level
had been used (see, e.g., references 39 and 40).
After the changes in the composition of FFM (and thus mass-
dependent effects) were accounted for, 72 kcal/d can be considered
“true” AT. The calculation of REE according to the observed de-
creases in function-related changes in the specific metabolic rates
of the heart (decrease in heart rate: −13%; −384 kcal/kg) and
kidneys (decrease in kidney function: −39%; −269 kcal/kg) to-
gether with the apparent increases in liver-specific metabolic rates
(206 kcal/kg as a result of increased gluconeogenesis as calculated
from urinary urea excretion) add up to 40 kcal/d, which leaves
32 kcal/d or 44% of “true” AT unexplained. Taking into account the
(nonsignificant) decline in body temperature (−0.3°C; Table 2)
and a temperature coefficient ($Q_{10}$) of 2 (= −38 kcal/d) would
explain AT.

**Impact of AT on regain of FM and VAT**

AT may contribute to weight regain (14). However, our data
showed that AT was reversible within 2 wk of refeeding (Figure 2;
Table 2). As regards body composition, contributions of FFM and
FM to either weight loss or weight gain were comparable (Table 1).
There were no disproportional increases in either FM or VAT (Figure
1A, Table 1). In the study of Keys et al. (19), the composition of body
weight regain differed from that of weight loss with disproportional
increases in FM and abdominal circumference during refeeding; this
was called “catch up fat” or a “fat overshooting” phenomenon (7).

**Study limitations and comparison with the Minnesota
Experiment**

In the Minnesota experiment as well as in our study, the energy
deficit was 50%, and 32 healthy, lean subjects were investigated.
## TABLE 3
SNS activity and plasma hormone concentrations during sequential overfeeding, underfeeding, and refeeding (n = 32; study 1)

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>7 d of overfeeding</th>
<th>21 d of caloric restriction</th>
<th>14 d of refeeding</th>
<th>7 d of overfeeding − basal</th>
<th>21 d of caloric restriction − basal</th>
<th>21 d of caloric restriction − 7 d of overfeeding</th>
<th>14 d of refeeding − 21 d of caloric restriction</th>
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<tbody>
<tr>
<td><strong>SNS activity (heart rate variability)</strong></td>
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<tr>
<td>RMSDD, ms</td>
<td>52.91 ± 27.938</td>
<td>42.20 ± 20.430</td>
<td>61.34 ± 28.717</td>
<td>38.28 ± 17.185</td>
<td>−10.72 ± 18.709*</td>
<td>−1.08 ± 20.313</td>
<td>19.38 ± 20.738*</td>
<td>−23.26 ± 22.609***</td>
</tr>
<tr>
<td>LF, ms</td>
<td>1545 ± 1300</td>
<td>1174 ± 836</td>
<td>1148 ± 850</td>
<td>1049 ± 619</td>
<td>−317 ± 917</td>
<td>−385 ± 945</td>
<td>−32 ± 786</td>
<td>−111 ± 801</td>
</tr>
<tr>
<td>HF, ms</td>
<td>848 ± 823</td>
<td>589 ± 546</td>
<td>1015 ± 802</td>
<td>464 ± 386</td>
<td>−259 ± 599</td>
<td>253 ± 655</td>
<td>436 ± 757*</td>
<td>−561 ± 673</td>
</tr>
<tr>
<td>LF:HF</td>
<td>2.53 ± 1.379</td>
<td>2.78 ± 1.550</td>
<td>1.57 ± 0.799</td>
<td>3.07 ± 1.723</td>
<td>0.26 ± 0.900</td>
<td>−1.01 ± 1.197*</td>
<td>−1.27 ± 1.350**</td>
<td>1.53 ± 1.458***</td>
</tr>
<tr>
<td><strong>Urinary excretion of catecholamines, mg/d</strong></td>
<td></td>
<td></td>
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<tr>
<td>Adrenaline</td>
<td>4.56 ± 2.301</td>
<td>5.25 ± 2.176</td>
<td>5.11 ± 2.385</td>
<td>5.78 ± 2.853</td>
<td>0.60 ± 1.865</td>
<td>0.46 ± 2.345</td>
<td>−0.14 ± 2.686</td>
<td>0.68 ± 2.719</td>
</tr>
<tr>
<td><strong>Plasma hormone concentrations</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TSH, mU/L</td>
<td>1.99 ± 1.064</td>
<td>1.83 ± 0.904</td>
<td>1.51 ± 0.736</td>
<td>2.17 ± 0.937</td>
<td>−0.21 ± 0.800</td>
<td>−0.56 ± 0.664***</td>
<td>−0.35 ± 0.452**</td>
<td>−0.71 ± 0.463***</td>
</tr>
<tr>
<td>Free T3, ng/mL</td>
<td>3.07 ± 0.362</td>
<td>3.01 ± 0.400</td>
<td>2.82 ± 0.400</td>
<td>3.19 ± 0.406</td>
<td>−0.07 ± 0.380</td>
<td>−0.27 ± 0.417**</td>
<td>−0.19 ± 0.369*</td>
<td>0.38 ± 0.354***</td>
</tr>
<tr>
<td>Free T4, ng/L</td>
<td>9.90 ± 1.238</td>
<td>9.23 ± 1.369</td>
<td>10.40 ± 1.172</td>
<td>9.01 ± 1.045</td>
<td>−0.66 ± 1.042**</td>
<td>0.45 ± 1.086</td>
<td>1.11 ± 1.100**</td>
<td>1.38 ± 1.035***</td>
</tr>
<tr>
<td>Ghrelin, ng/L</td>
<td>801.81 ± 296.576</td>
<td>681.75 ± 211.951</td>
<td>945.31 ± 451.050</td>
<td>683.31 ± 163.764</td>
<td>−120.06 ± 167.645</td>
<td>263.56 ± 346.227**</td>
<td>143.50 ± 246.617</td>
<td>−262.00 ± 356.634</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>4.07 ± 2.962</td>
<td>4.04 ± 2.745</td>
<td>2.26 ± 1.820</td>
<td>4.03 ± 2.578</td>
<td>−0.03 ± 0.750</td>
<td>−1.83 ± 2.395**</td>
<td>−1.77 ± 2.116**</td>
<td>1.77 ± 1.472***</td>
</tr>
<tr>
<td>Leptin:fat mass, ng·mL·−1·kg−1</td>
<td>0.27 ± 0.120</td>
<td>0.26 ± 0.104</td>
<td>0.18 ± 0.110</td>
<td>0.29 ± 0.106</td>
<td>−0.02 ± 0.062</td>
<td>−0.09 ± 0.167*</td>
<td>−0.08 ± 0.152*</td>
<td>0.11 ± 0.105***</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.94 ± 4.147</td>
<td>11.19 ± 6.626</td>
<td>5.60 ± 3.703</td>
<td>8.20 ± 4.355</td>
<td>4.05 ± 4.825***</td>
<td>−2.36 ± 2.861***</td>
<td>6.41 ± 4.147***</td>
<td>2.54 ± 2.265***</td>
</tr>
<tr>
<td>IGF-I, μg/L</td>
<td>226.96 ± 44.075</td>
<td>238.79 ± 51.149</td>
<td>190.04 ± 34.457</td>
<td>260.72 ± 61.211</td>
<td>−0.23 ± 29.811</td>
<td>−63.54 ± 37.409</td>
<td>−44.73 ± 38.963</td>
<td>71.11 ± 23.895</td>
</tr>
<tr>
<td>Adiponectin, μg/mL</td>
<td>8.15 ± 3.204</td>
<td>9.26 ± 3.072</td>
<td>4.71 ± 1.900</td>
<td>9.70 ± 3.232</td>
<td>1.11 ± 1.532**</td>
<td>−3.45 ± 2.177***</td>
<td>−4.56 ± 2.034***</td>
<td>5.00 ± 2.887***</td>
</tr>
<tr>
<td>Testosterone, μg/L</td>
<td>7.39 ± 2.064</td>
<td>7.09 ± 1.896</td>
<td>6.25 ± 2.300</td>
<td>6.31 ± 1.567</td>
<td>−0.45 ± 1.488</td>
<td>−2.18 ± 1.407*</td>
<td>−0.82 ± 1.695*</td>
<td>0.35 ± 1.693*</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>30.97 ± 12.185</td>
<td>28.49 ± 11.204</td>
<td>47.76 ± 15.174</td>
<td>27.08 ± 10.761</td>
<td>−4.30 ± 4.936</td>
<td>11.96 ± 4.759*</td>
<td>18.18 ± 6.212</td>
<td>−18.68 ± 6.779**</td>
</tr>
<tr>
<td>Insulin sensitivity (euglycemic hyperinsulinemic clamp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M, mg·kg−1·min−1</td>
<td>8.89 ± 2.859</td>
<td></td>
<td></td>
<td>8.71 ± 2.182</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant differences from preceding periods (repeated-measures ANOVA): ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001. bpm, beats per minute; HF, high-frequency range; IGF-I, insulin-like growth factor I; LF, low-frequency range; LF:HF, low-frequency high-frequency ratio; M, glucose metabolized; RMSDD, root mean square successive differences; SDNN, SD of all normal-to-normal intervals; SHBG, sex hormone binding globulin; SNS, sympathetic nervous system; TSH, thyroid-stimulating hormone; T3, 3,5,3′-tri-iodo-thyronine; T4, 3,5,3′,5′-tetra-iodo-thyronine.

1Reflects SNS activity and parasympathetic nervous system activity.
2Reflects parasympathetic nervous system activity.
3Reflects SNS activity.
However, CR lasted 3 wk in our protocol compared with 24 wk in the original study (19). Furthermore, refeeding was 2 wk in the current study compared with 12 wk of rehabilitation in the Minnesota Experiment. Our CR-study protocol could not be extended >3 wk because of ethical reasons.

The Minnesota semistarvation diet consisted of potatoes, cabbage, turnips, and cereals with only a few grams of animal protein per week; thus, the volunteers received an energy-restricted, very-low-fat (i.e., 27 g/d) and protein-restricted diet (55 g/d). By contrast, our subjects consumed a caloric-restricted but balanced diet (which varied only in carbohydrate and fat contents; see Methods). Despite reductions in food intake, Keys et al. (19) insisted that the men maintain their active lifestyle, including 22 miles of walking each week. By contrast, our subjects maintained a sedentary lifestyle throughout the whole study period.

In the Minnesota Experiment, volunteers lost 24% and 27.4% of their body weight and active tissue, respectively (19). At the end of the study, volunteers showed signs and symptoms of edema, anemia, polyuria, bradycardia, weakness, and depression (51). In the current study, 3 wk of CR decreased FFM (by 3.7%), body protein (by 2.0%), skeletal MM (by 4.7%), and internal organ masses (by 7.8%; Table 1) without clinical signs of malnutrition.

All foods were weighed, and energy contents were controlled with the use of regular analyses of duplicate portions. During meals, there was strict supervision, and any food left uneaten on the dishes was analyzed, and energy intakes were corrected accordingly. Contrary to the setting in the study of Keys et al. (19), our volunteers spent ~10 h/d in our metabolic ward (where they also had all of their meals) and were free during the remaining hours. Because we did continuous 24-h monitoring of interstitial glucose concentrations and physical activity, we did our best to control the study outside the institute. On the basis of the individual data, we had no evidence for any food intake or excessive physical activity outside of the metabolic ward.

In conclusion, AT is modest but reproducible. AT relates to early starvation and is associated with the fall in insulin secretion and extent of the FWCR. During a 3-wk controlled semistarvation, with a weight loss of ~6 kg, AT was manifest in ~60% of subjects. Changes in the composition of FFM (interrelations of organ and tissue masses) add to the explanation of AT. “True” AT is lower than what has been assumed previously (i.e., ~70 kcal). A reduced heart rate, kidney function, and body temperature together with increased hepatic gluconeogenesis add up to AT. CR-associated changes in leptin and T3 as well as in SNS activity reflect adaptations to weight loss, but they are not related to AT. Within the short term, AT has no impact on weight or fat regain.

We thank our dietitians, A Lindner, U Preuss, and B Rümpcker, for their help calculating, producing, and providing the diets.

The authors’ responsibilities were as follows—MJM and AB-W: designed the research and wrote and had primary responsibility for the final content of the manuscript; JE, BE, ML, DK, and AB-W: conducted the research; C-CG, JJK, and MP: provided essential reagents or materials; AB-W, MJM, JE, and WB: analyzed the data or performed the statistical analysis; and all authors: discussed the data and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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