

# Metabolic response to experimental overfeeding in lean and overweight healthy volunteers<sup>1,2</sup>

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**ABSTRACT** Possible adaptive mechanisms that may defend against weight gain during periods of excessive energy intake were investigated by overfeeding six lean and three overweight young men by 50% above baseline requirements with a mixed diet for 42 d [ $6.2 \pm 1.9$  MJ/d ( $\bar{x} \pm$  SD), or a total of  $265 \pm 45$  MJ]. Mean weight gain was  $7.6 \pm 1.6$  kg ( $58 \pm 18\%$  fat). The energy cost of tissue deposition ( $28.7 \pm 4.4$  MJ/kg) matched the theoretical cost (26.0 MJ/kg). Basal metabolic rate (BMR) increased by  $0.9 \pm 0.4$  MJ/d and daily energy expenditure assessed by whole-body calorimetry (CAL EE) increased by  $1.8 \pm 0.5$  MJ/d. Total free-living energy expenditure (TEE) measured by doubly labeled water increased by  $1.4 \pm 2.0$  MJ/d. Activity and thermogenesis (computed as CAL EE – BMR and TEE – BMR) increased by only  $0.9 \pm 0.4$  and  $0.9 \pm 2.1$  MJ/d, respectively. All outcomes were consistent with theoretical changes due to the increased fat-free mass, body weight, and energy intake. There was no evidence of any active energy-dissipating mechanisms. *Am J Clin Nutr* 1992;56:641–55.

**KEY WORDS** Adaptive thermogenesis, overfeeding, body weight, obesity, energy expenditure, whole-body calorimetry, doubly labeled water, set-point regulation

## Introduction

There appears to be significant variability in the response of different individuals to any energy excess or deficit in the diet. Research in this area has concentrated on the possible existence of a facultative mechanism for increasing energy expenditure via adaptive thermogenesis when overeating. Several biochemical mechanisms for this process have been suggested, such as a generalized uncoupling of oxidative phosphorylation (1), a specific uncoupling mechanism in brown adipose tissue (2), and an increased rate of substrate cycling (3).

According to Flatt (4) a generalized uncoupling is unlikely under physiological conditions. There is firm evidence in animals for a role for an uncoupling process in brown adipose tissue (2, 5), but studies in adult humans have indicated that brown adipose tissue is probably only present in small amounts under non-pathological conditions (6–8), and its energetic significance is questionable (8). Substrate cycling, however, is known to exist, for example, in the interconversion of fructose-diphosphate to fructose-6-phosphate, or in the hydrolysis of adipose tissue triglycerides followed by reesterification. Although it is now recognized that such cycles may play an important role in metabolic regulation, they were formerly referred to as futile cycles because

they consume ATP without any net change in other metabolic products. Such a facultative mechanism would represent expended energy that yields heat, but not work, and has been hypothesized to protect against obesity. Facultative thermogenesis might explain why some constitutionally lean people seem to be able to maintain a constant body weight over a wide range of energy intakes. Facultative thermogenesis might be faulty in obese people.

This aspect of human energy balance has been the subject of research since 1902, when Neumann (9) in Germany recorded the changes in body weight and nitrogen balance on himself during three periods in a year. He reported that his weight was almost constant despite intakes of 6.6, 8.0, and 11 MJ/d during the three periods. He proposed that the difference between consumption and energy needs was directly oxidized and dissipated as heat. Gulick (10) performed a similar long-term experiment on himself, varying his intake from 11.5, 13.4, or 14.6 MJ/d over 370 d, reportedly without any significant weight change. Gulick made meticulous measurements of his activity and was unable to attribute the differences in energy balance to either activity or basal metabolic rate (BMR). He attributed the cause to an extravagant energy expenditure, which earlier Neumann called *luxuskonsumption*. However, Forbes (11) reexamined the data of these two studies and found a linear relationship between changes in weight and modifications in energy intake, with a slope close to the predicted cost of weight gain. He concluded that both studies demonstrate that body weight responds to modest as well as profound alterations in energy intake in a predictable manner.

Since Gulick's study, there have been several other overfeeding experiments searching for *luxuskonsumption* and for metabolic differences between lean and overweight people (12–25). The duration of these studies varied from a few days to  $\geq 6$  wk and there has been a wide variation in the amount of weight gained by individuals on the same regime, for example, 0.1–3.4 kg on a food surplus of 293 MJ over 35 d (19), 3.2–10.4 kg on 469 MJ over 28 d (20, 21), 3.8–8.5 kg on 260 MJ over 42 d (18), and 4.3–13.3 kg on 353 MJ over 100 d (24). Although the variability may be due to real physiological differences among in-

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dividuals, other possible explanations include inadequate characterization of baseline requirements, as suggested by Webb and Annis (13); errors associated with calculated metabolizable energy (13, 18, 22); inaccuracy and imprecision in the estimation of energy stores (18, 22); extrapolation of total energy expenditure (TEE) in a calorimeter chamber to free-living conditions (18, 23); differences in the lean-fat ratio in the accrued tissue (24); or noncompliance by the subjects.

All the overfeeding studies that claim to have found evidence in support of *luxuskonsumtion* have been based on the demonstration of an apparent discrepancy between estimates of energy stored for a given energy excess and not on the actual measurement of an increased energy expenditure in free-living subjects (9, 10, 12–14). This failure to make direct measurements of the primary outcome variable in question (ie, heat production) also applies to many studies not supporting the existence of an adaptive thermogenic process. For instance, Ravussin et al (23) made estimates of free-living energy expenditure by increasing each individual's calorimeter energy expenditure by 25%. A slightly less indirect approach was used by Norgan and Durnin (18), whose subjects were asked to record all activities throughout the experiment, with the cost of each activity assessed by indirect calorimetry. As a result of these methodological shortcomings, there is no firm consensus about the existence of adaptive thermogenesis in humans.

Another long-disputed theory in obesity research is the suggestion, on the basis of animal studies, that body weight can be regulated around a "set point." This mechanism has been tested in rats that have been starved or force fed to radically alter their body weight. Once *refed ad libitum*, they rapidly return to their normal weight (26–29). In humans, the possible existence of such a mechanism remains controversial (30). It has been claimed that overweight people naturally tend to return to a high body weight after slimming, perhaps explaining why obese people cannot successfully maintain a reduced weight after dieting (31). The semistarvation studies by Keys et al (32) and the work of Sims and Horton (33) are often cited as supporting set-point theory. Counter evidence was published by Garrow (34).

The present study used a long-stay metabolic suite, whole-body calorimetry, and doubly labeled water (DLW) in an attempt to make direct measurements of any physiological or behavioral adaptive changes in response to experimental overfeeding. The ability of the subjects to return to their initial set point after substantial weight gain was also examined.

## Subjects and methods

### Subjects

This study was originally planned for six lean and six overweight male subjects. The study ultimately included seven lean and three overweight men because it was difficult to recruit overweight people who were prepared to be overfed. Forty potential volunteers were interviewed; most were lean but six were overweight, of whom three declined to be overfed.

The constitutionally lean subjects had low or normal weight and body fat ( $\leq 20\%$ ) and declared themselves to be good eaters and claimed to have difficulty gaining weight. The overweight subjects claimed to eat less than appetite, gain weight easily, and have difficulty losing weight when dieting.

The selection criteria for all subjects were good health, habitual alcohol consumption  $< 80$  g/wk, no food intolerances, and will-

ingness to complete fecal and urine collections. Subjects had to be prepared to reside in the area of the study for  $\geq 7$  mo, had to have no medical conditions that would be exacerbated by under- or overfeeding, had to have been assessed as being trustworthy and compliant, and had to have no personal or family history of diabetes. Obesity had an adult onset in the three overweight subjects. One subject (#805) had a significant family history of moderate obesity, one subject (#802) smoked occasionally, and two subjects (#805 and #809) smoked moderately. One subject was Palestinian, one was Lebanese, one was South African Caucasian, one was Australian Caucasian, and the remainder were British Caucasians.

Subjects were recruited by local advertisements and through an unemployment center. Unemployed volunteers were encouraged to obtain part-time employment before the study started in order to maximize their commitment to the study. Employment details were as follows (in subject order): part-time barman, office worker, theoretical physicist (desk work), part-time agricultural worker, author, hospital porter, postgraduate student, shop assistant, salesman, and unemployed. All selected subjects were fully informed about the study and signed a written consent form. The study was approved by the Dunn Nutrition Unit Ethical Committee.

During their stay in the unit, subjects were provided with food, accommodations, and a small honorarium. Each volunteer was assigned one of the rooms in the unit's metabolic suite.

### Protocol

The study lasted 7 mo for each subject and comprised five periods: baseline, overfeeding, free diet, underfeeding, and free diet. **Figure 1** outlines all measurements performed during the five study periods. This paper concerns only the first three periods. During the baseline period (3 wk), energy requirements were established and initial values of the different variables were measured. In the overfeeding period (6 wk), subjects were challenged with a diet supplying 50% more than the baseline energy requirements. The purpose of the postoverfeeding (or free-diet) period (6 wk) was to test each subject's ability to regain his hypothetical set point.

### Measurements

**Indirect calorimetry.** The two 11-m<sup>3</sup> whole-body calorimeters at the Dunn Clinical Nutrition Centre were described elsewhere (35, 36). They provide detailed minute-by-minute information on all components of energy expenditure with a very high degree of precision (37). Subjects were under constant supervision and followed a fixed timetable of activities, which remained the same throughout the study (**Fig 2**). Subjects were requested to have their evening meal 1 h before entering the calorimeter at 2000 h. There were periods of obligatory standing (a total of 96 min) consisting of two 30-min periods that included dressing, undressing, and washing and rearranging the furniture; a 5-min period before and a 10-min period after each exercise; and a 2–3-min period for collecting lunch or supper from the hatch. Subjects were asked to use these standing times to pass urine and feces if they so wished. Except where otherwise indicated, subjects remained seated and were only permitted very light activities such as television viewing, reading, writing, and eating. The purpose of such a rigid protocol was to eliminate behavioral noise that may have obscured the underlying physiological changes.

Weeks	3		9			15			21			27		
Period	Base line		Overfeeding			Free diet			Underfeeding			Free diet		
Measurement	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal	Cal
	Th			Th					Th					
	DLW			DLW			DLW		DLW			DLW		
	B	B	B	B	B	B	B	B	B	B	B	B	B	B

FIG 1. Study protocol: Cal, 36-h whole-body calorimetry at 26 °C; Th, 36-h whole-body calorimetry at 20 °C (thermal stress); DLW, 14-d doubly labeled-water measurement; B, body-composition assessment; UF, continuous urine and fecal collections.

Indirect calorimetry (37 h) was conducted at both thermo-neutrality ( $25 \pm 1$  °C) and below the thermoneutral range ( $20 \pm 1$  °C) to test for thermal stress, because animal experiments (2) have suggested that adaptive thermogenesis may be suppressed at higher temperatures because of a potential excess thermal load. At the lower temperature, subjects were required

to wear only thin cotton pajamas throughout the run and were not permitted to use bedclothes at night. Continuous indirect calorimetry was performed at least twice during the baseline period and after 2, 4, and 6 wk of overfeeding; thermal stress was tested once during the baseline period and at week 5 of the overfeeding period (Fig 1).

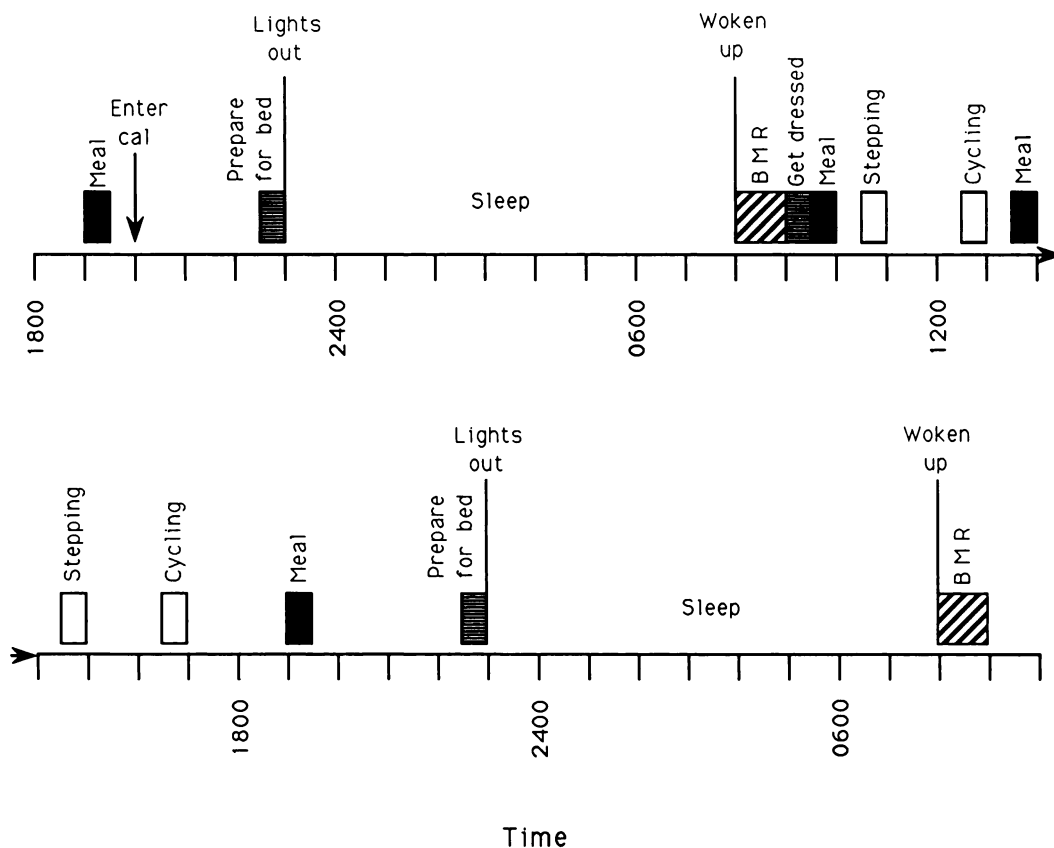


FIG 2. Calorimeter timetable. Subjects stand for 5 min before and 10 min after each exercise period. Cycle workload, 50 W at 50 rpm; stepping workload, 220 mm steps at 10 step-ups/min. Subjects remain sitting and constrained to light activities (reading, writing, watching television) except where indicated.

BMR was measured for 1 h, immediately on waking, between 12.5 and 13.5 h postabsorption, at thermoneutrality, and at complete physical rest. BMR was measured twice during each 37-h indirect-calorimetry run. In addition, BMR measurements alone were made after an overnight stay in the calorimeter during weeks 5–10 and 17–22, when 37-h calorimeter runs were not performed. Instrumentation noise ( $\pm 1$  SD) for 1-h measurements is typically  $\approx 0.04$  kJ/min. The CV for repeated measures within subjects is 1.6% (36).

Two 30-min periods of weight-dependent exercise (10 step-ups/min on 220-mm-high blocks), and two 30-min periods of weight-independent exercise (cycling at 50 W) were required. Exercise periods were closely supervised and rates were controlled by electronic metronome. The CVs for repeated 30-min measurements were 4.3% and 3.0%, respectively (36).

The value for activity plus thermogenesis (A+T) was calculated by subtracting BMR from the 24-h energy expenditure (CAL EE – BMR), where CAL EE is 24-h energy expenditure in the calorimeter.

Carbohydrate (CHO) and fat oxidation were calculated according to the method of King et al (38), assuming an energy content of 39.33 kJ/g for fat, 15.69 kJ/g for CHO, and 16.72 kJ/g for urinary nitrogen. The confidence limits ( $\pm 2$  SD) for our calorimeters are  $\approx 13$  g/d for fat oxidation and  $\approx 31$  g/d for CHO oxidation for a typical moderately active subject consuming an average of 490 L O<sub>2</sub>/d and producing 420 L CO<sub>2</sub>/d.

**Doubly labeled water.** To measure energy expenditure under truly free-living conditions, accurately weighed doses of deuterium (0.05 g/kg body wt) and <sup>18</sup>O (0.15 g/kg) were orally administered 14 d before the end of each period. Urine samples were collected on the dosing day and daily for 14 d thereafter. Isotope enrichments of the urine samples were analyzed by using an isotope-ratio mass spectrometer (Aqua Sira, Middlewich, Cheshire, UK). Theoretical considerations and calculations of energy expenditure by this method are described elsewhere (39–41). Propagation of error analysis as described by Cole et al (42) was performed on each TEE measurement to obtain individual errors. This propagated-error analysis aggregates the individual errors on the intercepts and slopes of both isotopes, before correcting for covariance between deuterium and <sup>18</sup>O. The computed errors, which include both measurement noise and day-to-day variation (biological noise), were on average  $5.0 \pm 3.6\%$  for baseline,  $4.5 \pm 2.2\%$  for overfeeding, and  $5.9 \pm 2.8\%$  for the postoverfeeding period.

One subject (#809) had no doubly labeled-water (DLW) data during overfeeding because of a malfunction of the scales used to weigh the dose. Subject #801 had no data during the post-overfeeding period because he left the study prematurely.

During their stay in the unit, subjects were free to perform their usual occupation and physical activities so that potential differences in the behavioral response to overfeeding between overweight and lean individuals could be assessed.

**Dietary intake.** Meals were prepared in the metabolic kitchen and were stored in each subject's own refrigerator, requiring only to be heated by microwave. Packed lunches were made available to subjects studying or working away from the unit. During the baseline and overfeeding periods, three meals were prepared per day on a 3-d rotating menu. The nine different meals had approximately the same nutrient composition (43) and, therefore, variability of the diet was reduced to a minimum. The composition of the diet reflected that of a typical British

diet (12% protein, 42% fat, and 46% CHO) and contained no artificial food.

The diet during the baseline period consisted of a standard 10-MJ diet plus any extra food the subject wanted to eat. (The extra food had to be selected from a number of food items made available in easily quantifiable amounts.) The food consumed was recorded in a food diary.

The energy content of the overfeeding diet was calculated (by DLW) as 150% of the mean intake and expenditure observed during baseline. As in the baseline period, the diet was provided as three equal meals to be eaten as breakfast, lunch, and supper. Because of the difficulty that many subjects experienced in consuming the overfeeding diet, subjects were permitted to extend meal times for as long as necessary. Accurately weighed and homogenized samples of each meal were freeze-dried for analysis of gross energy content by bomb calorimetry (Adiabatic Bomb, Gallenkamp, Crawley, Surrey, UK), and of nitrogen by Kjeldahl analysis (Tecator Kjeltec System, Tecator Ltd, Bristol, UK).

To calculate nitrogen balance and the metabolizable energy of the diet, total urine and fecal collections were made throughout the entire overfeeding period. Freeze-dried fecal samples were analyzed by bomb calorimetry for gross energy and by Kjeldahl analysis for nitrogen. Urine samples were analyzed by Kjeldahl analysis for nitrogen. Fecal collections were checked for completeness by using radioopaque markers as described by Branch and Cummings (44).

Dietary intake after the overfeeding period was not recorded, although most of the food was usually provided in the unit. In contrast to the controlled-diets conditions, subjects were free to eat and drink away from the unit according to their own preferences; however, subjects were fed the baseline maintenance diet during the calorimeter runs to maintain subjects in energy balance during the actual measurement.

**Anthropometry and body composition.** Body weight ( $\pm 1$  g) corrected to nude weight was measured with an integrating platform scale with a digital readout (type E1210; August Sauter, GmbH, Albstadt, Germany) daily after voiding and before breakfast. During the postoverfeeding period subjects were weighed blind to minimize cognitive cues when the set-point body-weight regulation was assessed.

Total body water (TBW) determined by DLW was calculated from the deuterium and <sup>18</sup>O spaces combined, as suggested by Schoeller et al (45). Fat-free mass was estimated by dividing TBW by 0.73 (46). Body fat was calculated by subtracting fat-free mass from body weight.

## Results

Physical characteristics of the subjects are shown in **Table 1**. Lean subjects were somewhat younger than the overweight subjects, although not significantly so; body weight and body fat were significantly lower in the lean group.

### Dietary intake

Subjects' compliance with diets, sample collections, and measurement procedures appeared to be very good despite the rigorous demands of the study protocol. The overfeeding diet was well tolerated although almost all volunteers found this part of the study difficult. Two lean subjects became ill during the overfeeding period, experiencing episodes of diarrhea and vomiting (probably of viral origin) for no more than 2 d, after which time

TABLE 1  
Initial characteristics of lean and overweight subjects

Subject	Age	Weight	Height	BMI*	Fat†
	<i>y</i>	<i>kg</i>	<i>m</i>		<i>%</i>
801	26	75.4	1.85	22.0	17.4
802	30	71.3	1.83	21.3	16.4
803	33	68.5	1.72	23.1	20.7
804	25	57.9	1.72	19.6	11.2
805‡	49	89.8	1.80	27.7	30.0
807	19	70.6	1.72	23.9	19.5
809‡	27	77.6	1.75	25.3	29.2
810‡	36	84.7	1.76	27.3	30.2
811	22	70.2	1.83	21.0	16.8
813	29	68.2	1.81	20.8	21.6
Lean					
$\bar{x}$	26.3	68.9	1.78	21.7	17.7
±SD	4.5	5.0	0.04	1.3	3.2
Overweight					
$\bar{x}$	37.3	84.0	1.77	26.8	29.7
±SD	9.0	5.0§	0.02	1.0§	0.5§
Total					
$\bar{x}$	29.6	73.4	1.78	23.2	21.3
±SD	8.0	8.6	0.05	2.6	6.1

\* Body mass index, weight/height<sup>2</sup> (kg/m<sup>2</sup>).

† Calculated from <sup>18</sup>O and deuterium dilution spaces.

‡ Overweight subjects.

§ Significantly different from the lean group, *P* < 0.001 (*t* test).

(with medical approval) they successfully finished the overfeeding. Although volunteers were told to eat everything given, there were a few days when some food was left over. This amount was discounted from the calculation of total energy intake during the period. Because some packed lunches were consumed away from the unit, total compliance could not be guaranteed. However, the most likely form of noncompliance would be failure to eat all that was provided; the high rates of weight gain achieved (*see* below) argue against such noncompliance.

The mean intraindividual CVs for intakes during the overfeeding period were 0.93% for energy, 1.0% for protein, 2.3% for fat, and 1.6% for carbohydrate. There was good agreement between metabolizable energy calculated from food tables (FME) and the measurements obtained from the gross, fecal, and urinary energy (MME). The ratio of MME to FME was close to 1.0 in most cases (Table 2). The 50% increase in energy intake during the overfeeding period, as compared with baseline, amounted to 6.2 MJ/d. The digestibility of the overfeeding diet was 93.9% and the metabolizable energy was 91.5% of the gross energy.

The mean percent of energy from protein during the baseline and overfeeding periods was, respectively, 11.7% and 12.7%; from fat, 39.0% and 41.5%; and from carbohydrate, 48.8% and 45.4%. The food quotient remained between 0.84 and 0.85 for both periods (47).

#### Changes in body weight during overfeeding

Figure 3 shows the mean and individual changes in body weight throughout the study. All subjects, except subject 807, gained weight almost linearly during the overfeeding. Subject 807 requires special mention because his weight curve might be considered evidence for luxuskonsumption. Activity question-

naires, observations by the investigators, and post hoc interviews all revealed a profound change in his activity pattern coincident with the start of overfeeding. At this time he had become employed as a hospital porter and had also started a daily regime of running and cycling for periods of 40–60 min. This change in activity pattern was consistent with the change in TEE from 12.5 MJ/d during baseline to 17.2 MJ/d during overfeeding and to 17.9 MJ/d in the postoverfeeding period. The values for A+T were 5.3, 9.3, and 10.0 MJ/d for the three periods, respectively. The failure of TEE to fall after overfeeding provides the best evidence that the change in activity was coincidental and was not related to the overfeeding. Note that the study design prevented the investigators from influencing this alteration in lifestyle or enquiring as to its reason at the time because this would have affected the remainder of the protocol. Except where indicated, data from subject 807 was excluded from subsequent analysis.

In the remainder of the subjects, the mean weekly weight-increase velocities during overfeeding were  $1.39 \pm 0.61$  ( $\bar{x} \pm$  SD),  $1.52 \pm 0.59$ ,  $1.23 \pm 0.52$ ,  $1.19 \pm 0.58$ ,  $1.12 \pm 0.62$ , and  $1.12 \pm 0.51$  kg/wk. After the second week there was a tendency for the average weight-gain curve to level off, as would be expected from the increase in energy requirements caused by the increased weight and FFM. The discontinuity between weight velocities in the first 2 and last 4 wk of overfeeding suggests that any changes in energy expenditure had virtually leveled off by the end of week 2. This was partially confirmed by the CAL EE results (*see* below).

There was considerable individual variability in the amount of weight gained during overfeeding (Table 3), with a range (even when subject 807 was excluded) from 5.0 to 10.5 kg ( $7.6 \pm 1.5$  kg, equivalent to 10.4% of initial body weight). This occurred despite the fact that all but one subject were fed  $51.0 \pm 1.9\%$  more energy during the overfeeding period than they were during baseline; subject 802 was overfed by only 36% because of an initial miscalculation of baseline energy expenditure (by DLW). The composition of the weight gained also varied markedly, with  $\delta$ FFM: $\delta$ BW ranging from 0.19 to 0.76 ( $\bar{x}$  0.42, *n* = 8). The

TABLE 2  
Dietary energy intake\*

Subject	Baseline		Overfeeding				MME/FME
	FME	GE	FME	GE	Fecal	Urinary	
	<i>MJ/d</i>						
801	14.2	23.6	25.3	1.3	0.6	23.4	0.99
802	13.7	18.1	19.8	1.3	0.6	17.9	0.99
803	14.6	21.2	23.4	1.5	0.6	21.3	1.00
804	14.8	21.4	23.2	1.4	0.6	21.2	0.99
805†	11.9	19.0	20.6	0.8	0.4	19.3	1.01
807	11.8	19.1	21.0	1.3	0.5	19.1	1.00
809†	11.1	18.3	21.4	1.8	0.6	19.0	1.04
810†	12.6	18.1	18.9	1.2	0.4	17.3	0.96
811	13.2	19.6	20.3	0.8	0.5	18.9	0.96
813	14.4	18.1	19.5	1.4	0.5	17.6	0.97
$\bar{x}$	13.3	19.6	21.3	1.3	0.5	19.5	0.99
±SD	±1.2	±1.7	±1.9	±0.3	±0.05	±1.8	±0.02

\* FME, metabolizable energy calculated from food-composition tables; GE, gross energy by bomb calorimetry; and MME, measured metabolizable energy.

† Overweight subjects.

variation in the amount of weight gained was surprisingly not explained by a greater proportion of FFM (with its lower energy density) in the subjects who gained the most weight. In fact the relationship was the reverse, with the ratio of  $\delta$ FFM to  $\delta$ BW being inversely correlated with weight gain ( $r = -0.703$ ,  $P < 0.05$ ).

#### Set-point regulation of body weight

After overfeeding, all subjects lost weight (Fig 3). Bereavement due to the death of a family member accounted for the exceptionally high weight loss of 9.3 kg in subject 805 during the postoverfeeding period. In the remaining subjects the total weight loss in the postoverfeeding period was  $4.16 \pm 1.52$  kg, or 55% (range 42–86%) of the amount gained ( $n = 8$ ). Mean weekly weight changes were  $-1.57 \pm 1.21$ ,  $-0.40 \pm 1.30$ ,  $-0.60 \pm 0.97$ ,  $-1.36 \pm 0.83$ ,  $-0.14 \pm 1.26$ , and  $-0.09 \pm 1.59$  kg, indicating that weight loss had virtually ceased by the fifth week after resuming a free diet.

#### Total energy expenditure by DLW

TEE measured by DLW is shown in Table 4. From the baseline average of  $13.2 \pm 1.1$  MJ/d, there was a  $1.4 \pm 2.0$  MJ/d (11%) increase in TEE with overfeeding in those subjects for whom estimates were available in both periods. Six subjects increased their TEE by 1–2 MJ/d whereas the other two subjects (#807 and #811, both lean) raised their TEE by 4–5 MJ/d. A significant change in the activity pattern was shown in both subjects. As discussed previously, the change in subject 807 was so marked that his data were excluded. Note that subject 811 showed the next lowest weight gain of those who were fed the true 50% energy excess. Another subject (#801) reduced his TEE when overfed because of a reduction in his physical activity (he played hockey during the baseline but the season ended at the beginning of the overfeeding period), causing a greater weight gain. Overall the changes in TEE between baseline and overfeeding explained 69% of the variance in the amount of weight gained ( $r = -0.827$ ,  $P < 0.01$  for  $n = 8$ , including subject 807 and excluding subject 802, who received only 36% excess).

TABLE 3

Changes in body weight and composition observed during the overfeeding and postoverfeeding periods

Subject	Overfeeding			Postoverfeeding		
	Weight	Fat	FFM*	Weight	Fat	FFM
	kg					
801	10.5	7.6	2.9	-6.3†	†	†
802	5.0	1.2	3.8	-2.2	+1.2	-3.4
803	8.0	4.5	3.5	-3.8	-3.6	-0.2
804	8.8	7.1	1.7	-4.8	-3.9	-0.9
805‡	7.5	5.2	2.3	-9.3§	-6.0	-3.3
807	1.3§	—	—	-3.2§	-2.9	-0.3
809‡	7.1§	—	—	-6.1	—	—
810‡	7.9	5.0	2.9	-3.3	-2.9	-0.4
811	5.9	3.5	2.4	-2.5	-3.9	+1.4
813	7.9	3.3	4.6	-4.3	-3.7	-0.6
$\bar{x}$	7.6	4.6	3.0	-3.9	-2.8	-0.7
$\pm$ SD	$\pm 1.5$	$\pm 1.9$	$\pm 0.9$	$\pm 1.2$	$\pm 1.8$	$\pm 1.4$

\* Fat-free mass.

† Subject 801 left the study prematurely.

‡ Overweight subjects.

§ Excluded from the mean because of the following: for #807, profound change in activity patterns (see text); #805, extra weight loss attributable to death of son; #809, no total-body-water data at end of overfeeding period.

The energy cost of activity shown by the A+T component increased by  $0.5 \pm 1.8$  MJ/d (10%) but the difference was not significant. Because of the changes in the denominator (BMR), the activity ratio (TEE/BMR) remained close to 1.8 in all three phases of the study.

In the postoverfeeding period, TEE was reduced toward the baseline values in all subjects except #807 and #809. A+T showed a similar pattern (Table 4).

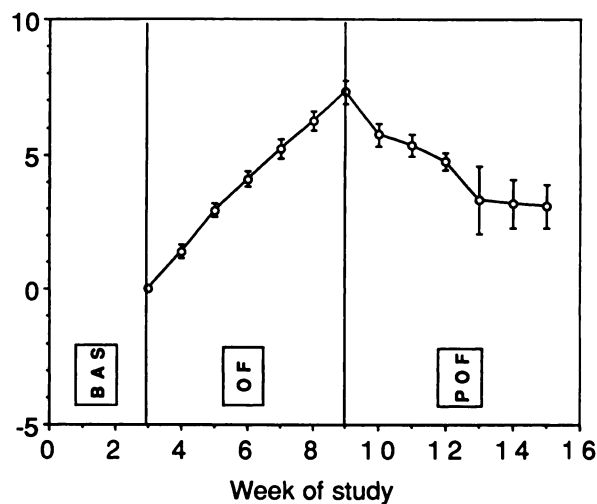
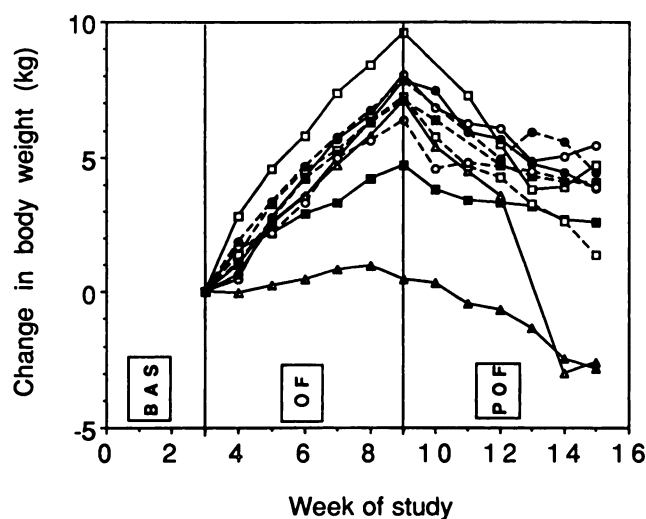


FIG 3. Changes in body weight during overfeeding and subsequent free diet. BAS, baseline; OF, overfeeding; POF, postoverfeeding. Right-hand panel shows  $\bar{x} \pm$  SE of changes relative to baseline excluding #807 during OF and 807 + 805 during POF. Subject symbols: 801 —□—, 802 —■—, 803 —○—, 804 —●—, 805 —△—, 807 —▲—, 809 —□—, 810 —■—, 811 —○—, 813 —●—.

TABLE 4  
Total energy expenditure by doubly labeled water\*

Subject	Baseline			Overfeeding			Postoverfeeding		
	TEE	A + T	TEE/BMR	TEE	A + T	TEE/BMR	TEE	A + T	TEE/BMR
	<i>MJ/d</i>								
801	15.8	8.4	2.0	14.8	6.8	1.8	ND	ND	ND
802	13.3	5.5	1.7	14.9	6.7	1.8	13.2	6.1	1.9
803	13.8	7.0	2.0	15.0	6.9	1.9	12.1	4.9	1.7
804	14.4	7.6	2.2	15.5	7.2	1.9	13.2	5.9	1.8
805†	12.4	4.6	1.6	15.0	6.3	1.7	11.0	3.1	1.4
807	12.5	5.3	1.7	17.2	9.3	2.2	17.9	10.0	2.3
809†	12.1	5.2	1.7	‡	‡	‡	10.4	3.6	1.5
810†	12.7	5.9	1.9	12.0	4.3	1.5	13.5	6.3	1.9
811	13.1	5.2	1.7	17.5	9.0	2.1	15.9	7.8	2.0
813	11.7	4.0	1.5	13.3	5.2	1.6	10.9	3.6	1.5
$\bar{x}$	13.2	5.9	1.8	15.0	6.9	1.8	13.1	5.7	1.8
±SD	±1.1	±1.4	±0.2	±1.6	±1.6	±0.2	±2.3	±2.2	±0.3

\* TEE, total energy expenditure; A + T, activity plus thermogenesis (derived as TEE minus BMR); BMR, basal metabolic rate; ND, not dosed.

† Overweight subjects. Baseline dosing at week 2, overfeeding at week 9, and postoverfeeding at week 15.

‡ See Methods (dosing).

#### Energy expenditure in the calorimeter

The 24-h energy expenditure in the calorimeter (Table 5) increased with overfeeding by an average of  $\approx 1.8 \pm 0.5$  MJ/d (17%); half of this amount was due to an increase in BMR. A+T, the component in which any putative luxuskonsumption should be most apparent, increased on average by only  $0.9 \pm 0.4$  MJ/d. The largest individual increase in A+T was only 1.4 MJ/d (subject 801). After overfeeding, 24-h energy expenditure, BMR, and A+T all returned to values very similar to those at baseline. The changes in CAL EE are shown on a weekly basis in Figure 4. There was a rapid increase in the energy expenditure, reaching in most cases a steady state after  $\approx 2$  wk, when the increment was usually within 1.5–2.5 MJ/d above baseline. Note that the energy excess when subjects were inside the chamber was actually > 50% because CAL EE was lower than the DLW TEE from which the 50% increment was calculated (10.6 vs 13.4 MJ/d during baseline). Thus, on days in the chamber, the excess averaged  $\approx 84\%$  at the beginning of overfeeding  $[(19.5/10.6) \times 100]$  and 57% at the end of overfeeding  $[(19.5/12.8) \times 100]$ . There were no detectable differences between the calorimeter measurements performed at 20 or 25 °C in any phase of the study.

The weekly individual changes in BMR are shown in Figure 5, which illustrates quite a wide variability in the rate at which BMR increased in response to the excess energy load. At the end of the overfeeding period BMR was  $0.9 \pm 0.4$  MJ/d ( $P < 0.001$ ), or  $9 \pm 10$  kJ · kg FFM<sup>-1</sup> · d<sup>-1</sup> ( $P < 0.05$ ) higher than baseline. The significant increase per kilogram FFM (equivalent to 0.5 MJ/d,  $P < 0.05$ ) may be partly explained by the fact that the thermic effect of the large evening meal had not completely disappeared by the time of the BMR measurement. The changes induced by overfeeding in all components of CAL EE are illustrated in Figure 6, which shows calorimeter traces from one subject during baseline and at the end of the overfeeding. The gradually diminishing thermic effect of food (TEF) is clearly

visible. After overfeeding, BMR was very similar to baseline, with most cases differing by no more than  $\pm 0.5$  MJ/d.

The energy cost of stepping and cycling increased during overfeeding (+11.4% and +9.0%) and decreased afterwards in absolute terms (Table 6), but not when expressed per kg body wt or as FFM (Table 7). The absolute energy cost of these activities was artificially increased by some TEF because the activities were always performed within 1–3 h after the meals (see Fig 2).

#### Substrate oxidation

Figure 7 shows the net fat and carbohydrate balances during the calorimeter measurements at baseline, at 6 wk overfeeding, and at the end of the postoverfeeding phase. Both carbohydrate and fat balance were accurately maintained during baseline and postoverfeeding. However, during overfeeding, the subjects were in slight positive carbohydrate balance and in massively positive fat balance.

We assume that the subjects must have reached a steady state with respect to carbohydrate shortly after commencing the overfeeding phase because glycogen stores would have been saturated. The slight positive balance during the calorimeter runs in the final week of overfeeding may be explained by the greater gap between energy intake (EI) and CAL EE than between EI and free-living TEE, which effectively increases the energy and substrate excess. The relative immobility in the calorimeter may also have suppressed muscle glycogen utilization. In absolute terms, carbohydrate oxidation increased from  $316 \pm 88$  g/d during baseline to  $491 \pm 90$  g/d.

Average intakes of fat were 131 g/d during baseline and 204 g/d during overfeeding. Oxidation rates in the calorimeter were  $126 \pm 24$  and  $71 \pm 112$  g/d, respectively. The extreme positive fat balance was therefore not simply due to a failure to oxidize the excess ingested. It was enhanced by a 46% suppression of fat oxidation secondary to the stimulated carbohydrate oxidation.

TABLE 5  
Calorimeter energy expenditure\*

Subject	Baseline			Overfeeding			Postoverfeeding		
	CAL EE	BMR	A + T	CAL EE	BMR	A + T	CAL EE	BMR	A + T
	<i>MJ/d</i>								
801	10.8	7.4	3.4	13.1	8.3	4.8	10.4	6.9	3.5
802	11.1	7.8	3.3	12.0	8.3	3.7	10.0	7.1	2.9
803	10.5	6.8	3.7	12.6	8.1	4.5	10.6	7.1	3.5
804	9.5	6.6	2.9	11.6	8.2	3.4	10.0	7.2	2.8
805†	11.0	7.8	3.2	13.3	8.8	4.5	11.1	7.7	3.4
807	10.0	7.2	2.8	11.6	8.0	3.6	10.1	7.7	2.4
809†	10.3	6.9	3.4	12.0	8.0	4.0	10.1	6.7	3.4
810†	10.2	6.8	3.4	12.2	8.0	4.2	10.7	7.2	3.5
811	11.3	7.9	3.4	12.8	8.4	4.4	11.8	8.1	3.7
813	10.8	7.7	3.1	12.4	8.1	4.3	11.1	7.3	3.8
$\bar{x}$	10.6	7.3	3.3	12.4	8.2	4.2	10.6	7.3	3.3
±SD	±0.6	±0.5	±0.3	±0.6	±0.2	±0.4	±0.6	±0.4	±0.4

\* CAL EE, calorimeter 24-h energy expenditure; BMR, basal metabolic rate; A + T, activity plus thermogenesis (derived as CAL EE minus BMR).  
† Overweight subjects.

#### Energy cost of tissue deposition

The energy cost of tissue deposition cannot be assessed precisely because the true energy excess must be calculated as the difference between EI and TEE during overfeeding and because DLW estimates of TEE were only obtained at baseline and for the last 2 wk of overfeeding. The best estimate of the aggregate excess can be calculated assuming a linear increase in TEE between baseline (group  $\bar{x}$ , 13.2 MJ/d) and the known DLW estimate for weeks 4–6 (group  $\bar{x}$ , 14.8 MJ/d). Use of this approach for each individual yields an average energy cost of tissue accretion of  $28.7 \pm 4.4$  MJ/kg (range 20.4–35.2 MJ/d). Because

the synthesis costs have already been accounted for in the measurement of TEE, the observed cost of deposition should be equivalent to the energy density of the accrued tissue. From the body-composition changes (Table 3), the gross energy of fat (39.6 kJ/g) and protein (23.6 kJ/g), and an assumed protein content of 20% for FFM, cost of deposition can be calculated as  $(0.61 \times 39.6) + (23.6 \times 0.20 \times 0.39) = 26$  MJ/kg. The observed mean value differed from this by only 10%.

#### Comparison between lean and overweight subjects

The extent to which valid comparisons can be made between the lean and overweight subjects is limited by our inability to

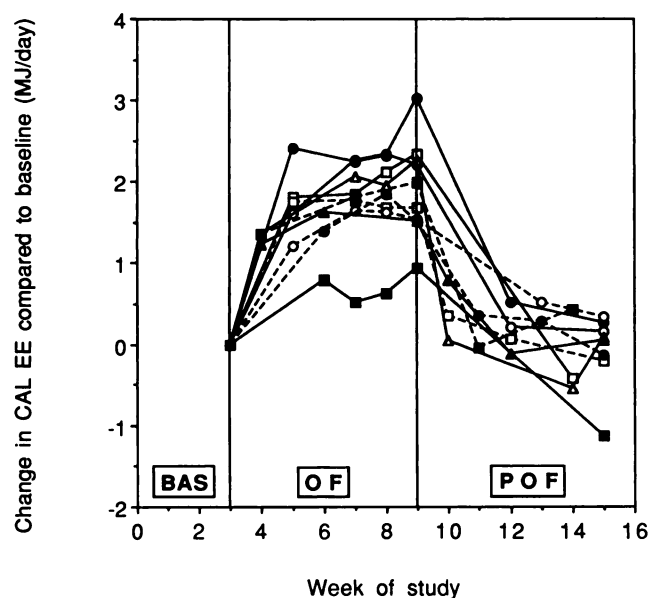


FIG 4. Changes in calorimeter energy expenditure (CAL EE) during overfeeding and subsequent free diet. Legend as for Figure 3.

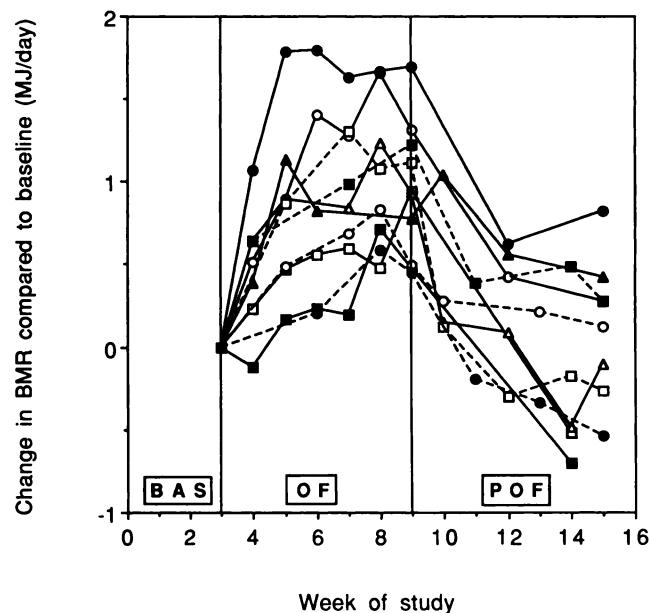


FIG 5. Changes in BMR during overfeeding and subsequent free diet. Legend as for Figure 3.



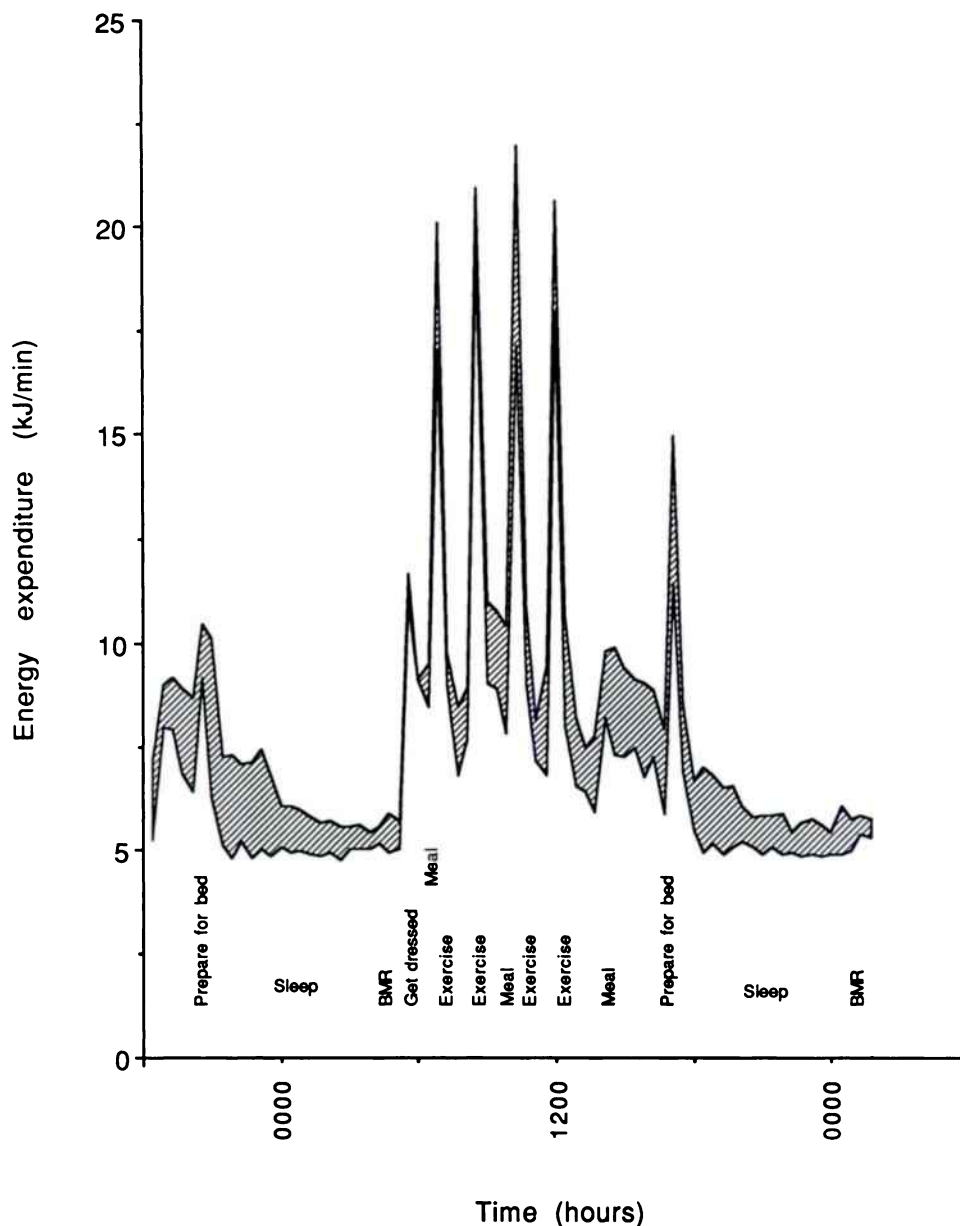


FIG 6. Calorimeter traces from a representative subject during baseline and at week 6 of overfeeding. Data from subject 801. Upper trace represents energy expenditure after overfeeding. Values are plotted as 30-min averages.

recruit other overweight subjects who were prepared to be intentionally overfed. Nonetheless it is noteworthy that the groups were virtually indistinguishable with regard to the major outcome variables studied. In particular, the amount of weight gained was very similar (lean,  $7.7 \pm 2.0$  kg; obese,  $7.5 \pm 0.4$  kg). The overweight and lean subjects showed a similar tendency to return (imprecisely) toward their initial body weight.

The increase in CAL EE in overweight subjects in response to overfeeding was actually slightly greater than it was in the lean subjects ( $2.0 \pm 0.3$  vs  $1.75 \pm 0.6$  MJ/d; NS). The increase in calorimeter A+T was identical ( $0.90 \pm 0.40$  vs  $0.90 \pm 0.40$  MJ/d; NS). It is not possible to make similar comparisons for the free-living TEE because data are missing from one overweight subject.

The main detectable difference between the two groups occurred in the substrate-oxidation results (Fig 8). Fat oxidation was remarkably low during overfeeding in overweight subjects compared with the lean group ( $19.4 \pm 4.9\%$  vs  $40.7 \pm 17.3\%$  of amount fed;  $P < 0.01$ ). Even during baseline and postoverfeeding, the overweight subjects tended to oxidize slightly less fat than they consumed. However, the most striking difference was that the overfeeding induced a 37% suppression of fat oxidation in the lean group compared with baseline (135 g reduced to 85 g) but induced a 64% suppression in the overweight group (105 g reduced to 38 g). Carbohydrate oxidation also differed but in the opposite direction, with overweight subjects tending to be in slight negative carbohydrate balance in the calorimeter during baseline and postoverfeeding, but in balance during overfeeding.

TABLE 6  
Energy cost of cycling and stepping

Subject	Stepping			Cycling		
	Baseline	Overfeeding	Postoverfeeding	Baseline	Overfeeding	Postoverfeeding
	<i>KJ/min</i>					
801	17.0	21.7	18.0	18.3	21.1	18.3
802	17.8	18.3	16.3	21.4	20.8	19.5
803	16.5	18.7	17.1	20.7	23.8	22.4
804	15.4	17.3	16.2	19.4	20.2	18.5
805*	19.6	22.9	18.4	21.8	23.8	21.0
807	16.6	18.3	16.3	20.0	20.8	18.9
809*	17.0	19.4	17.4	19.5	22.6	20.3
810*	20.3	20.6	19.1	18.1	21.2	19.5
811	18.1	19.3	18.2	21.0	22.8	22.3
813	16.8	18.9	17.7	19.3	21.3	20.5
$\bar{x}$	17.5	19.5	17.5	20.0	21.8	20.1
$\pm$ SD	$\pm 1.5$	$\pm 1.7\ddagger$	$\pm 1.0$	$\pm 1.3$	$\pm 1.3\ddagger$	$\pm 1.5$

\* Overweight subjects.

† Significantly different from baseline data,  $P < 0.001$  (paired  $t$  test).

## Discussion

The investigation of set-point regulation of body weight was a subsidiary part of this study and was limited to a 6-wk period in order not to compromise compliance with the remainder of the protocol. During this period there was a pronounced, but only partial, return toward initial weight over the first 4 wk (52%) and only a very gradual loss (a further 3%) in the next 2 wk. Subjects were given free access to food both inside and outside the metabolic unit and were permitted to consume alcohol. Body

weights were recorded by the investigators, and subjects were instructed not to weigh themselves so that cognitive cues could be minimized. However, it is recognized that other cues are inevitable, particularly from clothing. For instance, several subjects increased their waistlines to such an extent that we had to purchase new trousers for them. Tight-fitting clothes undoubtedly provided most subjects with powerful clues about their weight relative to baseline. Several of the subjects considered themselves overthin at baseline and were happy with their weight gain, but they still lost weight. Part of the reason for the rapid early weight

TABLE 7  
Summary of the changes induced by overfeeding compared with baseline\*

	Baseline	Overfeeding	Overfeeding minus baseline	$P$ †
Energy intake (MJ/d)	13.3 $\pm$ 1.3	19.5 $\pm$ 2.0	6.2 $\pm$ 1.9	<0.001
Body mass (kg)	73.7 $\pm$ 9.5	81.4 $\pm$ 9.6‡	7.6 $\pm$ 1.6‡	<0.001
Fat free mass (kg)	57.4 $\pm$ 4.0	60.7 $\pm$ 4.2‡	3.0 $\pm$ 0.9‡	<0.001
Fat mass (kg)	16.3 $\pm$ 7.0	20.3 $\pm$ 7.4‡	4.6 $\pm$ 2.1‡	<0.001
CAL EE (MJ/d)	10.6 $\pm$ 0.6	12.4 $\pm$ 0.6	1.8 $\pm$ 0.5	<0.001
CAL EE (kJ/kg/d)	146 $\pm$ 17	154 $\pm$ 15	9 $\pm$ 4	<0.001
CAL A + T (MJ/d)	3.3 $\pm$ 0.2	4.2 $\pm$ 0.4	0.9 $\pm$ 0.4	<0.001
BMR (MJ/d)	7.3 $\pm$ 0.5	8.2 $\pm$ 0.2	0.9 $\pm$ 0.4	<0.001
BMR (kJ $\cdot$ kg FFM <sup>-1</sup> $\cdot$ d <sup>-1</sup> )	128 $\pm$ 9	137 $\pm$ 9‡	9 $\pm$ 10‡	<0.05
Stepping (kJ $\cdot$ 30 min <sup>-1</sup> $\cdot$ kg <sup>-1</sup> )	7.2 $\pm$ 0.5	7.3 $\pm$ 0.4	0.1 $\pm$ 0.4	NS
Cycling (kJ $\cdot$ 30 min <sup>-1</sup> $\cdot$ kg <sup>-1</sup> )	8.2 $\pm$ 1.2	8.2 $\pm$ 0.9	0.0 $\pm$ 0.5	NS
DLW TEE (MJ/d)	13.2 $\pm$ 1.2	14.8 $\pm$ 1.6‡	1.4 $\pm$ 2.0‡	NS
DLW TEE (kJ $\cdot$ kg <sup>-1</sup> $\cdot$ d <sup>-1</sup> )	187 $\pm$ 35	186 $\pm$ 35‡	-1 $\pm$ 25†	NS
DLW A + T (MJ/d)	5.9 $\pm$ 1.4	6.5 $\pm$ 1.6‡	0.5 $\pm$ 1.8‡	NS
TEE/BMR	1.8 $\pm$ 0.3	1.8 $\pm$ 0.2‡	1.7 $\pm$ 0.2‡	NS
Energy/kg gained (MJ/kg)	NA	28.7 $\pm$ 4.4§	NA	—

\*  $\bar{x} \pm$  SD;  $n = 9$  (#807 excluded) unless indicated otherwise. CAL EE, calorimeter 24-h energy expenditure; BMR, basal metabolic rate; DLW TEE, doubly labeled-water total energy expenditure; A + T, activity plus thermogenesis (derived as CAL EE - BMR, or DLW TEE - BMR).

† Paired  $t$  test.

‡  $n = 8$  due to missing data.

§  $n = 7$  due to missing data (see earlier tables to identify missing points).

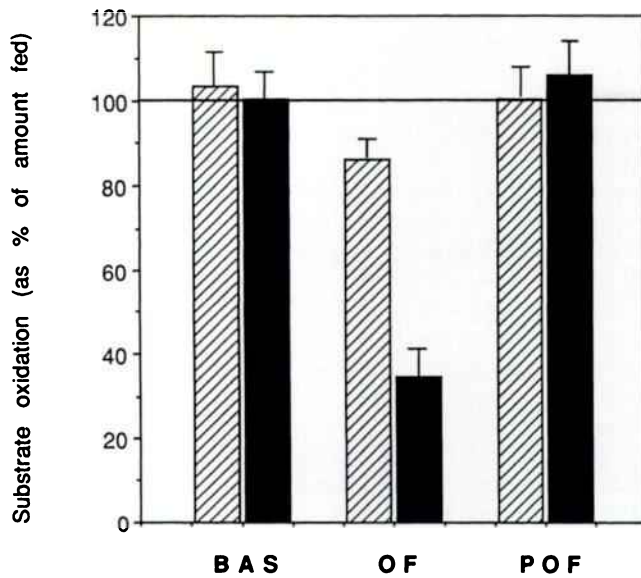


FIG 7. Effect of overfeeding on substrate oxidation assessed by whole-body calorimetry. Values for lean and overweight subjects combined. Hatched bars, carbohydrate oxidation; solid bars, fat oxidation.  $\bar{x} \pm SE$  for 24-h oxidation rates ( $n = 9$ ). Amount fed refers to diet in calorimeter [ie, averaging 10.5 MJ/d during baseline (BAS) and postoverfeeding (POF), and 19.5 MJ/d during overfeeding (OF)].

loss may have been a reaction to the unpleasant forced overfeeding that they had just endured. This may exaggerate the evidence for set-point regulation. On the other hand, the relief from a monotonous 3-d rotating diet would have been expected to stimulate food intake. On balance, we conclude that there was evidence that a physiological sensor was sensitive to the fact that body weight had been perturbed and was attempting to

reset it, but that this mechanism was imprecise because body weight remained > 3 kg higher than baseline.

The main purpose of the study was to search for possible autoregulatory mechanisms that defend the body against weight gain during periods of overeating. These could only occur in a limited number of ways: a reduction in the efficiency with which energy is extracted from the diet, an adaptive increase in thermogenesis designed to burn off energy as heat by increasing the metabolic flux through uncoupled or futile biochemical pathways, or a behavioral increase in physical activity. There are two further passive mechanisms that are an inevitable consequence of weight gain: a pro rata increase in basal metabolism as the amount of lean tissue increases and an increase in the energy cost of activity as body weight increases. Each of these components was assessed in the present study and will be discussed individually.

There was no perceptible reduction in the extraction rate of dietary energy. Measured metabolizable energy (19.5 MJ/d) during overfeeding was 91.5% of gross energy (21.3 MJ/d), which is entirely in line with the predicted value using Southgate and Durnin's (48) modified Atwater Factors derived in subjects fed in energy balance. As a consequence, the average metabolizable-energy value computed from food tables matched the measured value to within 1%. Therefore, we conclude that malabsorption is not invoked as a mechanism to protect against an overload of dietary energy, even when that overload was close to the limits of gastric tolerability for many of the subjects and persisted for 42 d.

Evaluation of the change in BMR must examine whether it can be explained purely in terms of the expected passive increment or whether there might also be a component of active heat dissipation. In absolute terms there was a highly significant increase in BMR during overfeeding from 7.3 to 8.2 MJ/d ( $P < 0.001$ ). FFM represents the best available denominator for BMR even though it only provides a crude average of all of the

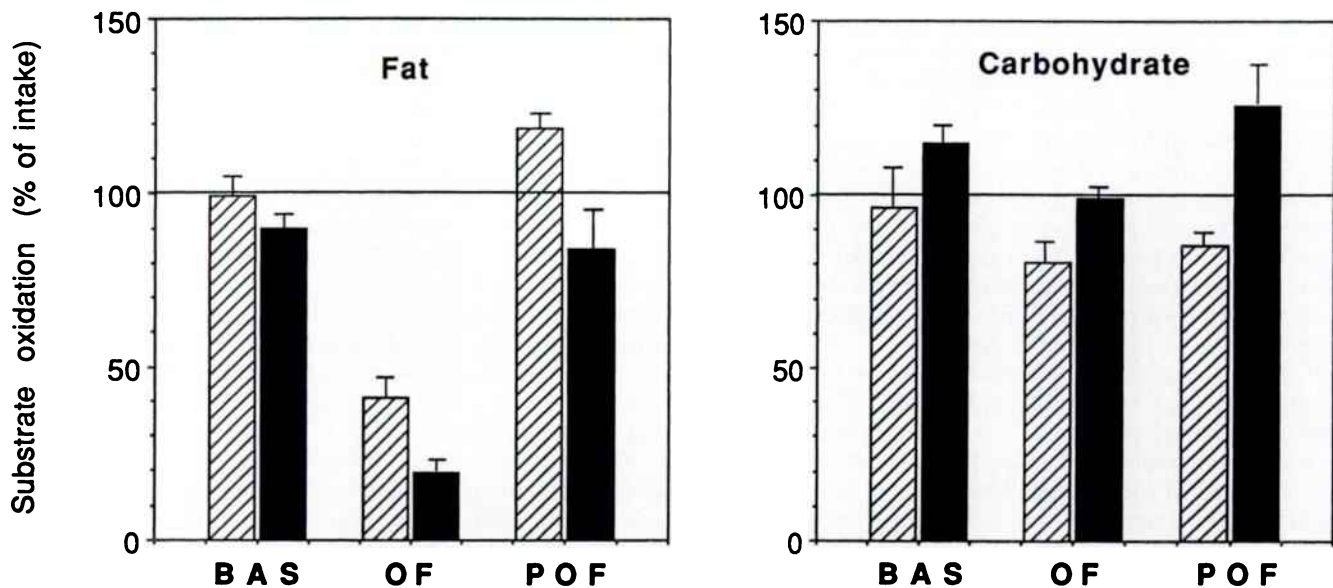


FIG 8. Differential fat and carbohydrate oxidation in lean and overweight subjects. Hatched bars, lean subjects ( $n = 6$ ); solid bars, obese subjects ( $n = 3$ ).  $\bar{x} \pm SE$  for 24-h oxidation rates. Amount fed refers to diet in calorimeter [ie, averaging 10.5 MJ/d during baseline (BAS) and postoverfeeding (POF), and 19.5 MJ/d during overfeeding (OF)].

metabolically active organs and in this study would not discriminate between gains of muscle and other organs with higher maintenance costs. BMR remained significantly raised by overfeeding when expressed per kg FFM ( $137$  vs  $128$   $\text{kJ} \cdot \text{kg FFM}^{-1} \cdot \text{d}^{-1}$ ;  $P < 0.05$ ), suggesting that there was a component of the increase over and above the anticipated passive rise. This conclusion would be strengthened if the correction for FFM was applied, allowing for the fact that there is a significant intercept in regressions of BMR on FFM, as recommended by Ravussin and Bogardus (49). By using this approach the predicted BMR at the end of overfeeding would be  $7.85$  MJ/d compared with the observed value of  $8.2$  MJ/d, a difference of  $4.4\%$ . However, these results are countered by the fact that the  $12.5$ -h postingestive interval between supper and BMR was probably insufficient to allow for the complete disappearance of the thermic effect of such a large meal (Fig 6). We believe that this can largely explain the  $4.4\%$  increase in BMR per kilogram FFM, indicating that if there is any persistent adaptive thermogenesis beyond the period associated with TEF it must be negligibly small.

The second anticipated passive alteration in energy requirements relates to the change in the energy cost of activity. The calorimeter data revealed a change in the energy cost of weight-bearing activity (stepping) of  $11.4\%$ , which is almost exactly in line with the  $10.4\%$  increase in body weight. The increase in the cost of non-weight-bearing activity (cycling) was  $9.0\%$ . In fact, the real increases in the energy cost of both activities must have been smaller than indicated by these figures because the four exercise periods were all performed within 3 h of a previous meal and included a component of TEF within the measurements. There is therefore no evidence for an adaptive increase in the energy cost of physical work or for a significant energy-dissipating interaction between the energy cost of exercise and TEF, as suggested previously (21, 50).

Resolution of the issue as to whether there is an active process of adaptive thermogenesis in response to overfeeding was the central aim of this study. As summarized in the Introduction, such a mechanism has often been inferred from the fact that observed weight gains were below those predicted for a given degree and duration of overfeeding (9, 10, 12–14). A similar analysis in the present study would not support the existence of luskonsumption because the average energy cost of weight gain ( $28.7$  MJ/kg) did not significantly differ from the predicted cost ( $26.0$  MJ/kg) based on the composition of tissue gained. Our observed value almost exactly matches the value of  $28$  MJ/kg reported by Forbes et al (22). Caution must clearly be exercised when drawing such a conclusion on the basis of mean values because there is significant heterogeneity between individuals. However, the heterogeneity suggests both higher- and lower-than-predicted costs of weight gain and will inevitably arise partly from errors within the measurements.

It was surprising to note a significant negative correlation ( $r = -0.703$ ,  $P < 0.05$ ) between the  $\delta\text{FFM}-\delta\text{BW}$  ratio and weight gain in this study, and we can provide no explanation. This association contributed to the high SD in the individual values for the computed energy costs of weight gain. Bouchard et al (24) found a contrary result, and their result seems more intuitively logical. They demonstrated that the variance in the energy cost of weight gain was reduced when allowance was made for differences in the  $\delta\text{FFM}-\delta\text{BW}$  ratio.

The absence of luskonsumption is further supported by the actual measurements of activity and thermogenesis (derived as

CAL EE – BMR, or TEE – BMR), which provide a much firmer refutation of the hypothesis. The calorimeter data are the most secure in view of the excellent precision of the measurements and the highly controlled experimental conditions. The strict protocol keeps minor behavioral and experimental noise to a minimum, and the timing and quantity of food intake can be absolutely controlled. Under these conditions, A+T had increased by only  $0.9$  MJ/d by the end of the overfeeding. The SD of  $0.4$  MJ/d was large as a proportion of the mean change, but small as a proportion of the amount of excess energy fed. The maximum value was only  $1.4$  MJ/d. At this stage the energy excess while in the calorimeter was  $7.1$  MJ/d. Thus, although the change in A+T includes changes in activity as well as changes in thermogenesis, it still only represents  $12.7\%$  of the energy excess.

Disaggregation of the lean and obese data yielded the following mean changes in CAL A+T: lean,  $11.8 \pm 6.0\%$  (range  $5.2$ – $23.1\%$ ), and obese,  $15.3 \pm 6.6\%$  (range  $8.6$ – $21.7\%$ ). This does not support the hypothesis that lean people have a more effective mechanism for dissipating excess energy. This increase in A+T cannot be completely corrected for the increased cost of exercise, but a partial correction can be made by subtracting the excess cost during the cycling and stepping periods. This amounts to  $0.23$  MJ/d, thus reducing the thermogenesis component to a maximum change of  $0.67$  MJ/d, or  $9.4\%$  of the energy excess. This maximal estimate is in agreement with classic estimates of TEF for mixed meals (51) and clearly demonstrates an absence of adaptive thermogenesis.

Attempts to compute the predicted costs of depositing the new tissue based on stoichiometric calculations and Flatt's (4) estimates of the ATP costs of the various synthetic pathways are not very useful in this context because the estimates of substrate balance in Figures 7 and 8 only represent net balances and provide no information on intermediary substrate handling and pathway fluxes. However, Figure 7 illustrates that carbohydrate oxidation balanced intake and that net fat oxidation was substantially lower than fat intake, making it possible that dietary fat was deposited directly at a minimal biochemical cost. The low value of A+T suggests that the costly process of de novo fat synthesis from carbohydrate was only minimally active, if at all. Data from Flatt et al (52) support this assumption.

It has been suggested that adaptive thermogenesis may be suppressed at high ambient temperatures because it may create a thermal overload (2). The cold calorimeter measurements performed at week 5 of overfeeding were not significantly different from the thermoneutral measurements at week 6, despite the fact that the subjects found  $20$  °C unpleasantly cold because of the minimal clothing allowed, the absence of bedding, and the high convective losses from the forced ventilation within the chamber. We believe that we successfully created conditions that would have been permissive of adaptive thermogenesis had it existed, although it may be argued that the  $36$ -h duration of the test was too short.

The final mechanism by which an energy excess could be dissipated is through an increase in the amount of physical activity, which could be particularly effective in view of the fact that the energy cost of each activity is also raised by the increased body weight. There are no previous reports of physical activity spontaneously increasing in response to overfeeding and it seems intuitively unlikely. Indeed the inclusion of DLW measurements in the present study was largely aimed at detecting possible leth-

argy and decreases in activity in the underfeeding phase of the protocol to be reported elsewhere.

On average, free-living TEE increased by only 1.4 MJ/d, but with a large SD of 2.0 MJ/d. The average is slightly lower than the increase of 1.8 MJ/d observed on the fixed calorimeter protocol, which accounts for changes in BMR and thermogenesis, and therefore suggests either no change in activity or a slight decrease in the group as a whole. However, the individual changes in TEE between baseline and overfeeding were an important determinant of the amount of weight gained ( $r = -0.827$ ,  $P < 0.01$ ). The strength of this relationship is remarkable in view of the inevitable imprecision in the TEE estimates, which is compounded when small within-subject differences are examined, and in view of the fact that TEE was only assessed for the last third of the overfeeding period. It demonstrates that most of the variance in weight gain was explained by spontaneous changes in TEE even though there was no consistent trend in the direction of these changes. The case of the single subject in whom weight gain was almost completely prevented by an exceptional increase in TEE (subject 807) was discussed in detail in the Results section. We are satisfied that the change in lifestyle was coincidental and does not represent a biological phenomenon.

For most of the variables discussed above there were no detectable differences between the responses of the lean and overweight subjects. This is evidence that the lean subjects' leanness was not attributable to differences in energy metabolism or luxusconsumption. The profound differences in the way that the two groups handled the substrate overload and the very marked suppression of fat oxidation in the overweight subjects may, however, point to alternative mechanisms related to fuel selection rather than to energetics. This is in agreement with much of our previous work (53).

One of the most critical issues to be considered in any discussion of studies that attempt to achieve substantial changes in human behavior is compliance. In studies that have failed to achieve predicted amounts of weight gain, the most obvious explanation is that the subjects have not eaten all of the excess food, although the great care taken in some of studies (especially the Vermont studies) makes this an unlikely explanation in these particular cases. However, in the present study very substantial weight gains were achieved and the energy cost of weight gain almost exactly matched the predicted cost after correction for changes in TEE. This is possibly the best evidence that the subjects had consumed all of the excess energy, and it removes the need for a detailed discussion of the precautions taken to ensure compliance and of the additional evidence that also indicates that compliance was good.

As discussed in the Introduction, there have been many previous attempts to test the luxusconsumption hypothesis (12–25), many of which are open to significant criticism on methodological or technical grounds. The four studies that are most closely comparable to the present study are those by Norgan and Durnin (18), Webb and Annis (13), Forbes et al (22), and Bouchard et al (24).

Norgan and Durnin (18) overfed six men by 6.2 MJ/d for 42 d and achieved a weight gain averaging 6.0 kg with 62% as fat (cf 7.6 kg and 58% fat in this study). They assessed energy expenditure by the factorial approach, for which they measured BMR and the energy cost of a limited number of activities. They concluded that resting metabolic rate increased by 12%, the cost

of sitting increased by 11%, and the cost of walking at 4.0 and 5.6 km/h increased by 9% and 12%, respectively. These values are extremely close to those observed in the present study (cf 11% for BMR, 11% for stepping, and 9% for cycling). Norgan and Durnin (18) concluded that “. . . no evidence was found for the characteristic of luxusconsumption. . . .” in their study.


Webb and Annis (13) overfed lean and overweight subjects (eight in total, some on two occasions) for periods of 30 d by an excess of 4.2 MJ/d on three different diets ranging from a high-carbohydrate (60% by energy), to an average American diet, to a high-protein and high-fat diet (fat 70% by energy). Weight gains were 2.73, 2.68, and 1.75 kg, respectively, compared with a predicted gain of 5 kg. The authors concluded that luxusconsumption accounted for the difference, but their actual measurements of daily metabolic rate over two sedentary periods of 24 h in a direct-indirect suit calorimeter showed a measured increase of only 7.4% averaged across all diets. We interpret the latter finding as refuting the existence of luxusconsumption and believe that the failure to gain the predicted amount of weight can be explained at least partly by their assumption of a low energy cost of adipose-tissue deposition, 25.08 kJ/g.

Forbes et al (22) overfed 13 women and 2 men by 79–159 MJ over 21 d. They gained an average of 4.4 kg, of which 49% was fat. The only measure of energy expenditure was BMR, which increased by 8.7%. The energy cost of tissue deposition was not significantly different from that predicted on the basis of the change in body composition. There is therefore no need to invoke luxusconsumption as an explanation for any discrepancy.

Bouchard et al (24) overfed 12 pairs of young adult male monozygous twins by a total of 353 MJ over 100 d. The incremental energy was fixed at 4.2 MJ/d in spite of the fact that the SD for FFM was 6.6 kg. The average weight gain was 8.1 kg, with 67% as fat, which is equivalent to 223 MJ of the 353-MJ excess. The 130 MJ unaccounted for would be equivalent to an increase in energy expenditure of 1.3 MJ/d. Although the baseline energy expenditure of the subjects is not listed, it is reasonable to assume that it would be similar to that measured in the present study (13.2 MJ/d). The inferred increase in expenditure would therefore represent an increase of only 10%, which is entirely in line with the passive increases observed in our study and once again refutes the existence of luxusconsumption.

In each of the studies cited above there was significant heterogeneity in the response of individuals and it might be claimed that certain subjects showed significant luxusconsumption. This cannot be entirely ruled out but can potentially be explained by noncompliance or measurement errors. In the present study the most robust test of luxusconsumption (CAL A+T) failed to reveal a single individual with a high level of adaptive thermogenesis.

Each of these studies has strengths and weaknesses. The aim of the present investigation has been to learn from the criticisms directed at previous overfeeding studies and to develop a more robust experimental design. Its strengths are that it achieved a very substantial energy excess over a period that would be long enough to allow for the induction of enzyme systems even if these responded very slowly [Garrow (34) considered that a total excess of  $\geq 83$  MJ would be necessary to test for the existence of adaptive thermogenesis]; exceptional care was taken to determine baseline requirements both by titration of intake to achieve energy balance and by DLW assessment of energy ex-

penditure; and the long-stay metabolic suite facilitated a high level of control of diets and of fecal and urine collections. Most importantly, the significant outcome variables of energy expenditure were directly measured by whole-body calorimetry and DLW. The results provide a very clear refutation of luxuskonsumption in support of the four studies summarized above. We therefore conclude that the balance of evidence is now heavily weighted against the existence of any adaptive mechanism to dissipate excess ingested calories in adult humans. Such a process has only ever been inferred and all attempts at proof by direct measurements have yielded negative results. 

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## References

- Edelman IS. Thyroid thermogenesis. *N Engl J Med* 1974;290:1303-8.
- Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet induced thermogenesis. *Nature* 1979;281:31-5.
- Newsholme EA. A possible metabolic basis for the control of body weight. *N Engl J Med* 1980;302:400-5.
- Flatt JP. The biochemistry of energy expenditure. In: Bray GA, ed. *Recent advances in obesity research*. Vol 2. London: Newman, 1978: 211-28.
- Nichols DG. The bioenergetics of brown adipose tissue mitochondria. *FEBS Lett* 1976;61:103-10.
- Huttunen P, Hirvonen J, Kinnula V. The occurrence of brown adipose tissue in outdoor workers. *Eur J Appl Physiol* 1981;46:339-45.
- Lean MEJ, James WPT. Uncoupling protein in human brown adipose tissue mitochondria: isolation and detection by specific antiserum. *FEBS Lett* 1983;163:235-40.
- Cunningham S, Leslie P, Hopwood D. The characterisation and energy potential of brown adipose tissue in man. *Clin Sci* 1985;69:343-8.
- Neumann RA. Experimental determination of human food requirements with particular consideration of essential protein needs. *Arch Hyg* 1902;45:1-87 (in German).
- Gulick A. A study of weight regulation in the adult human body during overnutrition. *Am J Physiol* 1922;60:371-95.
- Forbes GB. Energy intake and body weight: a re-examination of two "classic" studies. *Am J Clin Nutr* 1984;39:349-50.
- Zed C, James WPT. Dietary thermogenesis in obesity: fat feeding at different energy intakes. *Int J Obes* 1986;10:375-90.
- Webb P, Annis JF. Adaptation to overeating in lean and overweight women. *Hum Nutr Clin Nutr* 1983;37C:117-31.
- Dallosso HM, James WPT. Whole body calorimetry studies in adult men. 1: The effect of fat overfeeding on 24 h energy expenditure. *Br J Nutr* 1984;52:49-64.
- Miller DS, Mumford P. Gluttony 1: an experimental study of overeating on low or high protein diets. *Am J Clin Nutr* 1967;20:1212-22.
- Miller DS, Mumford P, Stock MJ. Gluttony 2: thermogenesis in overeating man. *Am J Clin Nutr* 1967;20:1223-9.
- Sims EAH, Danforth E, Horton ES, Bray GA, Glennon JA, Salans LB. Endocrine and metabolic effects of experimental obesity in man. *Recent Prog Horm Res* 1973;29:457-96.
- Norgan NG, Durnin JVA. The effect of 6 weeks of overfeeding on the body composition and energy metabolism of young men. *Am J Clin Nutr* 1980;33:978-88.
- Ashworth N, Creedy S, Hunt JN, Mahon S, Newland P. Effect of nightly food supplement on food intake in man. *Lancet* 1962;2:685-7.
- Bray GA, Whipp BJ, Koyal SN. The acute effects of food intake on energy expenditure during cycle ergometry. *Am J Clin Nutr* 1974;27:254-9.
- Whipp BJ, Bray GA, Koyal SN. Exercise energetics in normal man following acute weight gain. *Am J Clin Nutr* 1973;26:1284-6.
- Forbes GB, Brown MR, Welle SL, Lipinski BA. Deliberate overfeeding in women and men: energy cost and composition of the weight gain. *Br J Nutr* 1986;56:1-9.
- Ravussin E, Schutz Y, Acheson KJ, Dismewt M, Bourquin L, Jequier E. Short term mixed diet overfeeding in man: no evidence for "luxuskonsumtion." *Am J Physiol* 1985;249:E470-7.
- Bouchard C, Tremblay A, Depres J-P, et al. The response to long-term overfeeding in identical twins. *New Engl J Med* 1990;322:1477-82.
- Schutz Y, Acheson K, Jequier E. Twenty four hours energy expenditure and thermogenesis: response to progressive carbohydrate overfeeding in man. *Int J Obes* 1985;9(suppl 2):111-4.
- Cohn C, Joseph D. Influence of body weight and body fat on appetite of "normal," lean and obese rats. *Yale J Biol Med* 1962;34:598-607.
- Brooks C, Lambert EF. A study of the effect of limitation of food intake and the method of feeding on the rate of weight gain during hypothalamic obesity in the albino rats. *Am J Physiol* 1946;147:695-707.
- Powley TL, Keesey RE. Relationship of body weight to the lateral hypothalamic feeding syndrome. *J Comp Physiol Psychol* 1970;70:25-36.
- Teitelbaum P, Epstein AN. The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol Rev* 1962;69:74-94.
- Keesey RE. A set-point theory of obesity. In: *Handbook of eating disorders*. Brownell KD, Forey JP, eds. New York: Basic Books 1986:63-87.
- Stunkard AJ. *Behavioural self-management*. New York: Brunner/Mazel, 1977.
- Keys A, Brozek J, Henschel A, Michelson O, Taylor HL. *The biology of human starvation*. Minneapolis: University of Minnesota Press, 1950.
- Sims EAH, Horton ES. Endocrine and metabolic adaptation to obesity and starvation. *Am J Clin Nutr* 1968;21:1455-70.
- Garrow JS. *Obesity and related diseases*. Edinburgh: Churchill Livingstone, 1988.
- Brown D, Cole TJ, Dauncey MJ, Marrs RW, Murgatroyd PR. Analysis of gaseous exchange in open circuit indirect calorimetry. *Med Biol Eng Comput* 1984;22:333-8.
- Prentice AM, Goldberg GR, Davies HL, Murgatroyd PR, Scott W. Energy-sparing adaptations in human pregnancy assessed by whole-body calorimetry. *Br J Nutr* 1989;62:5-22.
- Dallosso HM, Murgatroyd PR, James WPT. Feeding frequency and energy balance in adult males. *Hum Nutr Clin Nutr* 1982;36C:25-9.
- King RFG, Almond DJ, Oxby CB, Holmfild JHL, McMahon MJ. Calculation of short term changes in body fat from measurements of respiratory gas exchange. *Metabolism* 1984;33:826-31.
- Lifson N, Gordon GB, McClintock R. Measurements of total carbon dioxide production by means of D<sub>2</sub>O<sup>18</sup>. *J Appl Physiol* 1955;7:704-10.
- Coward WA, Roberts SB, Prentice AM, Lucas A. The <sup>2</sup>H<sub>2</sub><sup>18</sup>O method for energy expenditure measurements—clinical possibilities necessary assumptions and limitations. In: *Clinical nutrition and metabolic research*. Proc 7th ESPEN Congress, Basel, Switzerland: Karger, 1986:169-77.
- Prentice AM, ed. *International Dietary Energy Consultancy Group. The doubly-labelled water method for measuring energy expenditure. Technical recommendations for use in humans*. Vienna: IDECG/IAEA, 1990.

42. Cole TJ, Franklin M, Coward WA. Estimates of error. In: Prentice AM, ed. The doubly-labelled water method for measuring energy expenditure. Technical recommendations for use in humans. Vienna: IDECG/IAEA, 1990.
43. Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. 4th ed. London: Her Majesty's Stationery Office, 1978.
44. Branch WJ, Cummings JH. Comparison of radio opaque pellets and chromium sesquioxide as inert markers in studies requiring accurate faecal collections. *Gut* 1978;19:371-6.
45. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspan J, Klein PD. Total body water measurements in humans with <sup>18</sup>O and <sup>2</sup>H labelled water. *Am J Clin Nutr* 1980;33:2686-93.
46. Pace N, Rathburn N. Studies on body composition in body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* 1945;158:685-91.
47. Black AE, Prentice AM, Coward WA. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr* 1986;40C: 381-91.
48. Southgate DAT, Durnin JVGA. Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets. *Br J Nutr* 1970;24:517-35.
49. Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 1989;49:968-75.
50. Miller DS, Wise A. Exercise and dietary induced thermogenesis. *Lancet* 1975;1:1290.
51. Jequier E, Schutz Y. New evidence for a thermogenic defect in human obesity. *Int J Obes* 1985;9(suppl 2):1-7.
52. Flatt JP, Ravussin E, Acheson KJ, Jequier E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest* 1985;76:1019-24.
53. Prentice AM, Black AE, Murgatroyd PR, Goldberg GR, Coward WA. Metabolism or appetite: questions of energy balance with particular reference to obesity. *J Hum Nutr Diet* 1989;2:95-104.