

# Energy metabolism after 2 y of energy restriction: the Biosphere 2 experiment<sup>1,2</sup>

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## ABSTRACT

**Background:** An adaptive decrease in energy expenditure (EE) in response to 6 mo of severely restricted energy intake was shown in a classic semistarvation study—the Minnesota experiment.

**Objective:** Our objective was to examine whether such adaptation also occurs in response to less severe but sustained energy restriction.

**Design:** Body composition, 1-wk total EE (TEE), 24-h sedentary EE, and spontaneous physical activity were measured in 8 healthy subjects (4 men and 4 women) at the end of a 2-y confinement inside Biosphere 2. Unexpectedly, the food supply was markedly restricted during most of the confinement and all subjects experienced a marked, sustained weight loss ( $9.1 \pm 6.6$  kg;  $P < 0.001$ ) from the low-energy (7000–11000 kJ/d), low-fat (9% of energy), but nutrient-dense, diet they consumed.

**Results:** The TEE inside Biosphere 2, assessed 3 wk before exit, averaged  $10700 \pm 560$  kJ/d ( $n = 8$ ). Within 1 wk after exit, the adjusted 24-h EE and spontaneous physical activity were lower in the biospherians ( $n = 5$ ) than in 152 control subjects (6% and 45%, respectively; both  $P < 0.01$ ). Six months after exit and return to an ad libitum diet, body weight had increased to preentry levels; however, adjusted 24-h EE and spontaneous physical activity were still significantly lower than in control subjects.

**Conclusions:** In lean humans, an adaptive decrease in EE appears to occur not only in states of life-threatening undernutrition, but also in response to less severe energy restriction sustained over several years. *Am J Clin Nutr* 2000;72:946–53.

**KEY WORDS** Metabolic rate, physical activity, energy restriction, aging, energy conservation, Biosphere 2

## INTRODUCTION

Whether humans are able to adapt to periods of limited energy intake with a decrease in energy expenditure (EE) beyond that predicted for the change in body size and composition has been controversial in nutritional sciences during much of the 20th century (1–5). Some 50 y ago, Keys et al (6–8) showed in a pioneering semistarvation study (the Minnesota experiment) that 6 mo of severe energy restriction in 32 lean men led to a marked reduction in EE. This was due to a reduction in both

physical activity and in the resting metabolic rate (RMR), which decreased not only in absolute terms (39%) but also when expressed per kilogram of metabolically active tissue (16%). This form of energy conservation, a biologically meaningful survival mechanism in the face of dangerously low energy supplies and stores, has been referred to as metabolic adaptation (6, 9). To date, the Minnesota experiment continues to be the most comprehensive underfeeding study in humans, and its findings—revisited recently in detail by Dulloo et al (10–12)—have provided important insights into our understanding of human energy metabolism and body weight regulation. However, it is important to remember that the diet in Keys et al's (6) study was designed to represent the severely energy-deficient diet in European famine areas during and after World War II. Consequently, the participating lean men rapidly lost large amounts of weight ( $\approx 25\%$  of body weight) and by the end of the study were severely undernourished with weakness, lethargy, and edema (6, 7). Clearly, despite the adaptive reduction in EE, such severe energy restriction would have soon led to death by starvation had the study not been terminated after 6 mo.

To examine whether a similar adaptive decrease in EE occurs when lean subjects are subjected to less severe energy restriction that is sustained over several years, we assessed energy metabolism in 8 volunteers who had been energy restricted for 2 y while confined inside Biosphere 2 (13–18). Biosphere 2 is a 12750-m<sup>2</sup> (3.15-acre), enclosed glass and steel structure near Tucson, AZ, that was constructed as a self-contained ecologic “miniworld” and prototype planetary habitat (13, 16). The enclosure contains 7 biomes (rain forest, savannah, ocean, marsh, desert, an agro-forest, and a human habitat), which are intended to provide sufficient food supply for humans confined therein (13, 16). From 26 September 1991 until 26 September 1993, 8 healthy subjects (4 men and 4 women) lived inside Biosphere 2, during which

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**TABLE 1**

Physical characteristics of the 8 biospherians at entry

Subject no. <sup>1</sup>	Age	Height	Weight	BMI
	<i>y</i>	<i>cm</i>	<i>kg</i>	<i>kg/m<sup>2</sup></i>
F1	39	171	55.9	19.1
F2	31	164	52.7	19.3
F3	29	168	59.1	20.9
F4	40	171	75.0	25.6
M1	30	179	67.3	21.0
M2	67	170	68.2	21.2
M3	41	166	67.3	23.7
M4	27	181	94.5	28.8

<sup>1</sup>F, female; M, male.

time the enclosure was materially sealed, ie, no material passed in or out except for small items required for research purposes.

To date, the Biosphere 2 experiment represents the longest sustained period of human isolation in a confined environment on record (14–18). Unexpectedly, the amount of food grown inside was lower than originally predicted and, although the quality of the diet was high, the total energy intake was insufficient during most of the 2-y period relative to the workload of the subjects (13–18). As a result, all 8 biospherians experienced a marked weight loss ( $14 \pm 5\%$  of body weight at 6 mo; range: 9–24%) that was maintained throughout the confinement. Because of these unforeseen circumstances, the Biosphere 2 project became a unique nutritional experiment that allowed study of the effects of prolonged energy restriction on human energy metabolism. For this reason, a series of studies was initiated in collaboration with the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, AZ, to comprehensively assess energy metabolism in the biospherians both at the end of their confinement (no preentry data were obtained because the weight loss was unexpected) and repeatedly after exit from Biosphere 2.

## SUBJECTS AND METHODS

### Subjects

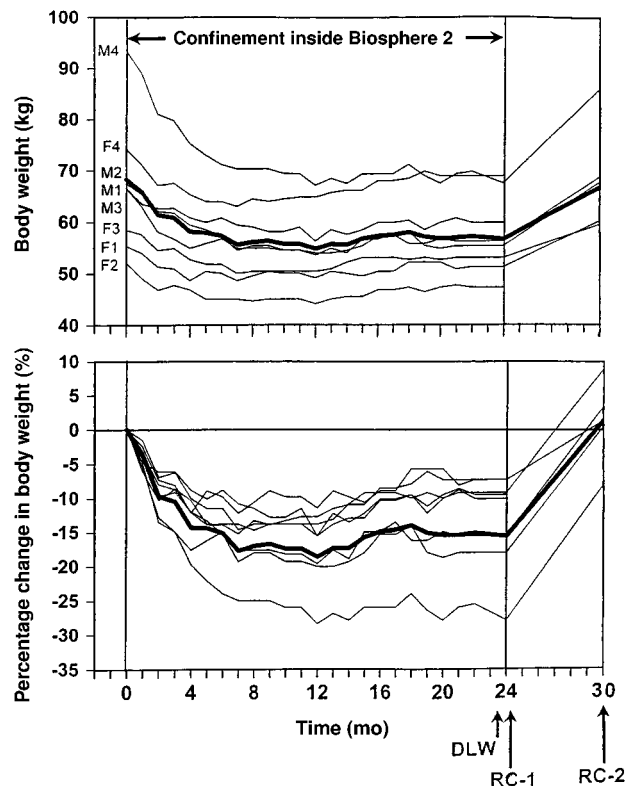
The baseline physical characteristics of the biospherians are given in **Table 1**. Before their confinement in Biosphere 2, all subjects were in good health as assessed by a comprehensive medical evaluation and all remained clinically healthy throughout the 2 y of confinement (13–18). All subjects were non-smokers and none was taking medication regularly, except for one woman (subject F1) who was receiving thyroid replacement therapy at a stable dose and was clinically euthyroid throughout the study. The study protocol was approved by the Human Use Committees of the Universities of Arizona, Tuscon, and California (Los Angeles), and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. All subjects provided written, informed consent before participation.

### Diet, physical activity, and body weight

The major foods available inside Biosphere 2 and the average dietary intake and habitual physical activities during the 2 y of confinement were reported in detail elsewhere (13). In brief, the diet was largely vegetarian, composed of large amounts of green and yellow vegetables and fruit, modest amounts of grain, and

small amounts of animal products. The diet was high in carbohydrate and low in fat ( $\approx 80\%$ ,  $9\%$ , and  $11\%$  of energy from carbohydrate, fat, and protein, respectively) and rich in vitamins, minerals, and trace elements (13–18). The food was consumed as 3 meals/d and was distributed equally among the 8 subjects regardless of age, sex, and body size (16). Computer-assisted nutritional analyses performed inside Biosphere 2 indicated a sufficient protein intake (63 g/d) and a sufficient supply of all essential nutrients, except for vitamin D (because of restricted ultraviolet radiation), vitamin B-12, and calcium (because of a low consumption of animal products) (16). To avoid chronic deficiencies in these nutrients, a vitamin-mineral supplement (Thompson Medical, West Palm Beach, FL) providing 50% of the recommended dietary allowance (19) of all essential vitamins and minerals was consumed daily by all subjects.

To maintain Biosphere 2 and earn their daily food throughout the 2 y of confinement, the 8 subjects had to sustain a relatively high workload, judged to be equivalent to 3–4 h of manual farming daily (16). Because of the unexpected agricultural problems caused by insufficient sunlight and insect pests, food availability was restricted during most of the 2-y period, most severely during the first 6 mo of confinement. During this time, the estimated 24-h energy intake averaged only 7460 kJ/d (13), leading to marked weight loss in all 8 subjects (**Figure 1**). Thereafter, food intake remained low (from 7000 to 11 000 kJ/d)



**FIGURE 1.** Absolute and relative changes in body weight in the 8 subjects (by sex and number) during the 2 y of confinement inside Biosphere 2 and 6 mo after exit. For the subgroup of 5 biospherians who were evaluated in the respiratory chamber within 1 wk (24 mo) and 6 mo (30 mo) after exit from Biosphere 2 (RC-1 and RC-2, respectively), the mean weight changes (thick, dark line) and body weights at 30 mo are also shown. DLW, doubly labeled water.

**TABLE 2**  
Plasma concentrations of thyroid hormones during and after confinement in Biosphere 2<sup>1</sup>

	Confinement in Biosphere 2			DLW and RC-1		
	5 mo (n = 7)	9 mo (n = 7)	16 mo (n = 7)	at 23 mo (n = 5)	RC-2 at 30 mo (n = 5)	39 mo (n = 7)
T <sub>4</sub> (nmol/L)	78 ± 10	81 ± 10	79 ± 8	81 ± 5	90 ± 11	85 ± 12 (64–141) <sup>2</sup>
fT <sub>4</sub> (nmol/L)	77 ± 10	80 ± 9	77 ± 6	79 ± 5	90 ± 8	84 ± 9 (64–141)
T <sub>3</sub> (nmol/L)	1.65 ± 0.34	1.37 ± 0.67 <sup>3</sup>	1.41 ± 0.12 <sup>3</sup>	1.69 ± 0.32	2.26 ± 0.62	1.78 ± 0.21 (1.15–2.69)
fT <sub>3</sub> (nmol/L)	1.61 ± 0.35 <sup>3</sup>	1.26 ± 0.24 <sup>3</sup>	1.40 ± 0.10 <sup>3</sup>	1.65 ± 0.34 <sup>3</sup>	2.23 ± 0.36	1.77 ± 0.14 (1.15–2.69)
TSH (μU/L)	2.4 ± 1.2	2.7 ± 1.6	2.0 ± 0.5	2.9 ± 1.5	2.1 ± 0.7	2.7 ± 1.3 (0.3–4.7)

<sup>1</sup> $\bar{x} \pm$  SD. DLW, doubly labeled water; RC-1 and RC-2, first and second evaluations in the respiratory chamber; T<sub>4</sub>, thyroxine; fT<sub>4</sub>, free T<sub>4</sub>; T<sub>3</sub>, triiodothyronine; fT<sub>3</sub>, free T<sub>3</sub>; TSH, thyroid-stimulating hormone.

<sup>2</sup>Normal range in parentheses; determined at the Endocrinology Laboratory, University of California, Los Angeles.

<sup>3</sup>Significantly different from RC-2 at 30 mo,  $P < 0.05$  (after Bonferroni correction).

and the reduced body weight was maintained for the rest of the 2-y period. After exiting Biosphere 2, the subjects resumed their original ad libitum diet and after 6 mo their mean body weights had returned to preentry values.

### Thyroid hormones

At 5, 9, 16, and 23 mo of confinement and at 6 and 15 mo after exit from Biosphere 2 (ie, at 30 and 39 mo), plasma concentrations of total and free thyroxine (T<sub>4</sub>), total and free triiodothyronine (T<sub>3</sub>), and thyroid-stimulating hormone were determined by radioimmunoassays (Table 2). Because none of these indexes were measured before entry, values 15 mo after exit (39 mo) were used as a retrospective control. Results from the woman receiving thyroid replacement therapy were excluded from this analysis.

### Energy metabolism

#### Total energy expenditure and physical activity inside Biosphere 2

To assess EE and physical activity during a typical workweek inside Biosphere 2, total EE (TEE) was measured over 7 d by the doubly labeled water method (20, 21) in all 8 subjects 3 wk before exit. For 3 consecutive days before dosing, baseline urine samples were collected in the morning. At 0700 on the day of dosing, each subject ingested a mixed dose containing 2.110 g 10.4% H<sub>2</sub><sup>18</sup>O and 0.111 g 99% <sup>2</sup>H<sub>2</sub>O/kg body wt. Urine samples were collected at 1000, 1300, 1600, and 1900 on the same day. After 7 d, subjects voided at 0700 and urine samples were collected at 1000, 1300, and 1600. A 40-mL aliquot of each urine sample was frozen in a sealed, 50-mL plastic container to limit fractionation. Separate samples were prepared for determination of <sup>2</sup>H enrichment by zinc reduction at 495°C and for determination of <sup>18</sup>O by equilibration with carbon dioxide as described previously (20). After the mean baseline value was subtracted, <sup>2</sup>H and <sup>18</sup>O isotopic elimination rates were calculated by using the 3 data points from the first day and the last 2 data points from the last day. EE was then calculated by the slope-intercept method, with isotope-dilution spaces calculated by extrapolation of the enrichments to time zero (20, 21).

During the 1-wk measurement period, all subjects consumed their habitual diet, providing an average of 11 330 kJ/d (range: 10 740–12 000 kJ/d), and maintained their routine physical activities. In 5 of the subjects (those who were later studied in the respiratory chamber; see below), RMR was measured during the

same week with a ventilated-hood indirect calorimetry system (Deltatrac; SensorMedics, Anaheim, CA). In the other 3 subjects, RMR was estimated according to the Harris-Benedict equation (22). The estimated physical activity EE (PAEE) was calculated as

$$\text{PAEE} = \text{TEE} - [\text{RMR} + (0.1 \times \text{TEE})] \quad (1)$$

where  $0.1 \times \text{TEE}$  is an estimate of the thermic effect of food (21). The physical activity level (PAL) was calculated as

$$\text{PAL} = \text{TEE}/[\text{RMR} + (0.1 \times \text{TEE})] \quad (2)$$

#### Body composition and 24-h energy metabolism after exit from Biosphere 2

Within the first week after exit from Biosphere 2 and again 6 mo after exit, when the subjects' body weights had reverted to preentry values, 5 of the 8 subjects were admitted to the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health for the assessment of body composition and 24-h energy metabolism. Body composition was measured by hydrodensitometry with simultaneous assessment of residual lung volume by helium dilution (23) and calculation of percentage body fat as described previously (24). Twenty-four-hour energy metabolism was assessed in a whole-body respiratory chamber (25). In brief, subjects entered the chamber at 0745 after an overnight fast and remained therein until 0700 the following morning. During the first evaluation in the respiratory chamber, within 1 wk after exit, the 5 subjects were fed a diet with a macronutrient composition similar to that of the diet consumed inside Biosphere 2 (80%, 9%, and 11% of energy from carbohydrate, fat, and protein, respectively; food quotient: 0.948).

After exiting Biosphere 2 and before the first evaluation in the respiratory chamber (between 3 and 8 d after exit), the subjects underwent a comprehensive series of tests while consuming an ad libitum diet, during which time they were asked to maintain their energy balances and body weights. During the second evaluation in the respiratory chamber, 6 mo after they resumed their usual ad libitum diet, the subjects consumed the standard diet provided in our respiratory chamber (50%, 30%, and 20% of energy from carbohydrate, fat, and protein, respectively; food quotient: 0.866) (26). On both occasions, the amount of food served to a given subject was calculated by using previously published equations (26) so that energy intake would match the estimated EE of that subject. Meals were provided at 0800, 1130, and 1700; an evening snack was provided at 2000.

TABLE 3

Physical characteristics of the control subjects and of the subgroup of 5 biospherians in whom 24-h energy metabolism was assessed in a respiratory chamber at within 1 wk and 6 mo after exit from Biosphere 2<sup>1</sup>

	Control subjects (n = 89 M, 63 F)	Biospherians (n = 3 M, 2 F)	
		1 wk after exit	6 mo after exit
Age (y)	35.3 ± 16.0	43.6 ± 16.0	44.1 ± 16.0
Height (cm)	170 ± 8	171 ± 7	171 ± 7
Body weight (kg) <sup>2</sup>	68.3 ± 8.7	59.7 ± 7.7 <sup>3</sup>	68.5 ± 10.6
BMI (kg/m <sup>2</sup> ) <sup>2</sup>	23.7 ± 3.4	19.8 ± 1.8 <sup>3</sup>	22.9 ± 2.0
Percentage body fat (%) <sup>4</sup>	21 ± 10	10 ± 3 <sup>5</sup>	21 ± 2
24-h energy expenditure (kJ/d)	8120 ± 990	7360 ± 970 <sup>6</sup>	7680 ± 1000
Sleeping metabolic rate (kJ/d)	5920 ± 730	5450 ± 520	5850 ± 720
Spontaneous physical activity (%)	7.5 ± 3.2	4.1 ± 1.3 <sup>6</sup>	4.1 ± 1.2 <sup>6</sup>
24-h Energy intake (kJ/d) <sup>4</sup>	8100 ± 880	6810 ± 820 <sup>3</sup>	8310 ± 1040
24-h Energy balance (kJ/d) <sup>7</sup>	-20 ± 620	-550 ± 420	630 ± 370
24-h Respiratory quotient <sup>7,8</sup>	0.863 ± 0.027	0.951 ± 0.035 <sup>5</sup>	0.888 ± 0.030

<sup>1</sup> $\bar{x} \pm SD$ .

<sup>2,4,7</sup>Significant time effect: <sup>2</sup> $P < 0.001$ , <sup>4</sup> $P < 0.01$ , <sup>7</sup> $P < 0.05$ .

<sup>3,5,6</sup>Significantly different from control subjects after adjustment for age and sex: <sup>3</sup> $P < 0.01$ , <sup>5</sup> $P < 0.001$ , <sup>6</sup> $P < 0.05$ .

<sup>8</sup>The 24-h respiratory quotient 1 wk after exit was measured under different dietary conditions (see Methods).

The rate of EE was measured continuously, calculated for each 15-min interval during the 23 h in the chamber, summed, and then extrapolated to 24 h (24-h EE). Spontaneous physical activity was assessed by radar sensors and expressed as a percentage of time over the 23-h period in which activity was detected (25). The sleeping metabolic rate (SMR) was defined as the average EE from all 15-min periods between 2330 and 0500 during which the spontaneous physical activity was <1.5%. Carbon dioxide production ( $\dot{V}CO_2$ ) and oxygen consumption ( $\dot{V}O_2$ ) were calculated for every 15-min interval of the 23-h chamber stay and then the values were extrapolated to 24 h. The 24-h respiratory quotient (24-h RQ) was calculated as the ratio of 24-h  $\dot{V}CO_2$  to 24-h  $\dot{V}O_2$ . The 24-h oxidation rates of fat, carbohydrate, and protein were calculated from  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and urinary nitrogen excretion as described previously (27).

### Statistical analyses

Statistical analyses were performed by using the procedures of the SAS Institute (Cary, NC). Results are given as means ± SDs. Changes in body weight and thyroid hormone concentrations over time were tested for statistical significance by repeated-measures analysis of variance (overall time effect) and paired *t* tests with Bonferroni correction for repeated comparisons (differences between selected time points). Measurements of 24-h energy metabolism in the 5 biospherians were compared with those of a control group of 152 healthy subjects (Table 3) chosen from the group of white subjects studied in our respiratory chamber who had similar heights and body weights, but who had been consuming an ad libitum diet before their evaluation. The physical characteristics of the 5 biospherians and of the 152 control subjects were compared by using general linear regression models, with simultaneous adjustment for age and sex. Measurements of 24-h energy metabolism in the 5 biospherians within 1 wk and 6 mo after exit from Biosphere 2 were compared with the control group by using general linear regression models with simultaneous adjustment for either age and sex alone (Table 3) or with adjustment for age, sex, fat-free mass, and fat mass. Paired *t* tests were used to assess whether the anthropometric and metabolic changes from within 1 wk to 6 mo after exit from Biosphere 2 were significant.

## RESULTS

### Physical characteristics

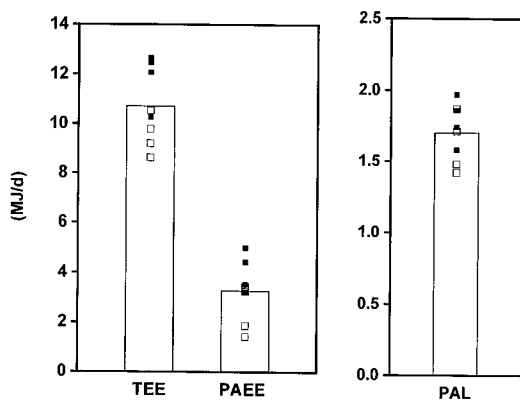
The physical characteristics of the 8 biospherians at the time of entry are given in Table 1. The absolute and relative changes in body weight during the 2 y of confinement and for the 5 subjects who were subsequently evaluated in the respiratory chamber for 6 mo after exit from Biosphere 2 are shown in Figure 1. During the first 6 mo of confinement in Biosphere 2, body weight decreased in all 8 subjects by an average of 14 ± 5% (9.8 ± 5.5 kg;  $P < 0.001$ ): by 16 ± 6% in men (from 73.6 ± 13.4 to 61.4 ± 6.7 kg;  $P < 0.001$ ) and by 12 ± 2% in women (from 60.1 ± 9.8 to 52.7 ± 8.0 kg;  $P < 0.001$ ). For the subsequent 18 mo of confinement, body weight was maintained at the reduced level but reverted to preentry levels 6 mo after the subjects had exited Biosphere 2 and resumed their ad libitum diets; the overall time effect was significant between 0 and 24 mo ( $P < 0.001$ ) but was not significant between 6 and 24 mo.

### Thyroid hormones

Compared with values shortly after weight regain (at 30 mo), plasma concentrations of free T<sub>3</sub> were lower by 26%, 39%, and 37% at 9, 16, and 23 mo of confinement, respectively (all  $P < 0.05$ ; Table 2). Compared with the value at 39 mo, however, the differences were less pronounced and were not significant. Similar results were obtained for total T<sub>3</sub>. Plasma concentrations of free and total T<sub>4</sub> also tended to be lower during confinement than after exit, but these changes were not significant.

### Total energy expenditure and physical activity inside Biosphere 2

The results of the doubly labeled water study are summarized in Figure 2. TEE during a typical workweek inside Biosphere 2 averaged 10700 ± 560 kJ/d 3 wk before exit, which was not significantly different from the average energy intake during this week (11330 ± 460 kJ/d), but was substantially higher than the average energy intake during the first 6 mo of confinement (7460 kJ/d) (18) and was also higher than the average energy intake during much of the 18 mo thereafter (21). The PAEE and PAL averaged 3270 ± 420 kJ/d and 1.70 ± 0.06, respectively.



**FIGURE 2.** Mean and individual total energy expenditure (TEE), physical activity energy expenditure (PAEE), and physical activity level (PAL) assessed by doubly labeled water in 4 male (■) and 4 female (□) biospherians during a typical workweek, 3 wk before exit from Biosphere 2.

### Body composition and 24-h energy metabolism after exit from Biosphere 2

The metabolic and physical characteristics of the 5 biospherians in whom body composition and 24-h energy metabolism were assessed within 1 wk and 6 mo after exit and of the 152 control subjects are summarized in Table 3 and in Figures 3 and 4.

One week after exit, fat-free mass in the 5 biospherians was not significantly different from that in the control group, but the biospherians had lower percentages of body fat and fat mass and thus lower total body weights (Table 3, Figure 3). When the 24-h EE of the biospherians was plotted against the relation between 24-h EE and fat-free mass in the 152 control subjects, all 5 biospherians fell below the prediction line for the control subjects (Figure 4). The unadjusted 24-h EE was  $760 \pm 330$  kJ/d ( $9.4 \pm 4.0\%$ ) lower in the biospherians than in the control group (Table 3). The 24-h EE remained significantly lower in the biospherians (by  $500 \pm 250$  kJ/d, or  $6.2 \pm 3.1\%$ ) than in the control group after adjustment for age, sex, fat-free mass, and fat mass (Figure 3), but not after additional adjustment for spontaneous physical activity in the chamber (lower by only  $230 \pm 210$  kJ/d, or  $2.9 \pm 2.7\%$ ).

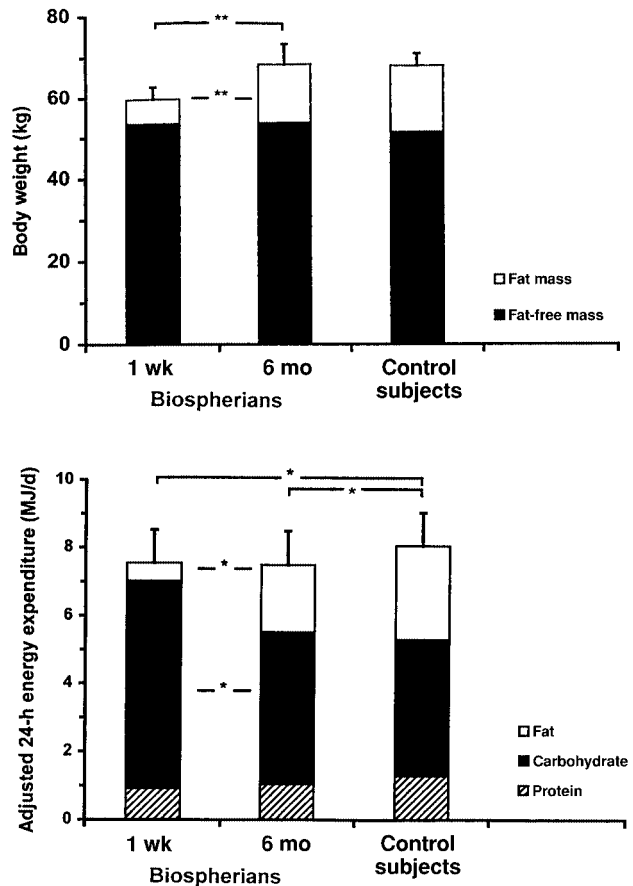
Spontaneous physical activity was significantly lower in the biospherians than in the control group (Table 3). The unadjusted SMR was  $470 \pm 270$  kJ/d ( $7.9 \pm 3.8\%$ ) lower in the biospherians than in the control group (NS; Table 3), a difference (of  $400 \pm 210$  kJ/d, or  $6.7 \pm 3.3\%$ ;  $P = 0.06$ ) that was nearly significant after adjustment for age, sex, fat-free mass, and fat mass. The mean 24-RQ of the 2 groups (Table 3) was similar to the food quotient of the different diets served (0.948 and 0.866, respectively).

Six months after exit and resumption of an ad libitum diet, mean total body weight in the 5 biospherians had increased by  $8.8 \pm 3.6$  kg ( $P < 0.001$ ) and was no longer different from the control group (Figure 3). The weight gain was almost entirely accounted for by an increase in fat mass of  $8.4 \pm 2.2$  kg ( $P < 0.01$ ); however, there was no significant change in fat-free mass ( $0.4 \pm 2.0$  kg), such that body composition became comparable between the 2 groups. The weight regain was not accompanied by a significant increase in 24-h EE ( $320 \pm 450$  kJ/d; Table 3). Consequently, 24-h EE remained lower in the biospherians than in the control subjects after adjustment for age, sex, fat-free mass, and fat mass (by  $540 \pm 240$  kJ/d, or  $6.7 \pm 3.2\%$ ;  $P < 0.05$ ; Figures 3 and 4). The spontaneous physical activity in the cham-

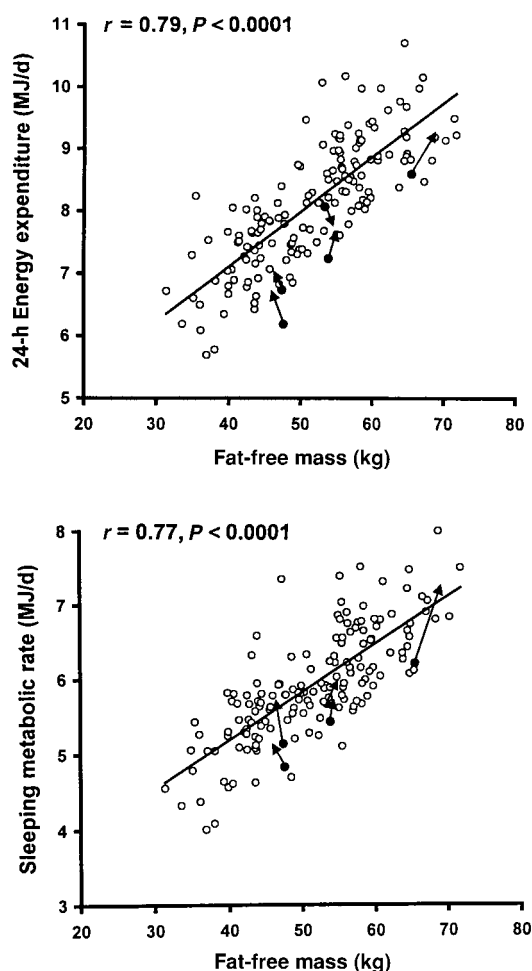
ber also remained significantly lower in the 5 biospherians than in the control group (Table 3) and, as within 1 wk after exit from Biosphere 2, the difference in 24-h EE ( $240 \pm 210$  kJ/d, or  $3.0 \pm 2.7\%$ ) was no longer significant after additional adjustment for spontaneous physical activity. The SMR at 6 mo was not significantly different between the biospherians and the control subjects ( $210 \pm 200$  kJ/d, or  $3.9 \pm 2.8\%$ ), nor was the 24-RQ (Table 3), which had now been measured while both groups were consuming the same diet (food quotient: 0.866) in the chamber.

### DISCUSSION

The results of the present study of participants in the Biosphere 2 experiment indicated that after 2 y of an energy-restricted, low-fat, but nutrient-dense, diet and a marked ( $\approx 15\%$ ) sustained weight loss, 24-h EE was significantly lower than predicted for age, sex, and body composition. The lower-than-predicted 24-h EE was in large part attributable to low spontaneous physical activity in the chamber, although the SMR was also lower than predicted ( $P = 0.06$ ). Six months after the subjects resumed an ad libitum



**FIGURE 3.** Least-squares mean ( $\pm$ SD) body weight and composition and adjusted 24-h energy expenditure and substrate oxidation rates in a subgroup of 5 biospherians evaluated in the respiratory chamber within 1 wk and 6 mo after exit from Biosphere 2 and in a control group of 152 weight-stable whites. The substrate oxidation rates and 24-h energy expenditure values were adjusted for age, sex, fat-free mass, and fat mass. \*\*\*Significant differences between groups: \* $P < 0.05$ , \*\* $P < 0.01$ .



**FIGURE 4.** Individual 24-h energy expenditures and sleeping metabolic rates in the subgroup of 5 biospherians evaluated in the respiratory chamber within 1 wk (●) and 6 mo after (top of arrow) exit from Biosphere 2 superimposed on the relation between fat-free mass and 24-h energy expenditure and sleeping metabolic rate in 152 control subjects (○). The lines represent the prediction lines in the control group.

diet, body weight had reverted to preentry values, almost entirely because of an increase in fat mass. Despite the complete recovery in body weight, adjusted 24-h EE and spontaneous physical activity remained lower than predicted. These findings suggest that in lean individuals, an adaptive reduction in EE can occur not only in states of life-threatening undernutrition (such as after several months of semistarvation), but also in response to less severe energy restriction that can be sustained over years.

Unlike the Minnesota experiment (6, 7), the Biosphere 2 project was not designed primarily as a nutritional experiment but unexpectedly became such when the amount of food grown inside was insufficient. Because of the unforeseen nature of the energy restriction, body composition and energy metabolism were not measured before entry, thus precluding the assessment of longitudinal changes. Instead, our findings are based on the comparison of energy metabolism between the biospherians immediately after exit and a large group of control

subjects who had been consuming an ad libitum diet. Another important difference between the Minnesota experiment (6, 7) and the Biosphere 2 project is that the energy restriction inside Biosphere 2 was less severe and the weight loss thus less pronounced (15% compared with 25% of initial body weight). Furthermore, because the diet consumed inside Biosphere 2 was of excellent nutritional quality, all 8 subjects not only remained healthy throughout the entire 2 y of confinement, but were even able to maintain the relatively high PAL required daily to grow and harvest the food inside the enclosure. This indicates that the adaptive reduction in EE was not just a secondary phenomenon caused by wasting or illness.

The doubly labeled water measurements, taken 3 wk before the subjects exited Biosphere 2, confirmed that the subjects maintained a relatively high PAL despite their confinement in a restricted environment. In fact, the average PAL inside Biosphere 2 was similar to values reported under free-living conditions (28, 29). At this late stage of the experiment, the mean TEEs of the 8 biospherians were roughly similar to their mean energy intakes (16), which explains why body weight had stabilized at the reduced level. Earlier in the experiment, energy intake was substantially lower (averaging 7460 kJ/d in the first 6 mo) (13), whereas TEE, at the same PAL, must have been higher because of the greater body weight. The gradual stabilization of body weight in the biospherians thus appeared to be the result of a steady increase in food supply and energy intake on the one hand and a steady decrease in TEE on the other, both factors gradually reducing the initial energy deficit.


Within 1 wk after exit, the percentage of body fat in the 5 biospherians who underwent further evaluation was very low ( $\approx 10\%$ ), suggesting that much of the preceding weight loss was attributable to a reduction in fat mass. When the 24-h EE was plotted against fat-free mass, which was similar in the 2 groups, values for all 5 biospherians were lower than predicted, and after adjustment for age, sex, fat-free mass and fat mass, their 24-h EE values were  $\approx 6\%$  (500 kJ/d) lower than those of the control subjects. This finding suggests that the subjects had adapted to the 2 y of energy restriction with a decrease in EE that was greater than predicted on account of the change in body weight and composition. The findings that the biospherians had low spontaneous physical activity in the chamber and that the difference in 24-h EE diminished after adjustment for spontaneous physical activity suggests that this adaptation was in large part attributable to a decrease in the amount of nonvolitional activities. This is interesting because the same type of activity, also referred to as fidgeting (30) or nonexercise activity thermogenesis (31), was shown recently to play a crucial role in the metabolic response to overfeeding (31). However, a reduction in spontaneous physical activity is probably not the only explanation for the adaptive decrease in 24-h EE because the SMR also decreased (by 400 kJ/d).

One possible mechanism for an adaptive decrease in the SMR in response to energy restriction is a decrease in thyroid hormone concentrations, which are known to decline as a result of short-term dieting in humans (32, 33). In the Biosphere 2 experiment,  $T_3$  concentrations were lower (by  $\leq 40\%$ ) during confinement than after weight regain (ie, at the second chamber evaluation). Other possible mechanisms underlying an adaptive decrease in the SMR could include changes in sympathetic tone (34–37), mitochondrial uncoupling (38, 39), and plasma insulin (40, 41) or leptin (42) concentrations, each of which was shown to be associated with EE in humans (34, 35,

38, 40, 42) and to be affected by energy restriction and weight loss (36, 37, 39, 41, 42). Because skeletal muscle is less metabolically active than other components of fat-free mass under resting conditions, it is also possible that a relative increase in the proportion of muscle mass contributed to the lower-than-predicted SMR in the biospherians. Finally, it was proposed recently by Dulloo et al (12), on the basis of a reanalysis of data from the Minnesota experiment, that the adaptive reduction in the SMR in response to food deprivation might be determined partly by an autoregulatory feedback system in which signals from the depleted fat stores suppress thermogenesis (12).

The finding that the body weight of the 5 biospherians returned to preentry values 6 mo after exit from Biosphere 2 agrees with the findings from previous underfeeding studies of shorter duration and suggests that even after 18 mo of weight-loss maintenance, body weight tends to return to its initial level. That the weight regain was almost exclusively accounted for by an increase in body fat stores appears to be a characteristic phenomenon that was observed previously in famine victims and emaciated prisoners of World War II (43, 44); in patients with anorexia nervosa (45), cancer (46), sepsis (47), and AIDS (48); and in subjects during the refeeding period of the Minnesota experiment (6, 12). The exact causes for this phenomenon, termed "poststarvation obesity" by Keys et al (6), remain elusive. Although it is well known that habitual energy intake increases during weight recovery (poststarvation hyperphagia) (12), this alone does not explain why the weight regain is directed so selectively toward the fat compartment. Dulloo et al (12) proposed a compartment model in which the pattern of lean and fat tissue deposition during weight recovery is determined by 2 autoregulatory control systems, one involving a reduction in thermogenesis and the other a change in energy partitioning.

The 24-h EE remained lower than predicted after weight recovery in the 5 biospherians, although body composition was no longer significantly different from that of the control subjects. This was attributable mainly to the persistence of a low spontaneous physical activity because the SMR was not reduced. Interestingly, evidence of a low level of nonvolitional activity after energy restriction was also found in previously undernourished Asian refugees (49). Normalization of the SMR after weight recovery in the biospherians may have been attributable in part to an increase in plasma free  $T_3$  concentrations.

As for the potential clinical implications of our findings, it is noteworthy that chronic energy restriction of mild severity sustained over prolonged periods, such as encountered inside Biosphere 2, was shown previously to increase the average and maximum life spans of rodents (50–52). It has been suggested by some authors that a decrease in metabolic rate may contribute to this effect, although this is debatable (53, 54). The Biosphere 2 project not only showed, once again, that even lean humans are able to sustain prolonged periods of energy restriction, but also that this kind of dietary restriction is associated with improvements in cardiovascular risk factors (16) and, as suggested by the present results, likely with a reduction in metabolic rate. Whether this ultimately increases longevity in humans remains to be examined. 

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