A comparative study of different means of assessing long-term energy expenditure in humans

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Rosenbaum, Michael, Eric Ravussin, Dwight E. Matthews, Chuck Gilker, Robert Ferraro, Steven B. Heymsfield, Jules Hirsch, and Rudolph L. Leibel. A comparative study of different means of assessing long-term energy expenditure in humans. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R496–R504, 1996.—We compared three independent techniques for measurement of total energy expenditure (TEE) in human subjects: *1*) weight-maintaining energy intake (dietTEE), *2*) 24-h chamber calorimetry (chamberTEE), and *3*) differential elimination rates ^2H_2O and $H_2{}^{18}O$ (isotopeTEE). Twenty-three healthy adult in-patients [19 never obese (NO), 2 obese (OB), and 2 formerly-obese (RO); 9 female, 14 male] ingested a liquid formula diet (40% of calories as fat, 45% carbohydrate, 15% protein), the volume of which was adjusted until body weight was stable for at least 14 days. Body composition was then determined by hydrodensitometry, isotope dilution, and dual photon beam absorptiometry (DXA). The thermic effect of feeding (TEF) and resting energy expenditure [REE; measured before arising (dietREE) and after arising (chamberREE)] were determined by indirect calorimetry. Non-resting energy expenditure (NREE) was calculated as NREE = TEE − (REE + TEF). Subjects then gained or lost 10% of their body weight and were restudied as described above. All measures of TEE were significantly correlated (dietTEE vs. chamberTEE r^2 = 0.75; dietTEE vs. isotopeTEE r^2 = 0.88; isotopeTEE vs. chamberTEE r^2 = 0.73; $P < 0.0001$). ChamberTEE (mean ± SE = 2,107 ± 64 kcal/day) was ~20% lower than either dietTEE (2,536 ± 94 kcal/day, $P < 0.0001$) or isotopeTEE (2,564 ± 83 kcal/day, $P < 0.0001$). When data were normalized to metabolic mass, weight gain of 10% was associated with significant increases in dietTEE ($P < 0.005$) and isotopeTEE ($P < 0.05$) but not chamberTEE; weight loss of 10% was associated with significant reductions in dietTEE ($P < 0.005$) and isotopeTEE ($P < 0.05$) but not chamberTEE. We conclude that measures of energy expenditure obtained in a highly controlled environment by caloric titration (dietTEE) or differential excretion rates of ^2H_2O and $H_2{}^{18}O$ (isotopeTEE) are not significantly different and that measurements of TEE obtained in a respiratory chamber (chamberTEE) are significantly lower than dietTEE or isotopeTEE, probably largely due to limitations on physical activity in the chamber.

obesity; body composition; caloric titration; chamber calorimetry; differential isotope excretion

OBESITY IS THE RESULT of a net positive imbalance of energy intake over energy output. For nearly 100 years, investigators have debated the primacy of one or the other in the pathogenesis of obesity. These arguments have been largely unresolvable because neither energy intake nor output could be measured with requisite accuracy over sufficiently long periods of time. Highly controlled long-term studies of energy expenditure are required, because small sustained imbalances of energy expenditure vs. energy intake can result in substantial changes in body composition. In a recent study of the effects of experimental weight perturbation on energy expenditure in obese (OB) and never-obese (NO) human subjects, we found that, during maintenance of a body weight 10% below "usual," there was a 15% decline in 24-h energy expenditure (measured by weight maintenance daily caloric requirements) normalized to metabolic mass, whereas during maintenance of a body weight 10% above usual there was a 16% increase in 24-h energy expenditure (11). These changes in energy expenditure are in a direction tending to restore body weight to its usual value. The present study was designed to compare the absolute measures of body composition and of long-term energy expenditure by different techniques and to validate our previous study (11) by comparing the effects of changes in body weight on energy expenditure measured by different techniques.

METHODOLOGY

Two OB (1 female, 1 male), 19 NO (12 male, 7 female), and two reduced-obese (RO) (1 male, 1 female) subjects were recruited by physician referral or through advertisement. These studies were approved by the Institutional Review Boards of the Rockefeller University Hospital, St. Luke's-Roosevelt Hospital Medical Center and the Clinical Research Center of the Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Phoenix, AZ, and written informed consent was obtained from all subjects before enrollment. All OB and NO subjects were at their maximal lifetime weight and had maintained this weight within a 2-kg range for at least 6 mo before enrollment. Subjects with body mass index (BMI) [[wt (kg)]/[ht (m)]^2] of at least 28 kg/m^2 were classified as OB and subjects with BMI <28 kg/m^2 were classified as NO (5). RO subjects were obese individuals who had undergone a controlled 10% reduction in body weight at the Rockefeller University Clinical Research Center (CRC) 3–9 mo before enrollment. None were taking any medication or special diet. Before enrollment, all subjects had normal findings on complete physical examination, including blood pressure and laboratory evaluations, including serum triiodothyronine, serum thyroxine, serum thyroid-stimulating hormone (TSH), a complete blood count, hepatitis A and B serologies, human immune virus serology, liver function tests, electrolytes, fasting serum glucose, urine analysis, and electrocardiogram.

Table 1. *Subject characteristics and body composition by different measures*

Plateau	All ($n = 45$)	Wt_{init} ($n = 21$)	Wt_{init} and $Wt_{+10\%}$ ($n = 10$)	$Wt_{+10\%}$	Wt_{init} and $Wt_{-10\%}$ ($n = 11$)	$Wt_{-10\%}$
Age, yr	24.6 ± 0.9	23.9 ± 1.1	23.5 ± 1.2		24.7 ± 1.2	
Height, cm	172.5 ± 1.6	171.0 ± 2.5	174.5 ± 2.3		167.9 ± 3.9	
Weight, kg	78.1 ± 4.6	75.3 ± 7.1	72.1 ± 8.8	79.3 ± 9.3*	84.7 ± 12.0	76.1 ± 9.5*
Body mass index, kg/m^2	26.1 ± 1.4	25.8 ± 3.5	23.8 + 3.1	25.7 ± 3.0†	29.8 ± 3.8	26.0 ± 3.1†
Body fat, %	24.8 ± 1.7	24.5 ± 2.9	19.9 ± 3.8	24.3 ± 3.5†	30.8 ± 3.9	25.8 ± 3.8†
Surface area, m^2	1.88 ± 0.04	1.85 ± 0.07	1.85 ± 0.09	1.94 ± 0.09	1.92 ± 0.12	1.86 ± 0.11
HydroFFM, kg	55.3 ± 1.8‡	54.5 ± 2.6	57.2 ± 3.3§	59.0 ± 3.3†§	55.4 ± 3.7‡	53.6 ± 3.2†§
IsotopeFFM, kg	53.2 ± 1.6	53.9 ± 3.2	54.2 ± 3.4	56.6 ± 3.3†	53.4 ± 4.2	51.2 ± 4.8

Values are means ± SE of all measurements in all subjects over all weight plateaus (All). Weight gain of 10% ($Wt_{+10\%}$) and weight loss of 10% ($Wt_{-10\%}$) were associated with significant increases or decreases, respectively, of fat-free mass (FFM). Wt_{init}, initial weight; hydroFFM, isotopeFFM, FFM studied by hydrodensitometry and isotope elimination, respectively. *$P < 0.001$ compared with same subjects at Wt_{init}; †$P < 0.05$ compared with same subjects studied at Wt_{init}; ‡$P < 0.001$ compared with same subjects studied by other measures (isotope); §$P < 0.05$ compared with same subjects studied by other measures.

Smokers were included in the study provided that no changes in daily consumption of cigarettes occurred during the study (5 subjects smoked; 1 RO, 4 NO; 4 male, 1 female). All smokers used fewer than 10 cigarettes/day. Subject characteristics are presented in Tables 1 and 2.

Experimental Design

The protocol for these studies is described in detail elsewhere (11). The plateau of usual weight for any subject is designated as $Wt_{initial}$. All NO and OB subjects were studied at $Wt_{initial}$, whereas RO subjects were studied only at reduced body weight. The weight plateau 10% above $Wt_{initial}$ is designated as $Wt_{+10\%}$, and 10% below $Wt_{initial}$ is designated as $Wt_{-10\%}$. The gender, somatotype, and number of subjects studied at each plateau are indicated in Tables 1 and 2.

Subjects were admitted to the CRC at Rockefeller University and allowed ad libitum physical activity while in the clinical research unit. Available activities included ping pong, billiards, and recreational art therapy; vigorous physical activity was prohibited. All subjects were fed a liquid formula diet [40% fat (corn oil), 45% carbohydrate (glucose polymer), 15% protein (casein hydrolysate)] supplemented with 5 g iodized NaCl, one multivitamin, 1,955 mg K^+ as a potassium salt, and 2.5 g calcium carbonate per day, 1 mg of folic acid twice weekly, and 36 mg ferrous iron every other day. The formula was prepared in the metabolic kitchen of the Rockefeller University CRC, and its caloric content was monitored by bomb calorimetry. The energy content of formula over 10 separate batches of formula was 1.36 ± 0.09 kcal/g (mean ± SD) by bomb calorimetry. Inter- and intra-assay coefficients of variability were 3.2 and 2.1%, respectively. Using digestibility quotients for each component of the formula (2), the mean ± SD metabolizable calories equaled 1.25 ± 0.06 kcal/g. Correction of caloric content of formula for digestibility coefficients accounts for the portion of ingested energy that is not absorbed, i.e., fecal energy loss (FEL). Subjects were in nitrogen balance as measured by comparison of urinary and stool nitrogen losses with the nitrogen content of the diet. We have reported previously that the percentage of ingested calories excreted as FEL and urinary nitrogen losses in subjects on this formula diet did not differ among weight plateaus (11). Urine analyses were obtained weekly in all subjects to ascertain that there were no further urinary caloric losses as proteinuria or glycosuria. Daily formula intake was adjusted until weight stability, defined as a slope of <0.01 kg/day in a 14-day plot of weight vs. days, was achieved. At this point, the metabolic testing described below was conducted over a period of ~14 days. Subjects continued to ingest formula during the 2 wk of metabolic testing. The degree of weight stability during testing was calculated by regressing the daily weight change of each subject in grams vs. *days 1-10* of testing: weight change (g) = 2.4 − 1.09 (day of testing) ($r^2 = 0.03$). Thus the average subject (23 subjects over 45 weight plateaus) lost 1.09 g/day over 10 days of testing. Studies during the testing period were *1*) resting energy expenditure (REE) and the thermic effect of feeding (TEF) by indirect calorimetry (6); *2*) total energy expenditure (TEE) by differential elimination rates of ^2H_2O and $H_2{}^{18}O$ (20); *3*) TEE by indirect calorimetry in a respiration chamber at the Clinical Diabetes and Nutrition Section, NIDDK in Phoenix, AZ ("chamber calorimetry") (15); *4*) body composition assessment by hydrodensitometry (8), isotopic dilution of ^2H_2O and $H_2{}^{18}O$ (16) and, in 13 subjects (10 males, 3 females), by dual photon beam absorptiometry (DXA) (12); and *5*) 8 day complete stool and urine collection to determine FEL and urine nitrogen loss.

After completion of studies at $Wt_{initial}$ (usual body weight), one OB subject and nine NO subjects were provided maximum tolerated intake of mixed solid self-selected foods (generally 5,000–8,000 kcal/day) until they had gained 10% of their initial ($Wt_{initial}$) body weight. No formula was ingested during the period of weight gain, which ranged from 5 to 9 wk. At the new weight plateau (i.e., 10% above $Wt_{initial}$ = "$Wt_{+10\%}$ plateau") weight was again maintained by exclusive feeding

Table 2. *FFM studied by hydroFFM, isotopeFFM, and DXAFFM*

Plateau	All ($n = 26$)	Wt_{init} ($n = 13$)	Wt_{init} and $Wt_{+10\%}$ ($n = 5$)	$Wt_{+10\%}$	Wt_{init} and $Wt_{-10\%}$ ($n = 8$)	$Wt_{-10\%}$
HydroFFM, kg	55.8 ± 1.7‡	56.0 ± 2.4§	60.9 ± 2.7	62.0 ± 2.2	53.0 ± 3.2§	51.5 ± 3.3†§
IsotopeFFM, kg	53.1 ± 1.8	54.0 ± 2.7	57.5 ± 2.8	59.3 + 2.3	51.4 + 3.8	47.5 ± 2.8†
DXAFFM, kg	53.2 ± 1.9	53.5 ± 2.7	58.0 ± 2.6	61.4 ± 2.3	50.6 ± 3.8	47.8 ± 3.2†

Values are means ± SE. FFM in 13 subjects over a total of 26 weight plateaus. DXAFFM, FFM measured by dual photon beam absorptiometry. †$P < 0.05$ compared with same subjects studied at Wt_{init}; ‡$P < 0.001$ compared with same subjects studied by other measures (isotope); §$P < 0.05$ compared with same subjects studied by other measures.

of the formula diet. When stable at $Wt_{+10\%}$ for at least 14 days, the energy expenditure studies described above were repeated.

Two OB subjects and nine NO subjects were fed 800 kcal/day of the liquid formula diet until body weight had been reduced 10% below usual weight ($Wt_{-10\%}$). One of the OB subjects had previously undergone studies at $Wt_{+10\%}$. At $Wt_{-10\%}$, as at $Wt_{+10\%}$, the exclusive formula feeding was reinstated and the amount titrated to maintain body weight. After maintenance of $Wt_{-10\%}$ for at least 14 days, the weight plateau studies described previously were repeated. RO subjects were studied only at $Wt_{-10\%}$.

Methods

TEE. CALORIC TITRATION. This is an indirect measure of TEE that relies upon the fact that during periods of constant body weight and composition, total absorbed energy intake must equal TEE. The degree of body weight stability achieved by caloric titration (dietTEE) is indicated by the fact that subjects, on average, lost only 1.09 g/day over the 2-wk testing period. Even if all of this weight loss were as fat (caloric density of 9 kcal/g), a weight loss of 1.09 g/day would represent only a 10 kcal/day deficit of caloric intake relative to expenditure.

DIFFERENTIAL ELIMINATION RATES OF ^2H_2O AND $H_2{}^{18}O$. At 9 A.M. on the first day of testing, our subjects received orally ~0.0125 mol of $H_2{}^{18}O$ and ~0.0076 mol of ^2H_2O per kg of estimated body water. Urine samples were collected at 0, 6, 12, and 24 h, and then daily for 10 days at 9 A.M. Subjects voided 1 h before each sample was collected. Therefore, each urine sample represented the isotopic enrichment of urine produced over 1 h plus whatever residual urine remained in the bladder. Relative enrichment of urine with these two isotopes was determined by mass spectrometry. Differential elimination rates of ^2H_2O and $H_2{}^{18}O$ (isotopeTEE) were calculated as described by Schoeller and Santen (20) using all data points, and compared with the same calculation using only four points (6 and 12 h and 9 and 10 days), to determine whether there were significant differences in the calculation based on the number of determinations used. The number of determinations necessary for accurate isotopeTEE assessment is an important point because of the costs of sample preparation and mass spectroscopy.

CHAMBER RESPIROMETRY. Chamber calorimetry was performed in an open-circuit indirect calorimeter (3.33 m long, 2.45 m wide, 2.39 m high) (15) at the Clinical Diabetes and Nutrition Section, NIDDK in Phoenix, AZ. Rates of O_2 consumption, CO_2 production, and energy expenditure were measured continuously for 23 h (0800–0700) and extrapolated to 24-h energy expenditure (chamberTEE). The intraindividual coefficient of variation for chamberTEE in this chamber has been reported as 2.4% (15). Activities of daily living, but no deliberate exercise, were permitted in the chamber. Aliquots of the formula diet were preshipped to this facility, and subjects ingested the same amount of this formula on the same schedule (local time) as they had while at the CRC at Rockefeller University. Because of the time zone difference between New York City and Phoenix, subjects were allowed at least 24–48 h of accommodation before receiving their formula and to adapt their sleep-wake cycle to mountain standard time before any testing was performed.

Resting energy expenditure, thermic effect of feeding, and non-resting energy expenditure. Indirect calorimetry was performed in the CRC at Rockefeller University with a Beckman MMC Horizon metabolic cart fitted with a ventilated hood to assess resting energy expenditure (REE) at 8 A.M. before arising (1). After calibration of the O_2 and CO_2 sensors with gas standards and the measurement of ambient concentrations of O_2 and CO_2, measurements of expired O_2 and CO_2 were made every 10 s for 60 min and stored in a microcomputer programmed to calculate metabolic rate from O_2 consumption corrected for respiratory quotient (RQ) and daily nitrogen excretion rate based on measurement of urinary nitrogen excretion rates. These data were downloaded to a computer and analyzed for the 15-min period of lowest metabolic rate and least variance in the rate of O_2 and CO_2. This period was used to calculate nonprotein RQ and REE for that study.

After the measurement of REE, the TEF was assessed by feeding the subject at 9 A.M. formula calories equal to 60% of the 24-h REE measured that morning, and then measuring the rates of oxygen consumption (rO_2) and CO_2 production (rCO_2) in the hood for 30-min intervals at time points 2 (11 A.M.) and 4 (1 P.M.) h after the ingestion of these calories. The area of the polygon whose base is the prefeeding value of REE and whose other vertices are energy expenditure at 9 A.M., 11 A.M., and 1 P.M. was used to calculate percentage of ingested calories oxidized after ingestion (11). TEF is expressed as percentage of ingested calories.

TEE assessed by any of the three methods described can be subdivided into three compartments: REE, TEF, and the energy expended as non-resting energy expenditure (NREE). NREE cannot be directly measured using current technology but can be calculated using dietTEE, isotopeTEE, or chamberTEE, as NREE = TEE − (TEF + REE).

Body composition. At the end of each weight plateau period, fat-free mass (FFM) was estimated by underwater weighing using the Siri equation (hydroFFM) (8). Subjects were instructed to exhale fully and were then submerged in the hydrodensitometry tank at St. Luke's-Roosevelt Hospital. Measurements were repeated until the subject was fully accommodated, as evidenced by reproducibility of measurement over three to five weighings.

Body composition was also measured by the isotope dilution space for both ^2H and ^{18}O. Isotope enrichment in urine samples obtained for isotopeTEE was used. Total body water was calculated by extrapolating the regression of isotope dilution vs. time from 6 h postisotope ingestion over the full 10-day study period back to *time 0*. To determine the impact of sampling frequency on this measure, this calculation was performed in two different ways: *1*) using all data points and *2*) extrapolating from only the first two data points (6 and 12 h) and the last two data points (9 and 10 days). Isotopic fat-free mass (isotopeFFM) was calculated according to the Pace-Rathbun equation as total body water/0.73 (8, 13). The FFM reported here (isotopeFFM) is the average of FFM calculated for ^2H_2O and $H_2{}^{18}O$ for each subject. The mean ± SE variability between FFM determined by ^2H vs. ^{18}O water spaces was 0.8 ± 1.1%. Surface area in all subjects was calculated from height and weight by the formula of DuBois and DuBois (4).

Measurement of body fat by DXA was compared with that obtained by hydrodensitometry to assess the possibility that FFM hydration status differed between weight plateaus. Thirteen NO subjects (10 male, 3 female) also underwent body composition determination by DXA (12) at more than one weight plateau (8 at $Wt_{initial}$ and $Wt_{-10\%}$; 5 at $Wt_{initial}$ and $Wt_{+10\%}$). This technique measures the attenuation of two electromagnetic beams of different energy levels by lean body tissues, fat, and bone, providing a direct measure of body fat mass (FM) which, unlike body composition assessment by hydrodensitometry or isotope dilution, is not dependent upon the hydration status of FFM (7).

Statistical analyses. Effects of methodology on assessments of energy expenditure and body composition were examined by paired *t*-testing. With the use of the Bonferroni adjustment (3), the significance level for P_α for these comparisons was set at $P_\alpha < 0.017$ to correct for multiple *t*-testing. For within-groups analyses, i.e., the same measures at initial weight vs. altered weight plateaus or calculated residual values vs. zero, groups were considered to be significantly different at $P_\alpha < 0.05$. RO subjects were not included in within-groups analyses because there were no available $Wt_{initial}$ data on these subjects.

In OB and NO individuals at normal body weight, REE is closely correlated with measures of metabolic FFM (15). Energy expenditure data are expressed in two ways. Energy expenditure per unit of FFM as determined by hydrodensitometry allows comparison of the same subjects studied at multiple weight plateaus by paired *t*-testing. However, regression equations relating energy expenditure to FFM do not necessarily have *y*-axis intercepts equaling zero (15). Thus, despite remaining on the regression line relating energy expenditure to FFM at $Wt_{initial}$, a weight-altered subject may show potentially misleading changes in the energy expenditure-to-FFM ratio (14). Therefore, regression equations of energy expenditure vs. FFM and FM for $Wt_{initial}$ were used to calculate residuals (actual energy expenditure − predicted energy expenditure based on $Wt_{initial}$ values) of the same subjects at other weight plateaus. FM was included in this calculation because it was found to vary significantly with some measures of energy expenditure and because FM could contribute to the energy cost of moving extra body weight. Residuals were then tested against the null hypothesis that residual equals zero to determine whether actual rates of energy expenditure differed significantly from that predicted in the same subjects studied at different weight plateaus. Effects on components of energy expenditure by sex, initial adiposity (OB vs. NO), and possible significant interactions between these variables and weight plateaus were assessed by univariate analysis of variance with repeated measures (17).

RESULTS

ChamberTEE, dietTEE, and isotopeTEE were significantly correlated ($P < 0.0001$) for all pairwise combinations (Table 3). However, chamberTEE was significantly lower than either of the other two methods over all measures and at all individual weight plateaus except for $Wt_{-10\%}$ (Table 4, Figs. 1 and 2). IsotopeNREE was significantly correlated with dietNREE and chamberNREE. The paired differences between measures of NREE must be the same as the paired differences between measures of TEE, because NREE was calculated by subtracting the same value (REE + TEF) from TEE determined by each method. The same absolute values for the paired differences between NREE and TEE represent a much larger fraction of NREE (which is roughly 30% of TEE) than of TEE. The larger fraction of NREE represented by these paired differences results in a much larger coefficient of variation when calculating correlations between different measures of NREE. The lower correlations among the three different measures of NREE compared with those noted between different measures of TEE are due to these larger coefficients of variation. ChamberNREE was significantly lower than dietNREE or isotopeNREE (Tables 1, 2, and 4).

Table 3. *Correlations between different measures*

Body Composition ($n = 45$)	IsotopeFFM	Surface Area
HydroFFM	(hydroFFM) − 2.1	0.03(hydroFFM) + 0.51
r^2	0.93	0.83
P	0.0001	0.0001
IsotopeFFM		0.02(isotope FFM) + 0.62
r^2		0.80
P		0.0001
TEE ($n = 45$)	**IsotopeTEE**	**ChamberTEE**
DietTEE	0.79(dietTEE) + 578	0.60(dietTEE) + 596
r^2	0.88	0.75
P	0.0001	0.0001
IsotopeTEE		0.66(isotope-TEE) + 421
r^2		0.73
P		0.0001
NREE ($n = 45$)	**IsotopeNREE**	**ChamberNREE**
DietNREE	0.49(dietNREE) + 452	0.16(dietNREE) + 261
r^2	0.29	0.08
P	0.001	NS
IsotopeNREE		0.28(isotope NREE) − 156
r^2		0.20
P		0.005
REE ($n = 45$)	**ChamberREE**	
DietREE	0.79(dietREE) + 453	
r^2	0.72	
P	0.0001	
Body Composition ($n = 26$)	IsotopeFFM	DXAFFM
HydroFFM	0.95(hydroFFM) − 0.16	1.05(hydroFFM) − 5.1
r^2	0.89	0.92
P	0.0001	0.0001
IsotopeFFM		0.99(isotopeFFM) + 0.8
r^2		0.89
P		0.0001

Equations, correlation coefficients, and probabilities relating different measures. TEE, total energy expenditure; chamberTEE, TEE measured by chamber calorimetry; NREE, non-resting energy expenditure; isotopeNREE, chamberNREE, NREE measured by stable isotope elimination and chamber calorimetry, respectively; REE, resting energy expenditure; chamberREE, REE measured after arising by indirect calorimetry.

All measures of TEE, REE, and TEF were significantly positively correlated with FFM. All measures of TEE and REE were significantly positively correlated with FM, calculated as body weight minus hydroFFM (Table 5). Weight gain of 10% was associated with significant increases in dietTEE (~20%), dietNREE (~42%), isotopeTEE (~16%), isotopeNREE (~30%), and TEF (~24%). No significant changes in REE, chamberTEE, or chamberNREE were noted at $Wt_{+10\%}$ (Table 4). Weight loss of 10% was associated with significant reductions in dietTEE (~18%), dietNREE (~35%), dietREE (~10%), isotopeTEE (~15%), and isotopeNREE (~23%). The absolute number of kilocalo-

Table 4. *Effect of weight change on components of energy expenditure*

Plateau	$Wt_{initial}$ and $Wt_{+10\%}$, kcal·kg FFM^{-1}·day^{-1} ($n = 10$)	$Wt_{+10\%}$, kcal·kg FFM^{-1}·day^{-1}	Residual, kcal/day	$Wt_{initial}$ and $Wt_{-10\%}$, kcal·kg FFM^{-1}·day^{-1} ($n = 10$)	$Wt_{-10\%}$, kcal·kg FFM^{-1}·day^{-1}	Residual, kcal/day
DietTEE	44.2 ± 1.3	53.7 ± 1.8*	593 ± 94	47.4 ± 2.0	38.4 ± 0.9*	−348 ± 87
P			0.0005			0.005
IsotopeTEE	43.8 ± 1.8	50.5 ± 1.6*	328 ± 73	49.4 ± 1.7	42.2 ± 1.5*	−378 ± 88
P			0.005			0.005
ChamberTEE	37.9 ± 1.9†	39.7 ± 1.4†	43 ± 72	38.6 ± 1.2†	35.8 ± 1.1†	−153 ± 74
P			NS			NS
DietNREE	14.4 + 1.0	21.5 ± 1.4*	469 ± 90	14.7 ± 1.3	9.2 ± 1.0*	−234 ± 85
P			0.001			0.05
IsotopeNREE	14.1 ± 1.1	18.2 ± 1.7‡	211 ± 79	16.7 ± 1.7	13.0 ± 1.7*	−214 ± 79
P			0.05			0.05
ChamberNREE	8.2 ± 1.5†	7.4 ± 1.0†	−133 ± 94	6.9 ± 0.7†	6.6 ± 1.3†	−36 ± 48
P			NS			NS
DietREE	26.7 ± 1.0	28.7 ± 1.2	82 ± 58	29.3 ± 1.4	26.4 ± 0.9‡	−86 ± 40
P			NS			NS
ChamberREE	29.4 ± 0.9§	32.4 ± 1.2§	97 ± 47	30.3 ± 1.5	28.6 ± 1.0	−84 ± 70
P			NS			NS
TEF	2.0 ± 0.2	2.5 ± 0.2‡	36 ± 13	2.3 ± 0.3	1.9 ± 0.1	−20 ± 11
P			0.05			NS

Values are means ± SE of energy expenditure (kcal·kg FFM^{-1}·day^{-1}) of different components of energy expenditure and residual values (kcal/day) of energy expenditure calculated by regression of energy expenditure vs. FFM and fat mass (FM) at $Wt_{initial}$ and applied to same subjects at $Wt_{+10\%}$ or $Wt_{-10\%}$. Residual values were then calculated as predicted value − actual value and tested against null hypothesis that residual values = 0, i.e., that the values obtained for subjects at altered body weight fall along same regression line of energy expenditure vs. body composition as they did at $Wt_{initial}$. *$P < 0.001$ compared with $Wt_{initial}$; †$P < 0.001$ compared with same subjects studied by other measures; ‡$P < 0.05$ compared with $Wt_{initial}$; §$P < 0.05$ compared with same subjects studied by other measures.

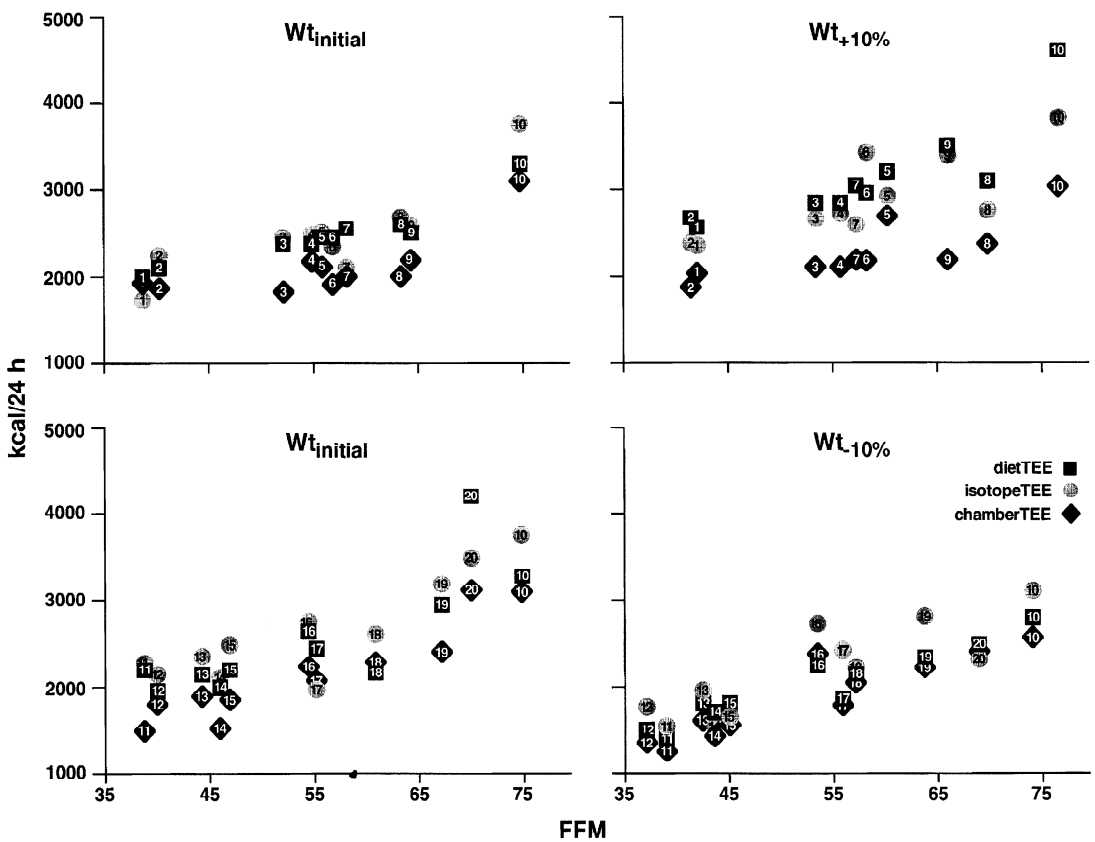

Fig. 1. Twenty-four hour energy expenditure (TEE) as measured by caloric titration (dietTEE, ■), isotope elimination (isotopeTEE, circles), and chamber calorimetry (chamberTEE, ◆) vs. fat-free mass (FFM) determined by hydrodensitometry at initial weight, weight plus 10%, and weight minus 10% ($Wt_{initial}$, $Wt_{+10\%}$, and $Wt_{-10\%}$, respectively). Numbers within symbols identify individual subjects.

ries per day represented by these changes in measures of energy expenditure after alteration of body weight are presented in Table 4 as residual values. No significant changes in TEF, chamberTEE, chamberREE, or chamberNREE were noted (Table 4). The same significant changes were detected when data were compared by paired *t*-testing of energy expenditure per kilogram FFM per day at different weight plateaus and when data were compared by calculation of residuals from those values predicted on the basis of the same subjects studied at $Wt_{initial}$ (Table 4), except that dietREE was significantly reduced at $Wt_{-10\%}$ when data were expressed as kilocalories per kilogram FFM per day, but residual values at $Wt_{-10\%}$ were not significantly different from zero. It is clear from the residual values that the majority of the changes in isotopeTEE and dietTEE after body weight gain or loss are due to respective increases or decreases in NREE.

Calculation of isotopeTEE was not significantly different when using only the first two (6 and 12 h) and last two (9 and 10 days) postisotope administration time points vs. a multipoint analysis using all available time points (total of 12 points per test) of differential rates of ^{18}O and ^{2}H excretion (mean ± SE difference = 0.1 ± 1.8% or 2.6 ± 46.7 kcal/day). The correlation between these two methods was $r^2 = 0.75$ ($P < 0.001$). The range of differences in isotopeTEE calculated by a four-point vs. a multipoint analysis was large: −31.9 to 33.4% (−933 to 820 kcal/day).

As expected, the various measures of metabolic mass (surface area, FFM by hydrodensitometry, and FFM by

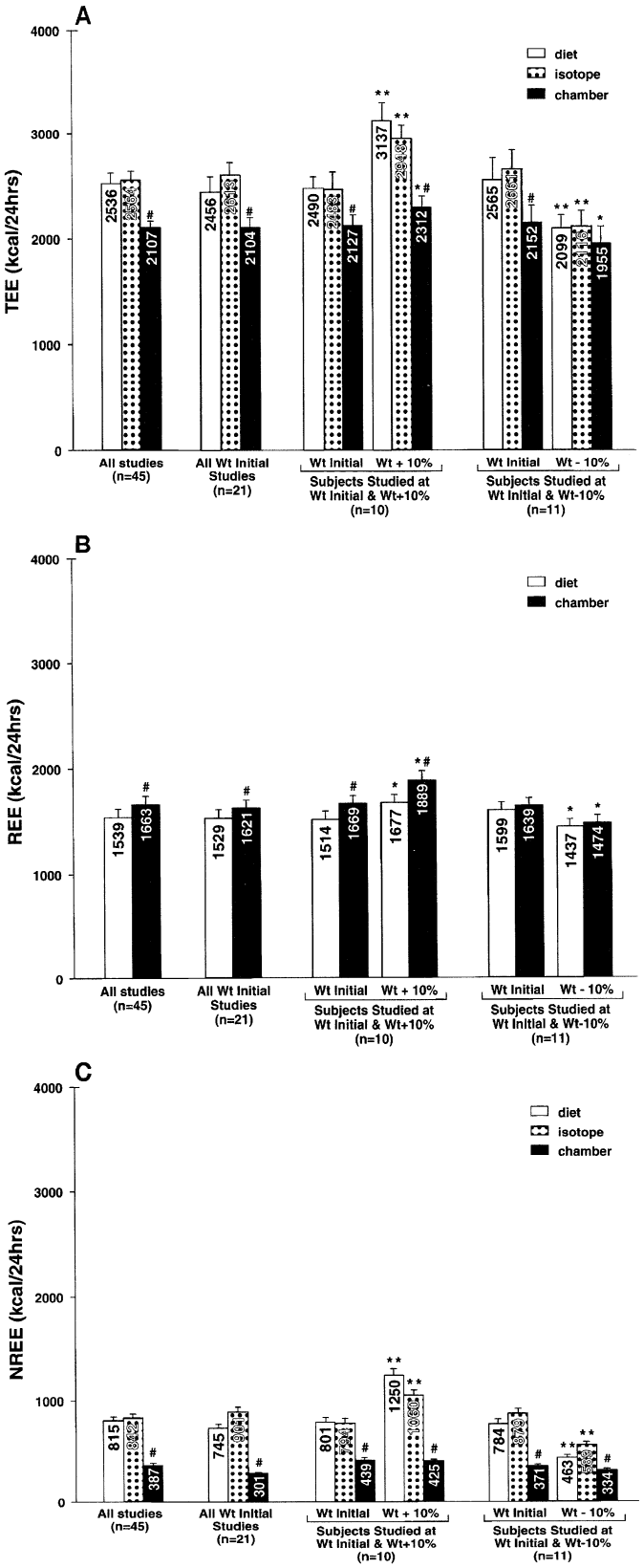

Table 5. *Multiple linear regression equations and correlation coefficients for body composition and directly measured components of energy expenditure in subjects studied at* $Wt_{initial}$

Measure	Multiple Linear Regression Equation	Multiple r^2	Partial r^2 for FFM	Partial r^2 for FM
DietTEE	24.0(FFM) + 14.5(FM)	0.88	0.68	0.60
P	+887	0.001	0.001	0.001
IsotopeTEE	24.2(FFM) + 11.2(FM)	0.78	0.59	0.53
P	+962	0.001	0.001	0.005
ChamberTEE	19.9(FFM) + 10.8(FM)	0.88	0.64	0.61
P	+787	0.001	0.001	0.001
DietREE	17.2(FFM) + 10.5(FM)	0.86	0.61	0.66
P	+375	0.001	0.001	0.001
ChamberREE	21.1(FFM) + 5.8(FM)	0.78	0.68	0.41
P	+348	0.001	0.001	0.01
TEF	1.9(FFM) + 0.4(FM)	0.29	0.29	0.14
P	+9	0.05	0.05	NS

Body composition refers to FFM and FM calculated from hydrodensitometry data. TEF, thermic effect of feeding by indirect calorimetry. $n = 21$ subjects.

Fig. 2. Mean ± SE measures of energy expenditure. *A*: 24-h energy expenditure measured by dietTEE, isotopeTEE, and chamberTEE at different weight plateaus. *B*: resting energy expenditure measured in subjects before rising (dietREE) or after rising (chamberREE) at different weight plateaus. *C*: non-resting energy expenditure calculated as (24-h energy expenditure) − (dietREE + TEF) using 24-h energy expenditure as measured by dietTEE (to calculate dietNREE), isotopeTEE (to calculate isotopeNREE), and chamberTEE (to calculate chamberNREE). $*P < 0.05$ compared with same subjects at $Wt_{initial}$; $**P < 0.0001$ compared with same subjects at $Wt_{initial}$; $\#P < 0.001$ compared with same subjects studied by other measures (isotope and chamber).

isotope dilution) were significantly intercorrelated (Table 3). FFM by hydrodensitometry was significantly greater than FFM by isotope dilution (~4%, Tables 1 and 2).

Thirteen subjects underwent metabolic mass assessment by hydrodensitometry, isotope dilution, and DXA at more than one weight plateau (total of 26 studies). The three methods were significantly intercorrelated, but FFM by hydrodensitometry was significantly greater than either of the other two methods (Tables 1–3). Paired differences in FFM determined by these three measures were not significantly different between weight plateaus, indicating that changes in body weight did not significantly affect the hydration status of FFM or FM.

Weight gain of 10% was associated with a significant increase in body fat expressed as a percentage of total body mass and in FFM by hydrodensitometry and by isotope dilution. Weight loss of 10% was associated with a significant loss of body fat, as well as a significant decrease in FFM as measured by hydrodensitometry and by isotope dilution (Fig. 2).

DISCUSSION

Energy Expenditure

In a controlled in-patient clinical research center environment, TEE measured by dietTEE and isotopeTEE methods was significantly correlated and did not differ significantly. In the present study, caloric intake and body weight were precisely monitored, permitting use of weight-maintenance caloric intake (dietTEE) as a measure of energy expenditure over an extended period (4–6 wk) of time. It is likely that TEE measured during shorter intervals (e.g., 1–2 days) or in a less controlled environment would not be as accurate or precise.

TEE measured by chamberTEE was significantly lower than isotopeTEE or dietTEE measured outside of the chamber. Seale et al. (22) measured dietTEE, chamberTEE, and isotopeTEE in four weight-stable free-living male subjects ingesting a weight-maintenance diet in a metabolic research unit. The formula quotient, i.e., the ratio of CO_2 to O_2 consumed in the combustion of the formula in a bomb calorimeter, of the diet was identical to that in the present study (0.85). Unlike the present study, chamber activities included a controlled exercise period of unspecified type intended to mimic normal activity. There was close agreement between dietTEE and isotopeTEE (mean ± SE difference = 1.04 ± 0.63%). However, chamberTEE was 21.9 ± 4.1% lower than isotopeTEE and 20.1 ± 4.4% lower than dietTEE. In contrast, Schoeller and Webb (21) measured isotopeTEE and chamberTEE simultaneously in five subjects and found that isotopeTEE was only 6 ± 8% greater than chamberTEE. When the data were reexamined in a subsequent analysis (19) this difference was reported to be only 1.7%.

The data in the present study do not contradict the findings of Seale et al. (22) or Schoeller and Webb (21). The similarities between dietTEE and isotopeTEE and between isotopeTEE and chamberTEE when these measurements are made simultaneously have been reported in many small studies recently reviewed by Schoeller (19). Because we measured dietTEE and isotopeTEE in subjects free to move about the CRC, it is not surprising that we, like Seale et al. (22), found that chamberTEE was significantly lower than isotopeTEE or dietTEE. The different conclusions regarding the relationship between isotopeTEE and chamberTEE reached by Schoeller and Webb and in the present study reflect differences in experimental design. All of these findings are consistent with the hypothesis that the differences between dietTEE or isotopeTEE and chamberTEE reported in the present study must be due to the relative confinement and associated limitation in physical activity that is imposed by the respiratory chamber. The support for this hypothesis can be summarized as follows.

When isotopeTEE and chamberTEE are both measured while subjects are in the respiratory chamber, they are not significantly different, whereas if, as in the present study, isotopeTEE or dietTEE are measured outside the chamber in a metabolic ward, they are significantly greater than chamberTEE. Therefore, the differences between isotopeTEE and dietTEE and chamberTEE noted in the present study must reflect differences in the testing environment or in the behavior of subjects in different environments rather than in the accuracy of testing methods themselves.

The effects of changes in body weight on TEE suggest that the major difference between the subjects in a metabolic ward and in a respiratory chamber is in the energy that they expend in physical activity. In the present study, experimentally induced changes in body weight altered energy expenditure measured by dietTEE or isotopeTEE per unit of FFM. These changes were not detected when TEE was measured by chamber respirometry. The change in TEE associated with the maintenance of an altered weight was due predominantly to changes in NREE. Because NREE is restricted by the confining environment of the chamber, the change in NREE associated with weight change was not evident using data obtained in the respiratory chamber. Because NREE is the major component of TEE that is altered after weight change, the differences between TEE and NREE measured by isotope elimination or by caloric titration and the same measures obtained by chamber respirometry were increased after weight gain (when NREE is increased; Ref. 11) and decreased after weight loss (when NREE is decreased).

The observations that there are significant increases in both dietTEE and isotopeTEE, normalized to metabolic mass, after weight gain and significant decreases in these variables normalized to metabolic mass after weight loss confirm the conclusions of our previous study (11) in which 41 subjects were studied at initial and altered weight plateaus, but weight-maintenance caloric intake was the only assessment of TEE reported. Because the same changes in energy expenditure, which constitute a metabolic opposition to the maintenance of an altered body weight, were observed

by differential isotope elimination and by dietary caloric titration, it is not possible that the subjects were "dumping" formula after weight gain (thus artificially inflating the measure of dietTEE) or consuming other "nonprescribed" calories after weight loss (thus depressing the measure of dietTEE). These phenomena would also have been reflected in a significantly greater dietTEE than isotopeTEE at $Wt_{+10\%}$ and a significantly lower dietTEE than isotopeTEE at $Wt_{-10\%}$. The possible mechanisms underlying this metabolic opposition and the implications of these findings for the clinical management of obesity are discussed elsewhere (11).

Body Composition

We found that hydrodensitometry yields significantly higher values for FFM than either isotope dilution or DXA. This result is in agreement with that of Wellens et al. (23) who noted that isotope dilution measures of FFM (calculated from deuterium dilution in saliva 2 h after an oral dose of ^2H_2O) were ~3% less than FFM measured by hydrodensitometry. However, in the same study, Wellens et al. found no significant differences between hydrodensitometry and DXA. Despite these differences, these measures are significantly intercorrelated and show similar changes after changes in body weight. As expected, body composition determined by hydrodensitometry, isotope dilution, and DXA were all significantly correlated with body surface area.

The estimation of total body water by extrapolating isotope dilution to *time 0* assumes single-compartment kinetics and immediate equilibration with extravascular and intravascular body water so that the isotope dilution at *time 0* does, in fact, reflect dilution in total body water. Initial isotopic enrichment at 6 and/or 12 h may be artifactually increased if isotope enrichment is higher in intravascular vs. extravascular water or may be artifactually decreased if the samples are diluted by residual urine in the bladder or if absorption of isotopes into the blood is delayed. The regression equation of isotopeFFM vs. time (after administration of isotope) was solved to determine the time at which isotopeFFM equaled hydroFFM. If hydrodensitometry is viewed as the "gold standard" of body composition measurement then, ideally, the time point on the regression lines of isotopeFFM vs. time at which isotopeFFM equaled hydroFFM would be zero. The average time after isotope administration at which isotopeFFM equaled hydroFFM was 13.2 ± 2.6 h ($P < 0.0001$ compared with zero). Such a discrepancy might be due to lack of equilibration between intravascular and extravascular water pools such that initial isotope enrichment was higher than would be found if isotopes were distributed evenly throughout body water. To examine this possibility, the actual deuterium and ^{18}O enrichments at the earliest available measure (6 h time point in 39 measures, 12 h time point in 6 measures) were compared with the values predicted based on the multipoint regression equations using all other time points over the 10 days of urine sampling. If these initial enrichment values were elevated due to lack of full body water equilibration or low due to slow isotope absorption or to dilution of the initial sample with residual urine in the bladder, then the actual and predicted isotope enrichments should differ significantly. Deuterium enrichments at the 6 or 12 h time point were 99.5 ± 0.2% of predicted, and ^{18}O enrichments were 99.7 ± 0.2% of predicted. These values were not significantly different from 100%, and therefore there is no evidence that enrichments of urine at these time points are in error due to an equilibration or dilution artifact.

Schoeller (18) reported intrasubject (same subjects studied on more than 1 occasion) and interassay (same samples assayed on different occasions) accuracy of 1–7% and precision of 3–11% using a two-point method (samples obtained at the start and at the end of the testing period). This variability is similar to that noted in the present study using a multipoint analysis. The wide variability (−31.9–33.4%) between four-point and multipoint measures of energy expenditure values suggests that the four-point method may be too imprecise for routine use when absolute measures of energy expenditure are required. Klein et al. (10) also reported increased agreement of a multipoint determination vs. a two-point determination of isotopeTEE with chamberTEE. Klein (9) suggested that this difference may be due to the effects of meal schedule, physical activity, and/or body composition on isotope elimination rates. A multipoint analysis integrates the variability of isotope flux cumulatively, reducing the impact of single aberrant points. In a two- or four-point analysis, an unusually high or low rate of isotope flux that is not reflective of average TEE will substantially alter the slope of the plot of isotope enrichment vs. time.

In summary, we have compared different techniques of assessing energy expenditure and body composition in human subjects undergoing experimental weight perturbation. Body composition assessment by hydrodensitometry yields a significantly higher (~3%) fractional FFBM than do either isotope dilution or DXA. The respiratory chamber yields values for TEE that are ~20% lower at initial weight, ~25% lower after weight gain, and ~7% lower after weight loss than measures of TEE by dietary caloric titration or differential elimination rates of ^2H_2O and $H_2{}^{18}O$. The lower values for chamber measures of TEE are apparently due to the restrictions that confinement in the chamber places on spontaneous physical activity. The conclusion that the majority of the differences in measured 24 h energy expenditure between chamberTEE and other measures are due to the limited physical activity permitted in the chamber is supported by other studies that demonstrate that isotope elimination, and indirect calorimetry measures of TEE do not differ significantly when both are measured while the subjects are in a respiratory chamber (21). Changes that occur in total energy expenditure as a result of weight perturbation are readily detected by caloric titration and differential elimination rates of ^2H_2O and $H_2{}^{18}O$. Comparison of TEE measured by a four-point vs. a multipoint analysis of differential elimination rates of ^2H_2O and $H_2{}^{18}O$ over a 10-day period demonstrates a wide variability in these measures and suggests that the simpler and less

costly four-point analysis may be prone to inaccuracies. On the basis of our experience with these measures of energy expenditure and body composition, we conclude that, in circumstances where energy expenditure related to physical activity is an important consideration, either caloric titration or differential elimination rates of ^2H_2O and $H_2{}^{18}O$ (multipoint analysis) be used as measures of TEE.

Perspectives

Obesity generally results from small positive imbalances in energy intake and expenditure integrated over long periods of time. To identify the mechanisms for such imbalances, very accurate measures of energy intake, output, and body composition are needed to quantify the respective components of this balance. This paper describes and contrasts the use of such techniques to document subtle changes in energy expenditure that occur as weight is experimentally perturbed in obese and never-obese subjects. It is clear that different means of assessing total energy expenditure vary in their sensitivity of detecting changes in individual components of energy expenditure, most notably, non-resting energy expenditure. It is also evident that the changes in energy expenditure that occur after experimental alterations in body weight are in a direction tending to restore body weight to its initial status, identifying one of the reasons for the very high recidivism rate to obesity among the formerly obese. The fact that most of the change in energy expenditure occurs in the component of energy expenditure that relates to physical activity (non-resting energy expenditure) suggests that there may be changes in the efficiency with which skeletal muscle converts chemical to mechanical energy. The biochemical basis for such changes is not clear but is under investigation using exercise physiology techniques in addition to direct measures of skeletal muscle morphology, enzyme content, and in vivo nuclear magnetic resonance spectroscopy.

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