

Relationship of resting metabolic rate to body composition and protein turnover

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WELLE, STEPHEN, AND K. S. NAIR. *Relationship of resting metabolic rate to body composition and protein turnover*. *Am. J. Physiol.* 258 (Endocrinol. Metab. 21): E990-E998, 1990.—This study examined whether variability among healthy young adults in resting metabolic rate, normalized for the amount of metabolically active tissue (assessed by total body potassium), is related to protein turnover. Resting metabolic rate was measured by indirect calorimetry for 2 h in 26 men and 21 women, 19–33 yr old, with simultaneous estimation of protein turnover during a 4-h infusion of L-[1-¹³C]leucine. After adjusting metabolic rate for total body potassium, the standard deviation was only 89 kcal/day, or 5.5% of the average value. There was a high correlation between leucine flux (an index of proteolysis) and metabolic rate ($r = 0.84$) and between the nonoxidized portion of leucine flux (an index of protein synthesis) and metabolic rate ($r = 0.83$). This relationship was weaker, but still significant, after adjusting leucine metabolism and metabolic rate for total body potassium ($r = 0.36$ for leucine flux vs. metabolic rate, $r = 0.33$ for nonoxidized portion of leucine flux vs. metabolic rate, $P < 0.05$). The regression analysis suggested that the contribution of protein turnover to resting metabolic rate was ~20% in an average subject. Metabolic rate and protein turnover were highest in the subjects with the greatest amount of body fat, even after accounting for differences in whole body potassium. Neither resting metabolic rate nor protein turnover was related to total or free concentrations of thyroxine or triiodothyronine, within the euthyroid range. We conclude that 1) resting metabolic rate is quite homogeneous in healthy young adults after accounting for differences in body composition, 2) differences in protein turnover explain a small but significant fraction of the variability in resting metabolic rate, 3) increased body fat is associated with elevated metabolic rate and protein turnover.

total body potassium; calorimetry; leucine flux

IT OFTEN HAS BEEN NOTED that the energy requirements of different individuals of similar size can be quite different (4, 27, 36, 39). Because resting energy expenditure generally accounts for two-thirds or more of total energy expenditure (35), resting metabolic rate obviously could be a major determinant of variations in energy expenditure. One of the processes that is thought to contribute substantially to resting energy expenditure is protein turnover, but there is no general agreement about exactly how much of the resting energy expenditure is attributable to this process. For example, the energy requirements of protein synthesis in humans have been estimated to account for 10% of the resting metabolic rate by Reeds et al. (37) and 25% by MacRae and Loble (25), based on the theoretical ATP requirements for

protein synthesis. When resting metabolic rate and whole body protein synthesis of lean and obese men were correlated, with no correction for weight or lean body mass, protein synthesis appeared to account for 50% of the resting metabolic rate (30). Moreover, protein turnover requires an unknown amount of energy for proteolysis, metabolism of amino acids not reincorporated into protein, protein transport across membranes, and RNA metabolism. Correlations between rates of energy expenditure and protein turnover in different species have been noted (37, 50, 51), and the decrease in protein turnover from infancy through old age in humans parallels the decrease in energy expenditure (51). Several conditions produce increases in both resting energy expenditure and protein turnover in humans, including uncontrolled diabetes (29, 31), hyperthyroidism (14), burn injury (23), leukemia (22), and overfeeding (48, 49). Undernutrition reduces both protein turnover and energy expenditure (18, 47). These studies suggest that protein turnover is an important component of the resting metabolic rate in humans. Thus the present study was undertaken to determine whether variations in protein turnover can explain the differences in resting energy expenditure that naturally occur among healthy adults.

Because whole body rates of all metabolic processes are highly dependent on the amount of active tissue, this variable must be included in the analysis of the relationship between protein and energy metabolism. In the present study, we have used whole body potassium content as an index of active tissue mass. Potassium is concentrated inside cells, where the processes of energy metabolism of protein turnover occur, is in very low concentrations in extracellular fluids and in organs with a slow metabolism such as bone and skin, and is not bound to triglyceride (9).

Some previous studies (2, 13, 17, 19) have suggested that resting metabolic rate is positively correlated with the amount of body fat, even after accounting for differences in lean body mass (in this paper the term lean body mass is the same as the term fat free mass and refers to the triglyceride-free mass, including the cytoplasm of adipocytes and the stromal component of adipose tissue). Other studies have not demonstrated such an effect (3, 20, 21, 42). Thus we examined whether resting metabolic rate, adjusted for body cell mass, was higher in the subjects with the most body fat and, if so, whether this effect could be explained by increased protein turnover (29). We also measured total and free serum levels of thyroid hormones [both thyroxine (T_4) and

triiodothyronine (T_3]) because thyroid hormones are important regulators of both resting metabolic rate and protein turnover (14) and plasma insulin concentrations because insulin inhibits protein turnover (12).

METHODS

Subjects. The subjects were 26 men and 21 women, 19–33 yr old, whose physical characteristics are described in Table 1. All subjects were healthy as determined by history and physical examination and had normal fasting plasma glucose concentrations. All of the women had regular menstrual cycles and were studied within 6 days of the most recent onset of menstrual bleeding. The subjects were recruited by advertisement. Verbal and written consent was obtained from each volunteer after the experimental procedures were explained verbally and in writing. The procedures were approved by the University of Rochester Research Subjects Review Board.

Determination of resting metabolic rate. The subjects were admitted to the University of Rochester Clinical Research Center for supervision of an overnight fast and to ensure that physical activity before the study was minimal. They were told to continue their normal activities and diets before admission.

Resting metabolic rate measurements were started at ~0900 h, 1 h after the start of the tracer infusions, which are described below. The measurements continued for a 2-h period, during which time there was no blood sampling or other procedures that might disturb the subjects. The subjects rested in bed, usually watching television or listening to music. The room temperature was ~25°C. Subjects were allowed to add or remove blankets or adjust the heating/cooling system in the room if they felt too warm or cold. During the measurements, an electric blower pulled air through a face mask at the rate of 30 l/min, as measured by a Rotameter (Fisher and Porter,

Warminster, PA). The transparent mask covered the subject's whole face. Different mask sizes were used to ensure a tight fit around the sides of the mask. The air pulled from the mask was sampled for oxygen concentrations (zirconia fuel cell) and carbon dioxide concentrations (infrared absorbance) with analyzers obtained from Ametek (Pittsburgh, PA). CO_2 and water vapor in the air entering the oxygen analyzer was removed with silica gel and soda lime to make the oxygen measurements independent of changes in moisture and CO_2 content of the expired air. The gas analyzers were calibrated before each measurement. The analyzers were interfaced to an IBM-PC for data processing through an analog-to-digital converter (Labmaster, Tecmar, Cleveland, OH). Every 10 min, the computer activated a cycle to flush the air from the mask out of the analyzers, measure room air gas concentrations, and flush room air from the analyzers with air from the mask. This cycle was included to continuously adjust results for electronic drift in the analyzers and real changes in room air gas concentrations. The difference in gas concentrations between room air and the mask, times air flow rate adjusted to standard temperature and pressure dry conditions, was used to calculate oxygen consumption and carbon dioxide production. During the 2-h measurement period, there were 12 measurement cycles that represented gas exchange over 5-min periods. The resting metabolic rate was calculated as described by Weir (46): $kcal/min = \dot{V}O_2 (l/min) \times [3.9 + (1.1 \times RQ)]$ where $\dot{V}O_2$ is oxygen consumption and RQ is respiratory quotient.

The consistency of the indirect calorimetry apparatus was assessed by burning ethanol in a hood attached to the system. The ethanol came from the same source and was infused into the combustion apparatus with the same pump and syringe each time. The coefficient of variation of six alcohol-burning tests done over the time period that this study was conducted was 0.4%, so that technical variability is a negligible source of error in these studies.

Reproducibility of the measurement of resting metabolic rate was determined in 6 subjects who had 2-h metabolic rate measurements done (without protein turnover measurements) two times within a 1-wk period and in 10 subjects who had measurements repeated 1–12 mo after the initial study.

Determination of protein turnover. At ~0800 h on the morning of the resting metabolic rate measurement, catheters were inserted into a dorsal hand vein and antecubital vein for blood sampling and tracer infusion. The hand used for blood sampling was kept in a warming box (70°C) so that the venous blood would approximate arterial blood more closely. The catheter was kept open with a slow drip of normal saline, which was thoroughly cleared from the sampling lines before blood collection. A primed (6 $\mu mol/kg$), continuous infusion of L-[1- ^{13}C]-leucine (6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) was given through an antecubital vein for 4 h. The bicarbonate pool was primed with $NaH^{13}CO_3$ (0.2 mg/kg) to allow rapid steady-state $^{13}CO_2$ levels for calculation of leucine oxidation. Plasma samples for analysis of ^{13}C enrichment of α -ketoisocaproate (KIC) were taken before the start of the tracer infusion and at 15-min intervals from 3–4 h of the infusion.

TABLE 1. Mean body composition, resting metabolic rate, and leucine kinetics

		Men	Women
Number		26	21
Age	Mean	23	25
	Range	19–26	19–33
Weight, kg	Mean	73.8	71.5
	Range	60.1–92.2	49.8–112.4
Lean body mass, kg*	Mean	63.6	51.3
	Range	49.2–78.1	42.4–63.7
Percent fat, †	Mean	13.8	26.5
	Range	0–31	0–44
Body mass index, kg/m ²	Mean	23.6	26.2
	Range	19.0–27.2	17.1–35.1
Resting metabolic rate, kcal/day	Mean	1,731	1,467
	Range	1,469–2,030	1,224–1,714
Leucine flux, $\mu mol/min$	Mean	155	120
	Range	111–188	96–168
Leucine oxidation, $\mu mol/min$	Mean	29	22
	Range	17–45	15–33

* Assumes 68.1 mmol K/kg lean body mass in men, 64.2 mmol K/kg lean body mass in women. † Two men and 1 woman had a calculated fat mass slightly <0, which is due to errors in total body potassium measurement and variability in potassium content of fat-free tissues. All 3 also had fat mass close to 0 when lean body mass was estimated from creatinine excretion (see text for equation). These subjects had their fat mass entered as 0.

Breath samples were collected in 20-ml Vacutainer tubes at the blood-collection times for analysis of $^{13}\text{CO}_2$ enrichment.

The trimethylsilyl quinoxalinol derivative of KIC (26) was separated on a capillary column (Hewlett-Packard, HP-1), and analyzed under electron impact conditions with a Hewlett-Packard 5988A GC-mass spectrometer. $^{13}\text{CO}_2$ enrichment was measured with an isotope ratio mass spectrometer (VG Isogas SIRA 12). Steady enrichments of ~ 5 atom % excess for [^{13}C]KIC and 0.01 atom % excess for $^{13}\text{CO}_2$ were observed during the final hour of the tracer infusions.

Total leucine flux was calculated as infusion rate of [^{13}C]leucine/mean [^{13}C]KIC enrichment during the final hour of tracer infusion. This value includes the [^{13}C]leucine infused. Endogenous leucine flux, which is referred to simply as leucine flux in the remainder of the paper, was calculated as total leucine flux minus the leucine infusion rate. This value is an index of proteolysis when subjects are in the postabsorptive state, since leucine is an essential amino acid. KIC enrichment rather than leucine enrichment was used to estimate leucine flux, because plasma KIC is more likely to reflect intracellular leucine enrichment than plasma leucine (24, 26, 41), and KIC enrichment is less sensitive to sampling site when the tracer is infused intravenously (24). Leucine oxidation was calculated as (total CO_2 production \times $^{13}\text{CO}_2$ enrichment during the final h of tracer infusion)/([^{13}C]KIC enrichment \times 0.8). The 0.8 is included to correct for the incomplete recovery of $^{13}\text{CO}_2$. Although there is disagreement in the literature about what percentage of the $^{13}\text{CO}_2$ is recovered in breath, studies in normal volunteers in our laboratory give values near 80% (45). The labeled carboxyl group in the tracer can only be liberated as $^{13}\text{CO}_2$ or incorporated into protein. Thus the nonoxidized portion of leucine flux is an index of protein synthesis and was calculated as total leucine flux-leucine oxidation.

Total body potassium and body fat. Each subject underwent a 40-min determination of total body potassium in the University of Rochester ^{40}K counter, as described in detail by Forbes (9, 10). Sixteen subjects had the total body potassium determination repeated immediately after the first measurement, to determine the technical variation in the potassium measurement. The amount of body fat was calculated as the total weight minus the lean body mass, assuming 68.1 mmol K/kg lean body mass in men and 64.2 mmol K/kg lean body mass in women (10). Percent fat refers to (fat mass/total weight) \times 100.

Urinary creatinine and nitrogen excretion. A timed (~ 5 h) urine collection was obtained on the morning of the metabolic studies while the subjects were in the postabsorptive state. Total volume was recorded, and an aliquot was frozen for analysis of creatinine and nitrogen concentrations. The relationship between creatinine excretion and lean body mass (estimated from whole body potassium) with this single postabsorptive collection [lean body mass = $22.8 + 0.021$ (mg creatinine/d), $r = 0.91$] was very similar to the relationship previously observed (9) with 24-h creatinine excretion in adults on

a meat-free diet [lean body mass = $20.7 + 0.024$ (mg creatinine/d), $r = 0.95$].

Thyroid hormone and insulin concentrations. Blood was drawn from the arterialized hand vein during the final hour of the tracer infusion for analysis of serum T_3 , T_4 , free T_4 , and plasma insulin concentrations by radioimmunoassay. Free T_3 was calculated by multiplying total T_3 concentrations by the free fraction, determined by equilibrium dialysis (40).

Data analysis. Simple correlations and stepwise multiple regression analyses were performed with BMDP Statistical Software (8). With this method, the investigator controls the order of entry of the variables into the multiple regression equation. The data were analyzed by first entering whole body potassium (or creatinine excretion) as the independent variable and metabolic rate as the dependent variable. The regression of metabolic rate against potassium yielded a residual value for each subject, which refers to the deviation of the observed resting metabolic rate from the value expected on the basis of whole body potassium. The expected value was determined from the regression line relating metabolic rate to whole body potassium in the whole set of subjects. Similar analyses were done using leucine flux or leucine flux minus oxidation as the dependent variables. After entering whole body potassium (or creatinine excretion) into the regression equation, partial correlations of metabolic rate with other variables (e.g., leucine flux, T_3 levels, sex, percent fat, and so forth) were calculated. Partial correlations refer to the correlations between the residual values for one variable and the residual values for another, with both variables being predicted from a third variable (usually whole body potassium in this study) or a set of variables. For example, the partial correlation between resting metabolic rate and leucine flux is the correlation between the residual metabolic rate and the residual leucine flux, after residuals for each variable were calculated using whole body potassium. In some cases, the simple correlations between residuals and other variables, not adjusted for whole body potassium or creatinine excretion, also were computed. At each step of the analysis, the standard error of the estimate was calculated. This term represents the amount of scatter of the observed values around the regression line and is analogous to the standard deviation around the mean. Sex was entered as a numeric value (male = 1, female = 2). Further analysis of possible gender differences was done by performing separate regression analyses on the data from the men and women.

RESULTS

Reproducibility of metabolic rate, leucine flux, and potassium measurements. The within-day coefficient of variation of the 5-min resting metabolic rate values for the 47 subjects averaged 3.2% (range 1.5–5.9%). When resting metabolic rate was measured twice within the same week, the second value was within 7% of the initial value in all six subjects, and the mean deviation from the initial value was 47 kcal/day (2.7%). When the measurement was repeated within 1–12 mo in 10 subjects, the second value differed from the first by 0.9–

12.1% (mean 7.2%) or 14–178 kcal/day (mean 104 kcal/day). However, one of these subjects had lost 8.4 kg and four others had gained 2.8–4.9 kg. Including only the five subjects with stable weights (within 1.7 kg of the initial weight), the second resting metabolic rate differed from the first by an average of 5.4% (78 kcal/day).

No data on reproducibility of leucine flux were obtained in the present study. However, in a previous study we found that when the basal leucine flux determination was repeated in six subjects (after 3 days constant diet before each study), the second value was within 7% of the initial value in all subjects, with a mean difference of 3.8% (32).

In the 16 subjects in whom the total body potassium measurement was repeated on the same day, the second value differed from the first by 0.1–5.8% (mean 2.4%).

Relationship of metabolic rate to body composition. Total body potassium was an excellent predictor of resting metabolic rate [resting metabolic rate (kcal/min) = $0.41 + 0.183$ total body potassium (mol), $r = 0.88$, $P < 0.01$]. The standard error of the estimate (SEE) of resting metabolic rate after adjusting for potassium was 89 kcal/day or 5.5% of the mean value. The relationship between metabolic rate and total body potassium was not significantly different in men and women, as indicated by the fact that separate regression analyses of men and women did not have significantly different intercepts or slopes and did not result in lower SEE values. Moreover, there was no correlation between sex and metabolic rate after entering whole body potassium into the regression analysis when data from both sexes were analyzed together. Figure 1 shows the distribution of the deviations of observed resting metabolic rate from the values expected on the basis of whole body potassium. Over 80% of the subjects had metabolic rates within 100 kcal/day of the expected value, and all had metabolic rates within 180 kcal/day of the expected value.

After adjusting the variables for total body potassium, there were significant ($P < 0.01$) partial correlations of resting metabolic rate with indices of fatness, including percent fat ($r = 0.45$), fat mass ($r = 0.44$), body mass index ($r = 0.42$), and body weight ($r = 0.46$). The simple correlations between residual resting metabolic rate (predicted from whole body potassium) and the indices of fatness also were significant ($P < 0.01$, $r = 0.39$ for percent fat, $r = 0.42$ for fat mass, $r = 0.42$ for weight, $r = 0.41$ for body mass index). Figure 2 shows the relation-

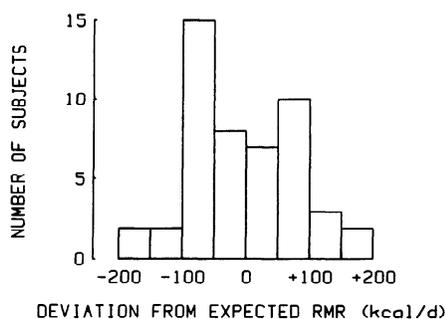


FIG. 1. Frequency distribution of residual (deviation from predicted) resting metabolic rate (RMR) in 47 healthy young subjects, after adjustment for total body potassium content.

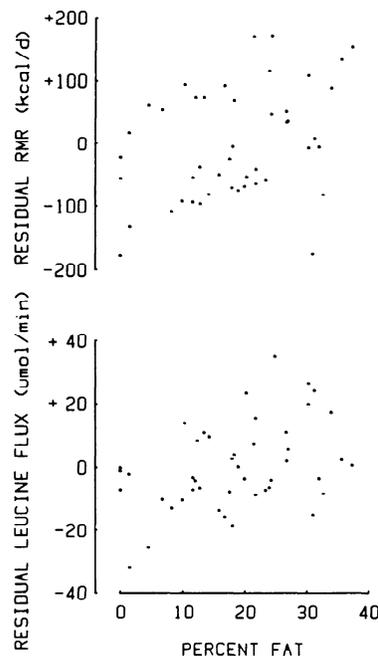


FIG. 2. Deviation from predicted (residual) resting metabolic rate (RMR) and leucine flux as a function of percent body fat in 47 healthy young subjects. RMR and leucine flux were predicted from total body potassium content.

ship between percent fat and the residual resting metabolic rate.

Prediction of resting metabolic rate from creatinine excretion (Cr_u) was less accurate [resting metabolic rate (kcal/min) = $0.63 + 0.0069 Cr_u$ (mg/h), $r = 0.81$, $P < 0.01$, SEE = 112 kcal/day] than prediction from whole body potassium. However, partial correlations between metabolic rate and indexes of fatness after adjusting for Cr_u were similar to partial correlations after adjusting for potassium ($P < 0.05$, for percent fat $r = 0.32$; for fat mass $r = 0.37$; for body mass index $r = 0.41$; for weight $r = 0.56$).

Relationship between body composition and leucine metabolism. Leucine flux was closely related to whole body potassium [leucine flux ($\mu\text{mol}/\text{min}$) = $7 + 34.1$ total body potassium (mol), $r = 0.85$, $P < 0.01$]. The SEE of leucine flux after adjusting for whole body potassium was 14 $\mu\text{mol}/\text{min}$, 10% of the mean value. There was no significant difference between men and women in leucine flux after accounting for differences in whole body potassium (using the same criteria employed to conclude that resting metabolic rate was not different between men and women, as discussed above). After adjusting the variables for whole body potassium, there were significant partial correlations ($P < 0.05$) between leucine flux and various indexes of fatness, including percent fat ($r = 0.46$), fat mass ($r = 0.42$), weight ($r = 0.39$), and body mass index ($r = 0.36$). Simple correlations between residual leucine flux (based on whole body potassium) and the indices of fatness also were significant ($P < 0.05$, $r = 0.41$ for percent fat; $r = 0.40$ for fat mass; $r = 0.36$ for body mass index; $r = 0.35$ for weight). Figure 2 shows the relationship between percent fat and the residual leucine flux.

The correlation between leucine flux and Cr_u [leucine

flux ($\mu\text{mol}/\text{min}$) = $47 + 1.3 \text{ Cr}_u$ (mg/h), $r = 0.79$, $P < 0.01$, $\text{SEE} = 16 \mu\text{mol}/\text{min}$] was not quite as strong as the correlation between whole body potassium and leucine flux. The partial correlations between leucine flux and indices of fatness, after adjusting for Cr_u , were similar to the partial correlations after adjusting for whole body potassium ($P < 0.05$; for percent fat $r = 0.37$; for fat mass $r = 0.39$; for body mass index $r = 0.38$; for weight $r = 0.50$).

The portion of leucine flux that was oxidized did not vary much among the subjects (range 14–23%), so that the nonoxidized portion of leucine flux was highly correlated with leucine flux ($r = 0.98$, $P < 0.01$). Thus the relationship between the nonoxidized portion of leucine flux and whole body potassium was nearly identical to the relationship between leucine flux and whole body potassium.

Thyroid hormones and insulin. Total T_4 concentrations ranged from 5.0 to 9.7 $\mu\text{g}/\text{dl}$ (mean 6.8) and total T_3 concentrations ranged from 63 to 168 ng/dl (mean 112). All of these values were within normal limits. There was no significant correlation between residual resting metabolic rate (based on whole body potassium) and concentrations of either T_4 ($r = 0.05$) or T_3 ($r = 0.04$). Correlations between residual leucine flux and T_4 ($r = 0.09$) and T_3 ($r = 0.23$) concentrations were slightly higher but not statistically significant ($P > 0.05$). Free T_4 concentrations ranged from 1.07 to 1.81 ng/dl (mean 1.44), and free T_3 concentrations ranged from 149 to 642 pg/dl , (mean 307). Residual resting metabolic rate did not correlate ($P > 0.05$) with either free T_4 ($r = -0.05$) or free T_3 concentrations ($r = 0.02$). The residual leucine flux also did not correlate with concentrations of free T_4 ($r = 0.05$) or free T_3 ($r = 0.10$).

Plasma insulin concentrations ranged from 5 to 30 $\mu\text{U}/\text{ml}$ (mean 10). After adjusting for whole body potassium, there was no significant correlation between resting metabolic rate and insulin levels ($r = 0.13$), but there was a positive correlation between leucine flux and insulin levels ($r = 0.30$, $P < 0.05$). However, after adjusting for both whole body potassium and percent fat, the correlation between leucine flux and insulin levels was no longer significant ($r = 0.19$).

Relationship between metabolic rate and leucine flux. Resting metabolic rate and leucine flux were closely related ($r = 0.84$, $P < 0.01$, Fig. 3). They were still

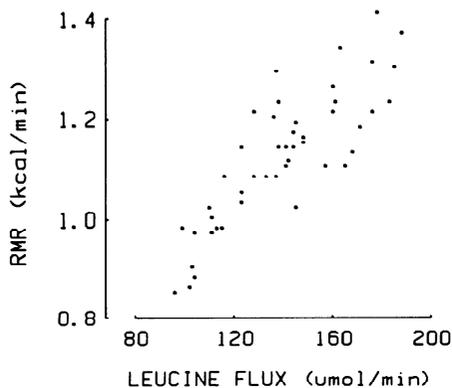


FIG. 3. Correlation between leucine flux and resting metabolic rate (RMR) in 47 healthy young subjects.

significantly correlated after adjusting for weight ($r = 0.77$, $P < 0.01$), Cr_u ($r = 0.56$, $P < 0.01$), or whole body potassium ($r = 0.36$, $P < 0.05$, Fig. 4). The regression coefficients indicated that metabolic rate increased an average of 6.2 kcal/day per 1 $\mu\text{mol}/\text{min}$ increase in leucine flux, when no adjustment was made for the amount of active tissue. However, potassium-adjusted metabolic rate increased an average of only 2.45 kcal/day for each 1 $\mu\text{mol}/\text{min}$ increase in potassium-adjusted leucine flux.

The relationships with metabolic rate were very similar for leucine flux and the nonoxidized portion of leucine flux ($r = 0.83$, $P < 0.01$, before adjusting the variables for total body potassium, $r = 0.33$, $P < 0.05$, after adjusting both variables for potassium). Before adjusting the variables for potassium content, both leucine oxidation ($r = 0.69$) and total nitrogen excretion ($r = 0.61$) correlated with resting metabolic rate ($P < 0.01$). After adjusting for potassium content, metabolic rate was marginally related to leucine oxidation ($r = 0.29$, $P = 0.05$) and was not significantly related to nitrogen excretion ($r = 0.09$).

DISCUSSION

Variability of resting metabolic rate. Several recent studies have suggested considerably more intersubject variability in resting metabolic rate, after accounting for lean body mass, than what we observed in the present study. For example, Bogardus et al. (3) reported a residual standard deviation of 141 kcal/day after adjusting resting metabolic rate for lean body mass, sex, and age. Owen et al. (33, 34) found residual standard deviations of 152 kcal/day in women and 215 kcal/day in men after adjusting resting metabolic rate for weight, which was done because weight was better than lean body mass in predicting resting metabolic rate in their studies. Foster et al. (11) reported a residual standard deviation of 226 kcal/day after adjusting resting metabolic rate for lean body mass in moderately obese women. In contrast, in the present study the residual standard deviation of resting metabolic rate after adjusting for whole body potassium was only 89 kcal/day (the upper limit of the 95% confidence interval for the standard deviation of potassium-adjusted metabolic rate was 114 kcal/day). The deviations of the observed metabolic rates from the

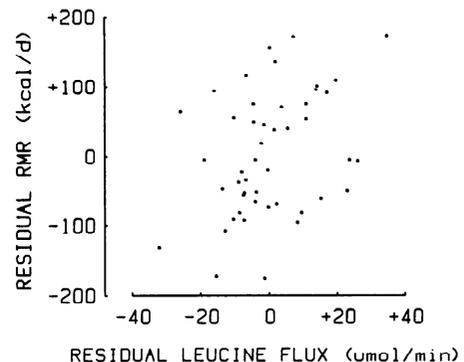


FIG. 4. Residual (deviation from predicted) resting metabolic rate (RMR) as a function of residual leucine flux in 47 healthy young subjects. RMR and leucine flux were predicted from total body potassium content.

values predicted by the regression line were not much greater than changes in metabolic rate over time in the same subject. The mean deviation from the predicted resting metabolic rate was only 75 kcal/day, whereas the short-term (1 wk or less) change in metabolic rate from the first to the second measurement on the same subject averaged 47 kcal/day, and the change in metabolic rate from the first to the second measurement over longer periods (1–12 mo) in weight-stable subjects averaged 78 kcal/day. These data suggest that the differences between individuals in energy requirements are largely the result of differences in body composition and the amount of physical activity or thermic responses to food or other stimuli and that differences in basal metabolic efficiency (i.e., the resting metabolic rate per amount of active tissue) are small.

It is not clear why we observed less variability of metabolic rate in this study than what has been reported previously. One factor contributing to the small inter-subject variation may be the long period of measurement of 2 h. Resting metabolic rate was measured for <1 h in the studies cited above. Moreover, all subjects in the present study stayed in the research ward the night before the study and had been resting in bed for >1 h before measurements started. Another reason for the low variability in metabolic rate may be the choice of potassium as the index of the amount of active tissue. Potassium is concentrated inside the cells and therefore is theoretically a better measure of active cell mass than the fat free mass as determined with other techniques. However, other laboratories using potassium counting have not reported such high correlations between potassium and metabolic rate (e.g., 11, 21). Another potential reason for the low variability of resting metabolic rate in the present study is that the need to insert intravenous catheters could have deterred anxious individuals from volunteering to participate. Anxious subjects would probably have higher metabolic rates, which would increase the variability.

Many investigators calculate the standard deviation of metabolic rate after dividing by lean body mass, which assumes that resting metabolic rate is directly proportional to lean body mass. However, in the present study metabolic rate was not directly proportional to whole body potassium. Instead, the metabolic rate per millimole of potassium was lower in individuals with a high whole body potassium than in subjects with a low whole body potassium. For example, the man with the highest potassium content in this study had 59% more potassium than the man with the lowest whole body potassium, but his metabolic rate was only 34% higher. This effect is probably related to the large difference in metabolic rate between resting muscle and other organs (15). Much of the increase in lean body mass in the subjects with the highest whole body potassium contents was caused by increased mass of muscle (based on creatinine excretion, muscle mass ranged from 18 to 46 kg). Because of the much lower metabolic rate (per kg) in resting muscle than in many other organs (e.g., brain, liver, kidney), an increase in total cell mass caused by an elevated muscle mass should not increase metabolic rate as much as the

same increase in cell mass distributed over many organs.

Increase in resting metabolic rate and protein turnover in obesity. Resting metabolic rate and protein turnover were positively correlated with various indices of adiposity. The data indicate that a person who is 40% fat would tend to have a metabolic rate 8% higher and a protein turnover rate 13% higher than a person with the same lean body mass who is only 10% fat. This relationship between adiposity and metabolic rate has been noted in several previous studies (2, 13, 17, 19) but not in others (3, 20, 21, 42). One could argue that this is partly a statistical problem of nonindependence between the measure of fatness and the adjustment of metabolic rate and protein turnover for potassium content. For example, if potassium content were underestimated, then the amount of fat would be overestimated and the residual metabolic rate and leucine flux both would tend to be positive. However, this cannot explain the relationship completely because the estimated amount of body fat varied by >40 kg, whereas the maximum error in the estimation of body fat (relative to the value obtained from deuterium oxide dilution) is <10 kg (10). Moreover, other indexes of adiposity unrelated to the potassium measurements, such as total weight and body mass index, also had significant positive correlations with potassium-adjusted metabolic rate and leucine flux. The fact that similar correlations between percent fat and metabolic rate or protein turnover were obtained using creatinine excretion as the index of lean body mass (which is independent of the potassium measurement) also indicates that the relationship between adiposity and metabolism is not simply a statistical artefact.

Several energy-consuming processes may be elevated in obese subjects, including the increase in protein turnover noted in the present study. Based on the energy cost of protein turnover discussed later, it appears that about a third of the increase in resting metabolic rate associated with increasing adiposity might be attributed to the corresponding increase in protein turnover. Increased Na-K-ATPase activity, another major energy-consuming process, has been observed in liver and skeletal muscle of obese subjects (5, 6). It is worth noting that adipocytes from obese subjects have lower *in vitro* rates of heat production (per gram of lipid) than adipocytes from lean subjects (16, 43), which would attenuate the contribution of adipose tissue itself to the elevated metabolic rate in obese subjects. We also considered the possibility that the ratio of visceral to muscle mass is greater in obese subjects, which would increase the metabolic rate per kilogram active cell mass as discussed above. However, this idea was not supported by the fact that the mean ratio of creatinine excretion (mg/h) to total body potassium (mol) was the same in the fattest men (18.9 in men >20% fat) and women (17.5 in women >30% fat) as in the lean men (18.8 in men <20% fat) and women (17.6 in women <27% fat).

Increased protein turnover per kilogram of fat-free mass has been observed previously in a small group of obese subjects (29). Because insulin inhibits protein breakdown, this effect was attributed to insulin resistance in the obese subjects. However, Staten et al. (44)

found that obese subjects with type II diabetes did not have higher rates of leucine flux than nondiabetic obese subjects, suggesting that resistance to insulin's effects on glucose metabolism does not necessarily correspond to resistance to insulin's effects on protein metabolism. Thus further work is needed to elucidate why protein turnover is elevated in obese subjects.

Relationship between metabolic rate and protein turnover. When no adjustment was made for the amount of active tissue, there was a very strong correlation between resting metabolic rate and protein turnover. When metabolic rate and protein turnover were adjusted for various indices of active tissue mass (weight, creatinine, and potassium), there was a decrease in the strength of the correlation between metabolic rate and protein turnover. Thus, to some extent, the strong relationship between metabolic rate and protein turnover reflects their mutual dependence on the amount of active tissue. Nevertheless, protein turnover and metabolic rate still were significantly correlated even after adjusting for active cell mass (whole body potassium).

Some caution must be exercised when interpreting correlations between variables that are not entirely interdependent. Because metabolic rate and protein turnover both were adjusted for whole body potassium, we must consider the possibility that their correlation is caused by errors in the potassium measurement. For example, if we underestimated the potassium content of an average-sized subject by 5% (which according to the repeated measurements is the maximum error that would occur), we would have overestimated his residual metabolic rate by ~50 kcal/day and his residual leucine flux by about 7 $\mu\text{mol}/\text{min}$. Examination of Fig. 4 clearly shows that such errors would not contribute very much to the observed correlation between residual leucine flux and residual metabolic rate. One also might argue that resting metabolic rate is a better index of active cell mass than whole body potassium, which could lead to a positive correlation between leucine flux and metabolic rate even after adjusting for whole body potassium. However, this explanation is not supported by the fact that the correlation between leucine flux and metabolic rate ($r = 0.84$) was not stronger than the correlation between leucine flux and whole body potassium ($r = 0.85$).

The relationship between metabolic rate and protein breakdown (i.e., leucine flux) was nearly identical to the relationship between metabolic rate and protein synthesis (i.e., the nonoxidized portion of leucine flux). It should be noted that this does not prove that protein synthesis is entirely responsible for the relationship between protein turnover and resting metabolic rate because leucine flux and the nonoxidized portion of leucine flux were highly correlated ($r = 0.98$). Thus on statistical grounds alone it is not possible to determine to what degree the relationship between protein turnover and resting metabolic rate is mediated by protein synthesis.

Some mention should be made of our choice of [^{13}C] leucine as a tracer to study whole body protein metabolism, instead of other commonly used tracers such as [^{15}N]glycine or [^{15}N]lysine. The advantages of [^{13}C] leucine are the following: 1) it is diluted in a small free

pool of leucine so that a rapid steady state can be achieved; 2) it has an intracellular metabolite (KIC) that retains the label so that the intracellular enrichment can be more accurately estimated; 3) it is an essential amino acid so that its flux represents proteolysis in the post-absorptive state; 4) the only two fates of the label are incorporation into protein or loss as CO_2 , so that whole body protein synthesis can be estimated from the difference between flux and oxidation; 5) it is abundant in many proteins, amounting to ~7–8% of the total mass of protein. From a practical standpoint, [^{13}C]leucine is inexpensive and is currently the most commonly used tracer to study whole body protein metabolism in humans. Hence we believe that this tracer was an appropriate one for this study, even though we recognize that results obtained with other tracers of protein metabolism might not have given identical results.

We have interpreted the relationship between energy expenditure and protein metabolism as one in which the energy requirements of higher rates of protein turnover lead to higher metabolic rates. An alternate interpretation would be that a higher metabolic rate leads to a higher rate of protein breakdown, to provide amino acids as energy substrates. However, this interpretation was not supported by the fact that nitrogen excretion (which reflects total amino acid oxidation) did not correlate with metabolic rate after adjusting the variables for whole body potassium. Nevertheless, because nitrogen excretion was not corrected for changes in total body urea or for excretion of nitrogen from sources other than amino acids, the use of nitrogen excretion as an index of total amino acid oxidation is only a rough approximation.

Our data indicated that, at a constant body cell mass, metabolic rate increased 2.45 kcal/day for each 1 $\mu\text{mol}/\text{min}$ increase in leucine flux. Thus in the average subject with a leucine flux of 140 $\mu\text{mol}/\text{min}$, the apparent energy expenditure for protein turnover was ~340 kcal/day or 20% of the resting metabolic rate. This value is considerably lower than an earlier estimate, using a similar approach, that protein turnover can account for ~50% of resting metabolic rate (30). The reason for the discrepancy is that in the previous study the data were not adjusted for active cell mass (30). If the data from the present study had not been adjusted for whole body potassium, then the slope of the regression line relating metabolic rate to protein turnover would have again suggested that protein turnover accounts for ~50% of resting metabolic rate.

If the assumption is made that leucine constitutes 8% of protein by weight, 1 $\mu\text{mol}/\text{min}$ of leucine flux corresponds to 2.36 g/day of protein turnover. Thus, based on the value of 2.45 kcal/day per μmol leucine flux (as discussed above), the energy expenditure associated with protein turnover in the present study was 1.04 kcal/g protein. This value is similar to often-quoted theoretical values for the energy requirements of protein synthesis of 0.85–1.08 kcal/g protein (1, 7, 25, 28, 38).

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