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Postprandial Resting Metabolic Rate and Body Composition in the Moderately Obese and Normal-Weight Adult Subjects at Sitting Posture

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Summary A reduced metabolic rate in the etiology of obesity has been a subject of controversy. The prediction of the energy requirements for the obese using reference values may therefore be distorted. In order to examine this possibility, resting metabolic rate (RMR) while the subject was sitting comfortably in a chair was measured in a total of 134 moderately obese and normal-weight subjects (68 women aged 20 to 71 with a mean of 53.1 and 66 men aged 20 to 63 with a mean of 36.5). RMR per kg of body weight was significantly lower in the female obese subjects, but not in the male obese subjects. There was no evidence of difference in RMR between obese and normal-weight subjects in either sex when RMR was indexed with fat-free mass (FFM), indicating no substantial decrease in the metabolism due to obesity. Multiple regression analyses indicate that standardization of RMR by FFM eliminates the apparent difference in RMR between the sexes, and the diminution of RMR with age was not observed. While the best and logical prediction of RMR is to use FFM, regression analyses suggest an alternative way of predicting RMR by an incorporation of subscapular skinfold thickness to adjust the different body composition in lean and obese subjects. Prediction equations of postprandial RMR (kcal/24-h) while sitting are $RMR = 24.5 \times FFM(kg) + 303.7$, and $RMR = 22.7 \times weight(kg) - 13.6 \times SSF$ (subscapular skinfold: mm) $+ 350.6$. Problems in predicting RMR are discussed.

Key Words resting metabolism, obesity, adaptive metabolic change, index of metabolism, BMR standards

In the previous paper, we found a large discrepancy between the measured and the predicted values of resting metabolic rate (RMR) in the middle-aged moderately obese Japanese housewives (*1*). In that, the RMR determined by oxygen con-

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sumption while the subject was sitting in a chair was 15% lower than the RMR predicted from the Japanese BMR (basal metabolic rate) standard, sitting postprandial RMR being predicted by assuming a 20% increase above the BMR (*2, 3*). The discrepancy was not negligible because their daily physical activities accounted for only 20% of the 24-h energy expenditure of free living conditions (*1*). If this applies generally to any adult population, the prediction of energy requirements using the reference values may result in an erroneous conclusion regarding the energy balance of people, which would thus prove to be a serious obstacle to the prevention and treatment of obesity.

The majority of the female subjects (70%) in our previous study were obese with a fat mass more than 30% of body weight. One possibility for the discrepancy is a metabolic change in response to obesity. Some recent studies suggested an adaptive metabolic change, but other studies indicated no such evidence (*4–9*). The conclusions differ among the reports depending on the body parameters used as the index of metabolism; *i.e.*, per whole body, body surface area (BSA), kg weight, or kg fat-free mass (FFM).

In order to find contributing factors for the discrepancy observed in the previous paper, we further measured the RMR for male and female subjects with different degrees of obesity. The present analyses were made to examine whether there was an obesity-related reduction in the metabolism, and to investigate the best index and prediction model of the RMR using anthropometric measurements.

SUBJECTS AND METHOD

The measurements of the RMR were carried out by means of indirect calorimetry as a part of the study to investigate daily energy expenditure under free living conditions. The studies were done in June/July and October/November in 1982 and January/February in 1983 at the Ikebukuro Health Center for the female subjects engaging exclusively in household tasks, and September/October in 1983 for the male subjects working at a factory of Mitsubishi Electric Co. in Kamakura where computer and electronic machines are manufactured. Details of the methods for recruiting subjects are given in the previous papers (*1,10*). A total of 134 individuals, 68 women and 66 men, took part in this study. The subjects were apparently healthy at the time of the study. There were no subjects who were extremely lean or obese.

For determination of oxygen consumption, the subjects were asked to come to the laboratories at the respective locations at least 1 h after a morning or noon meal, and to avoid substantial exercise on the previous day. Before the measurements, every subject was familiarized with the apparatus and measurement procedures, and informed consent of the subjects was obtained. Heart rate at rest sitting comfortably in a chair was monitored on a chart with a portable ECG (model FD16, Fukuda Denshi, Tokyo) in order to check whether the resting condition was maintained. The mean heart rates (HR) of the female and male subjects in the steady state were

74.9 ± 8.8 and 70.3 ± 10.2. The measurements of oxygen consumption were performed by collecting the last 2-min sample of expired air in a Douglas bag with the subjects at rest sitting comfortably in a chair for 10 min. The room temperature at measurement was between 20 and 25 °C. The volume of expired air was measured with the certified gas meter (Shinagawa Seisakusho, Tokyo) as accurate as 10 ml. The expired air of 200 ml was sampled for the determination of O_2 and CO_2 concentration, using room air as the standard, with the gas analyzer (Expired Gas Monitor, model 1H21, San-Ei Sokki, Tokyo). The analyzer was calibrated with a certified gas mixture (Nihon Sanso Inc., Tokyo). The volume of oxygen consumed was converted to STPD, and the RMR was calculated using the energy equivalence of oxygen for the measured respiratory quotient.

Anthropometric measurements were made by one investigator using the standard techniques (*11*)—with an Anthropometer of Martin for the stature to the nearest 0.1 cm, and with a scale (Digital Speed Scale, Yagami, Tokyo) for the weight to the nearest 0.1 kg. The subscapular and triceps skinfold thicknesses were measured on the left side of the body using Holtain skinfold calipers. Fat mass was estimated from the following equations: for the body density, [D = 1.0897 − 0.00133 × (the sum of the subscapular and triceps skinfolds) for female, and D = 1.0913 − 0.00116 × (the sum of the subscapular and triceps skinfolds) for male] as described by Nagamine and Suzuki (*12*); and for the fat mass, {fat mass = weight × [(4.57/D) − 4.142]} by Brozek *et al.* (*13*). Fat free mass (FFM) was determined by subtracting fat mass from the body weight. Body surface area (BSA) was calculated as described by Fujimoto *et al.* (*14*).

For the comparison of RMR between obese and normal-weight subjects, obesity and normal-weight were defined by the criteria of fat mass percent (*15*, *16*): obese (female, 30% or greater; male, 20% or greater); normal-weight (female, less than 25%; male, less than 15%).

Data were analyzed using the Statistical Package for the Social Sciences (*17*). Differences in the measurements were examined using ANOVA or Student's *t*-test techniques. When the ANOVA test was significant, means were compared by the least-significant difference test. Pearson's correlation coefficients were computed for RMR with several variables, and multiple regression analyses of RMR were performed stepwise against the sets of the variables representing body size or components. Differences are regarded as significant if the probability is less than 0.05. Values in the text are presented as mean and standard deviation, unless otherwise stated.

RESULTS

Table 1 shows the physical characteristics of the subjects. Male subjects were significantly younger than female subjects. Within each sex, physical characteristics were compared between the moderately obese and normal-weight subjects. In either sex, there were no differences in age and height between obese and normal-weight

Table 1. Physical characteristics of the subjects.

	Male			Female		
	Total	Normal	Obese	Total	Normal	Obese
No. of subjects	66	36	12	68	23	33
Age (yr)	36.5 ± 10.4 (20–63)	35.2 ± 9.9^{a}	37.7 ± 9.5^{a}	53.1 ± 8.6 (20–71)	52.7 ± 10.1^{b}	52.5 ± 7.9^{b}
Height (cm)	167.3 ± 6.0 (156.0–182.3)	166.9 ± 5.8^{a}	169.7 ± 7.1^{a}	150.8 ± 5.3 (137.0–163.1)	150.7 ± 4.6^{b}	151.7 ± 5.2^{b}
Weight (kg)	66.1 ± 10.1 (49.5–102.5)	60.8 ± 6.9^{a}	78.5 ± 9.4^{b}	54.6 ± 8.1 (37.0–74.5)	48.0 ± 5.2^{c}	60.0 ± 6.7^{a}
FFM[1] (kg)	55.5 ± 6.6 (43.9–78.8)	53.2 ± 5.5^{a}	61.0 ± 7.2^{b}	38.2 ± 3.7 (29.6–46.5)	37.7 ± 3.4^{c}	38.5 ± 4.0^{c}
Fat mass (kg)	10.6 ± 4.2 (5.0–23.7)	7.6 ± 1.7^{a}	17.6 ± 2.5^{b}	16.5 ± 6.3 (3.9–34.9)	10.3 ± 2.3^{c}	21.5 ± 4.8^{d}
Fat (%)	15.6 ± 4.1 (9.1–24.6)	12.5 ± 1.6^{a}	22.4 ± 1.5^{b}	29.3 ± 7.6 (10.5–51.3)	21.3 ± 3.3^{b}	35.5 ± 4.9^{c}
BSA[2] (m^2)	1.70 ± 0.14 (1.46–2.10)	1.64 ± 0.11^{a}	1.85 ± 0.13^{b}	1.46 ± 0.11 (1.23–1.69)	1.38 ± 0.09^{c}	1.53 ± 0.09^{d}
BMI[3]	23.6 ± 3.1 (17.9–34.8)	21.8 ± 1.9^{a}	27.3 ± 3.1^{b}	24.0 ± 3.2 (16.3–32.0)	21.1 ± 1.8^{a}	26.1 ± 2.8^{b}

[1] FFM, fat-free mass; [2] BSA, body surface area; [3] BMI, body mass index as defined body weight/height^2 (kg/m^2). Different superscripts in the same row indicate the mean values differ significantly ($p < 0.05$). The ranges are shown in the parentheses.

Table 2. Postprandial RMR by sex and body composition.

RMR	Male		
	Total	Normal	Obese
Whole body (kcal/min)	1.15 ± 0.20^{A}	1.09 ± 0.16^{a}	1.30 ± 0.21^{b}
Per kg weight (kcal/day)	25.3 ± 3.6^{A}	25.9 ± 3.7^{a}	23.8 ± 2.9^{a}
Per kg FFM (kcal/day)	30.0 ± 4.1^{A}	29.6 ± 4.1^{a}	30.7 ± 3.8^{a}
Per m² BSA (kcal/day)	976.1 ± 131.7^{A}	957.1 ± 117.6^{a}	$1{,}007.0 \pm 134.0^{a}$

Table 2. (continued)

RMR	Female		
	Total	Normal	Obese
Whole body (kcal/min)	0.86 ± 0.13^{B}	0.84 ± 0.13^{c}	0.88 ± 0.13^{c}
Per kg weight (kcal/day)	23.0 ± 4.0^{B}	25.5 ± 4.4^{a}	21.3 ± 3.3^{b}
Per kg FFM (kcal/day)	32.6 ± 4.8^{B}	32.4 ± 5.2^{b}	33.2 ± 5.0^{b}
Per m² BSA (kcal/day)	852.7 ± 120.6^{B}	884.3 ± 140.7^{b}	833.6 ± 118.2^{b}

Data indicate mean and standard deviation. Different superscripts in the same row indicate the mean value differs significantly ($p < 0.05$, t-test for the upper-case letters, and least-significant difference test for the lower-case letters).

subjects, but the other measurements differ significantly. Among the females, no significant difference was detected in FFM between obese and control subjects.

The RMR per whole body, per kg of body weight, per BSA, and per kg of FFM were compared between the sexes, and the obese and normal-weight subjects (Table 2). Sex differences existed in the RMR indexed with any of the body indices. In the male subjects, the RMR expressed as an absolute value was found to be significantly higher in the moderately obese subjects than in the normal-weight subjects, but no significant differences were found between the obese and normal-weight subjects in the RMR indexed with any of the body indices. In the female subjects, RMR of the whole body value, RMR per FFM, and RMR per BSA did not differ significantly between the obese and normal-weight subjects. The RMR per body weight was the

Table 3. Correlations of RMR in normal-weight and obese subjects.

	Male			Female		
	Total	Normal	Obese	Total	Normal	Obese
No.	66	36	12	68	23	33
Age	−0.189	−0.377*	0.072	0.119	0.212	0.115
Height	0.401**	0.665**	0.169	0.198	−0.076	0.267
Weight	0.636**	0.403*	0.687*	0.356**	0.157	0.408*
FFM	0.626**	0.420*	0.664*	0.349**	0.176	0.412*
BSA	0.633**	0.533**	0.592*	0.354**	0.098	0.425*

* $p<0.05$, ** $p<0.01$.

only variable which differed significantly between the female obese and normal-weight subjects.

Univariate correlation coefficients between the body indices and RMR are shown in Table 3. In male subjects, RMR correlated strongly with weight, FFM, and BSA, and less strongly with height and fat %. The same variables in female subjects were poorly correlated with RMR, although they were significantly different from zero. Within both sexes, weight was the best correlated variable with RMR. BSA was intermediate, and FFM was somewhat weakly correlated with RMR. In both sexes, separate examination for the correlations in the normal-weight and obese subjects showed that RMR correlated less strongly or insignificantly with the body indices in the normal-weight subjects as compared to that in the obese subjects. As can be seen in Table 3, correlation analyses of RMR with body indices by sex and obesity did not yield any conclusive relations.

When the male and female subjects were combined, the extent of relation in weight, FFM and BSA with RMR increased, and the order of importance changed (Fig. 1). FFM showed the best relation to RMR ($r=0.779$, $p<0.001$), followed by BSA ($r=0.746$, $p<0.001$) and body weight ($r=0.694$, $p<0.001$). Data pooled for males and females indicate that RMR significantly correlated with age ($r=-0.481$, $p<0.01$), height ($r=0.684$, $p<0.01$), and fat % ($r=-0.371$, $p<0.01$).

To find the best linear model for predicting RMR, multiple regression analyses were performed using a stepwise procedure (Table 4). The first run was made using a set of variables comprising age, height, weight, FFM, BSA, BMI, and sex (a dummy variable; male=1, female=0). By the stepwise procedure, only FFM was selected as the significant predicting variable. Age, sex, and other body indices did not contribute to the prediction of RMR when FFM was used.

Fig. 1. Correlations of postprandial resting metabolism with fat-free mass, body surface area, and body weight. ●, male; ○, female; FFM, fat-free mass; BSA, body surface area; WEIGHT, gross body weight.

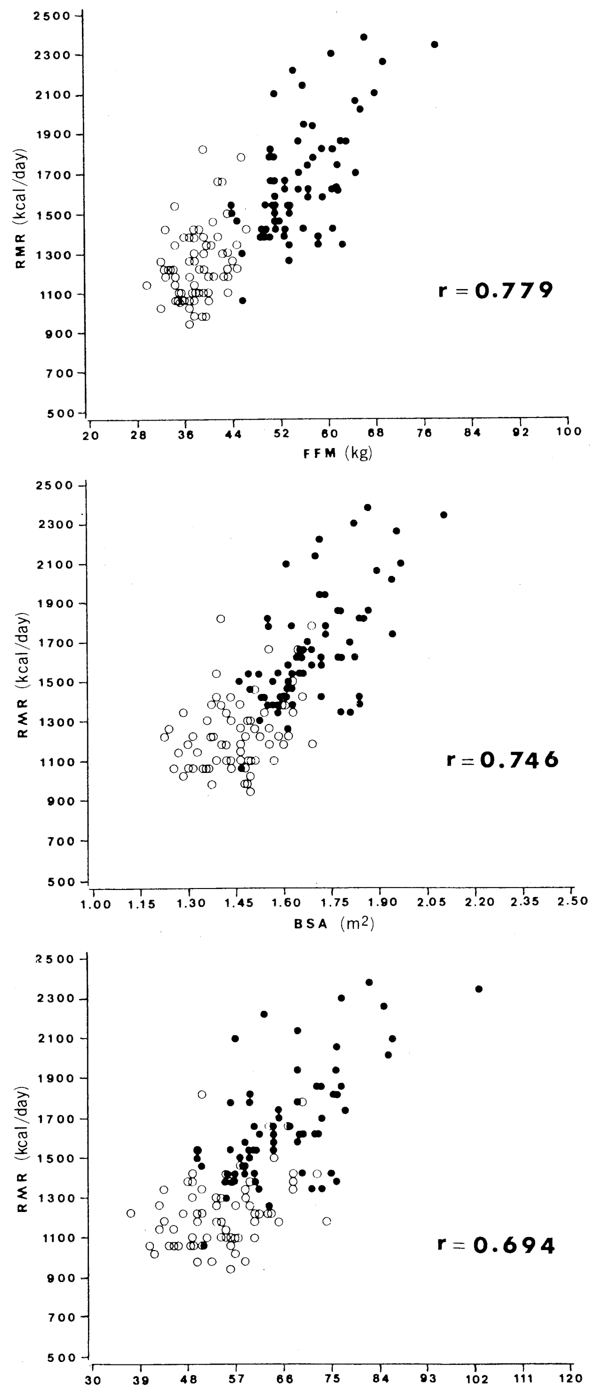
RMR (kcal/day)
2500
2300
2100
1900
1700
1500
1300
1100
900
700
500
r = 0.779
20 28 36 44 52 60 68 76 84 92 100
FFM (kg)
r = 0.746
1.00 1.15 1.30 1.45 1.60 1.75 1.90 2.05 2.20 2.35 2.50
BSA (m2)
r = 0.694
30 39 48 57 66 75 84 93 102 111 120
WEIGHT (kg)

Fig. 1.

Table 4. Multiple regression equations for postprandial RMR (kcal/24-h).

Equations	Sets of predictor variables	B	Beta	t-value	R^2 (%)
1	FFM[a,c,e]	24.476	0.779	14.256**	60.6
	constant	303.689		3.701**	
2	Weight[b]	14.141	0.476	7.269**	48.1
	Sex	259.661	0.407	6.224**	60.0
	constant	466.737		4.278**	
3	BSA[b,d]	1,010.549	0.552	7.162**	55.6
	Sex	177.538	0.279	3.611**	59.6
	constant	−232.630		1.124	
4	Weight[d]	22.671	0.763	13.754**	48.1
	SF-SUB	−13.592	−0.367	6.622**	61.1
	constant	350.590		3.452**	
5	Weight[f]	20.544	0.691	12.756**	48.1
	Fat %	−12.750	−0.366	6.761**	61.5
	constant	495.756		4.606**	
6	BSA[f]	1,275.824	0.697	12.278**	55.6
	Fat %	−7.415	−0.213	3.751**	59.9
	constant	−396.186		2.201*	

*$p<0.05$, **$p<0.01$. R^2 (%), cumulative percentage of variance explained. FFM, fat-free mass (kg); weight (kg); sex, a dummy variable (male = 1, female = 0); BSA, body surface area (m^2); SF-SUB, subscapular skinfold (mm). [a–f] Set of variables used to test the best equation: [a] Age, sex, weight, height, BMI, FFM, BSA. [b] Exclusion of FFM from the set of variables "a". [c] Inclusion of subscapular and triceps skinfolds to the set "a". [d] Exclusion of FFM from the set "c". [e] Inclusion of fat % to the set "c". [f] Exclusion of FFM from the set "e".

In the next run, weight or BSA instead of FFM was entered into the equation in the first place to examine the extent of explanation by these and other predictor variables. By this procedure, sex alone was selected as being significant after inclusion of weight or BSA. Age was again an insignificant predictor. Inclusion of sex with weight or BSA explained more the variance for the RMR in comparison with the linear equation using single predictor variable of weight or BSA. However, these equations did explain still no better than that of the equation using FFM alone.

The third run was made by adding skinfold thicknesses (subscapular and triceps skinfolds) or fat % to the set of variables. When FFM was included in the first place into the equation, fat % or skinfold thickness was not selected as the significant predictor variable. When weight or BSA entered in the first place into the equation, subscapular skinfold and fat % became the significant predictors instead of sex (weight with subscapular skinfold or fat %, and BSA with fat %). The final regression equations by these procedures indicate that any set of the predictor

variables does not have a conspicuously greater extent of explanation than FFM alone. These equations explain less than 61% of the variance for RMR.

DISCUSSION

1. Metabolic change due to obesity. There was no substantial difference in the RMR per kg of FFM between the obese and normal-weight subjects in either sex, indicating no substantial decrease in metabolism in the obese subjects. The changes in metabolic rate in response to weight gain or loss have been a subject of many studies of RMR. Ravussin *et al.* (*18*) recently showed that the obese subjects had significantly higher RMR than the control subjects. However, when RMR was expressed on the basis of BSA or FFM, there was no significant difference between control and obese subjects. In another recent observation of RMRs before and after weight loss in obese subjects, they found that the decrease of RMR in absolute value was the same magnitude as that of the FFM, and the RMR on the basis of FFM remained unchanged (*19*). They concluded that no adaptive mechanism had been involved in the overall energy metabolism during obesity and caloric restriction. From the present data and those of the literature, it is unlikely that aging or obesity has been the major factor contributing to a great difference in metabolism between obese and normal-weight subjects. Rather, the problem lies in the indexing of RMR with body size. This may have caused the discrepancy between the predicted and the measured RMR as indicated in our previous study for the female obese subjects (*1*).

2. Prediction of resting energy expenditure using BMR standards. The BMR standards are frequently used for the prediction of resting metabolism or of energy expenditure of daily activities, and are an important basis for the assessment

Table 5. Comparison between measured and predicted resting energy expenditure (kcal/day).

	No.	Measured	Predicted	Percent difference[a]
Male				
Total	66	1,661.1 ± 287.0	1,776.7 ± 163.8**	7.0
Normal	36	1,566.7 ± 227.3	1,719.3 ± 138.9**	9.7
Obese	12	1,868.0 ± 297.7	1,930.9 ± 156.8	3.4
Female				
Total	68	1,239.1 ± 184.9	1,346.0 ± 110.5**	8.6
Normal	23	1,213.9 ± 188.8	1,273.7 ± 86.0	4.9
Obese	33	1,272.2 ± 199.5	1,411.8 ± 93.0**	11.0
Total	134	1,446.9 ± 319.9	1,558.1 ± 256.9**	7.7

[a] [(predicted − measured value)/measured value] × 100. ** Predicted value differs significantly from the measured value ($p < 0.01$, by paired t-test).

of adequate energy requirements. An accurate prediction of the RMR is particularly important because the energy expenditure during resting state or sitting state is the major component of total energy expenditure of a sedentary population. Recent reports showed unexpected low levels of energy expenditure in healthy women (*20*), and overestimation in the predicted RMR using the available BMR standards compared to the measured RMR (*1, 21–23*). Table 5 shows predicted postprandial sitting RMR for the subjects of the present study which was computed, using the Japanese BMR standards indexed with BSA by age and sex, with an assumption of a 20% increase above the BMR. Statistically significant differences between the measured and predicted RMRs were also observed in the present study. As a whole, the predicted RMR was 7.7% greater than the measured RMR. The extent of overestimation did not differ substantially between male and female subjects, but differed between obese and normal-weight subjects. No clear tendency towards an increase of difference with obesity was observed: overestimation was greater in normal-weight subjects for males, but in obese subjects for females. A possibility remains that the error comes from the assumption of the RMR being a 20% increase above the BMR. But nonsystematic differences betweeen the measured and predicted RMRs suggest that this is not the primary cause. The previous and the present studies suggest that the RMR or BMR should be appropriately indexed to reflect the metabolically active tissue mass.

3. Active tissue mass and RMR. Despite the criticism that the theoretical basis for BSA was discredited largely due to the lack of a physiological base (*24, 25*), BSA has been used for many years as a reference unit for expressing the RMR. Theoretically the RMR is determined by the amount of metabolically active tissue mass. But due to the difficulty of estimating active tissue mass, BSA, weight, and FFM are used to index the RMR. A vast volume of literature has contributed to investigation of the best relation between the RMR and these variables. Many recent studies found FFM to be the body component with the greatest influence on metabolic rate, regardless of the method used to assess it (*25–27*). The present study agrees with these recent studies. The multiple regression analyses indicate that FFM is the best single predictor of RMR. The results clearly show that RMR expressed by BSA or weight differs by sex and body fatness. The differences in RMR by age and sex just reflect energy metabolism that differs by size of FFM (*27–29*). The effect of age on RMR or BMR was small and negligible as in other studies (*27, 29*). Standardization of RMR by FFM has eliminated the apparent diminution of RMR with age (*25–27*).

If FFM is a proper index of active tissue mass that determines the metabolic level of sedentary activity (*15*), it is physiologically logical to use FFM as the unit for the BMR or RMR standards. However, at the present time we cannot determine the true fat percentage or FFM in humans as can be accomplished with animal studies. We must estimate FFM by the methods of densitometry, potassium 40 counting, or use of an equation on skinfold thicknesses. In view of our data that there was statistically significant difference between males and females in RMR

indexed with estimated FFM, the estimation method of FFM needs further investigation because women may have a different density of the FFM from that of men (*30*) and the previous studies assumed that adipose tissue was metabolically inert. Until a better method for estimating active tissue mass is developed, the use of RMR indexed with estimated FFM is the most reasonable way to avoid the confusion and erroneous conclusion regarding the difference in metabolism by sex, age, and obesity.

The present study suggests the importance of the use of simple anthropometric measurements, *i.e.*, weight and subscapular skinfold. The prediction equation of postprandial sitting RMR is expressed as $\text{RMR(kcal/24-h)} = 22.7 \times \text{weight(kg)} - 13.6 \times \text{SSF(subscapular skinfold: mm)} + 350.6$ which has the same predictive power of the equation using FFM alone: $\text{RMR (kcal/24-h)} = 24.5 \times \text{FFM(kg)} + 303.7$. Although the predictive power of less than 61% of the variance for RMR may limit the practical application of these equations, our results suggest that weight and skinfold thicknesses are essential information in RMR measurements until a better and practical method of indexing RMR is established. These measurements are easily accomplished by the usual anthropometric procedures without using sophisticated equipment. Information on skinfold thicknesses is essential not only in adjusting different body composition in obese and lean population but also for the future studies on preventive measures of obesity-related diseases. Recent studies indicate the importance of knowledge about body fat localization—centrally located body fat such as subscapular, suprailiac, and subcostal skinfold thicknesses being more strongly related to increased levels of serum triglyceride and blood pressure, and increased prevalence of diabetes than peripheral skinfold thicknesses such as that at the triceps (*31*). In this connection, our finding on the relation of RMR with centrally located body fat (subscapular skinfold thickness) is particularly important.

4. *Conclusions.* There was no obesity-related reduction in resting metabolism when RMR was indexed with FFM. Our analyses suggest that the problem in establishing the proper index of RMR may have been responsible for the discrepancy between the predicted and the measured RMR. Our data also indicate that the best and logical index of RMR is FFM. Finally we proposed, by multiple regression analyses, an alternative way of predicting RMR using gross body weight and subscapular skinfold thickness. However, some reservations should be noted on our results before a general conclusion is drawn, because our data are based on measurements of the postprandial sitting RMR. Interpretation of the results is limited to sitting RMR 1 to 3 h after a meal, but some advantageous aspects of measuring postprandial RMR rather than the post-absorptive RMR or BMR are worth consideration: the RMR at least an hour after the usual meal does not vary substantially for the next 3 h; the measurements do not require the subject to be present in an overnight fast nor to postpone breakfast until mid-morning, and limiting inconvenience to the subjects facilitates the examination of a large body of RMR data (*21*). In addition to these, the RMR (not fasting RMR) is a realistic state

of metabolism under free living conditions, and comprises 60–80% of total 24-h energy expenditure. Recent findings on the variability of the fasting RMR within the range of thermal comfort zone may pose a serious problem in establishing a reference value for BMR or RMR under post-absorptive condition. Postprandial RMR does not vary within the thermal comfort zone (22–28°C), whereas the fasting RMR differs significantly between the upper and lower limits of the thermal comfort zone (*32, 33*). These points, together with diet-induced thermogenesis, require careful examination in future studies.

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