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ORIGINAL ARTICLE

Low resting energy expenditure in postmenopausal Japanese women with type 2 diabetes mellitus

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Abstract

Objective Resting energy expenditure (REE) is an important tool in nutrition management, especially in type 2 diabetes mellitus (T2DM). The predicted REE (pREE) was reported to be inaccurate, compared with measured REE (mREE) in Japanese T2DM patients. Despite the accuracy of REE, measured via indirect calorimetry (mREE), the technique is demanding. This study evaluated the associated clinical factors of the difference between pREE and mREE in Japanese patients with T2DM.

Methods Forty-nine Japanese patients with T2DM but no severe complications (32 men and 17 women) were enrolled. mREE was determined via indirect calorimetry.

Results Participants average age was 56.3 ± 11.0 years, body mass index was 25.2 ± 3.6 kg/m^2, and HbA1c was 9.6 ± 1.6%. The mean mREE was 1099 ± 212 kcal/day. Age, body mass index, hemoglobin, and uric acid levels were all associated with mREE by simple regression; of these, body weight was the significant factor in the multiple regression analysis. When the patients were divided into tertiles, the average mREE values were lower than the pREE values for each group. The difference between mREE and pREE was largest in the lowest value group, whose subjects were mostly women aged over 50 years. This group of women showed significantly lower mREE (904 ± 121 kcal) in comparison with men in the same age group, with 26% overestimation of pREE, even when the equation that yielded the closest mREE value was used.

Conclusion The previously reported pREE overestimates mREE in Japanese patients with T2DM, especially in postmenopausal women.

Keywords Type 2 diabetes mellitus · Resting energy expenditure · Basal metabolic rate · Postmenopausal

Introduction

In the management of nutrition-related diseases, evaluation of energy expenditure is necessary. The basal metabolic rate (BMR) is the minimum energy requirement for survival, accounting for > 70% of the total energy expenditure [1]. Since diet therapy is an important approach in managing patients with type 2 diabetes mellitus (T2DM), it is critical to evaluate the resting energy expenditure (REE) in these patients.

In practice, the REE can replace the BMR if it is measured under standard conditions such as a fasting state in the morning (≥ 12 h after the last meal), with the patient in a supine position after more than 20 min of rest. Indirect calorimetry is the most precise and non-invasive technique for the measurement of REE; however, its use may be limited by the requirement of patient preparation and the efforts involved on the part of the clinical staff. Therefore, predictive REE (pREE) values (Table 1) are often used in

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Table 1 Equations for predicting resting energy expenditure

Equation	Sex	Formula	Data source	References
Harris-Benedict	Male	$66.47 + 13.75 \times W + 5.0 \times H - 6.75 \times A$	Healthy Caucasians	[2]
	Female	$655.1 + 9.75 \times W + 1.85 \times H - 4.68 \times A$		
Ganpule	Male	$(0.0481 \times W + 0.023 \times H - 0.0138 \times A - 0.4235) \times 1000/4.186$	Healthy Japanese	[4]
	Female	$(0.0481 \times W + 0.023 \times H - 0.0138 \times A - 0.9708) \times 1000/4.186$		
Kyoto University		$10 \times W - 3 \times A + 125$ (if male) $+ 750$	Japanese diabetic patients	[6]

W weight in kg, *H* height in cm, *A* age in years

clinical practice. The Harris–Benedict (HB) equation [2] is frequently used throughout the world to calculate pREE, although the value is overestimated by approximately 100 kcal among Asians, especially in women, when using this method [3], since the HB equation was formulated using data from healthy Caucasian subjects, and therefore, it might not give an accurate estimate of REE for healthy Japanese individuals. Accordingly, Ganpule et al. developed the Ganpule predictive equation (GN equation) [4], which considers the individual's age, sex, height, and body weight for predicting BMR among healthy Japanese individuals [5].

The Kyoto University (KU) equation is the only equation developed using Japanese patients with diabetes mellitus; the subjects included patients with both type 1 (T1DM) and type 2 (T2DM) diabetes [6]. Patients with T1DM and T2DM are known to differ phenotypically in terms of insulin secretion and visceral fat accumulation. Individuals with metabolic syndrome have been reported to exhibit a significantly lower resting metabolic rate adjusted for fat-free mass than controls and obese individuals without metabolic syndrome [7]. Similarly, obese patients with T2DM have been shown to have equal or higher resting energy expenditure than obese patients without T2DM [8]. Among Japanese patients with well-controlled T2DM (through regular monitoring), many are not obese, suggesting that there must be some variation in the REE of these patients. In a recent report, the pREE in Japanese patients with T2DM was revealed to have large random errors and significant proposal errors even when the data were adjusted for bone mineral content and lean soft-tissue mass [9].

However, there has been no study about the factor which causes the discrepancy of REE with the general biochemical examination of the Japanese type 2 diabetes patients, without complications, and under strict conditions of diet and blood glucose control.

The present study aimed to determine the influence of clinical background characteristics on REE in patients with T2DM, as determined by indirect calorimetry. These clinical factors may be responsible for the difference in the pREE values among Japanese patients with T2DM.

Methods

Subjects

Japanese patients with T2DM with no overt complications were recruited. They were admitted at our clinic (Diabetes Center, Tokyo Women's Medical University Hospital, Tokyo, Japan), to a 1-week program, which sought to examine the complications in patients, in addition to providing diabetes education, from September 2008 to April 2009. Patients were considered eligible for the study if they were able to walk independently, had an estimated glomerular filtration rate of ≥ 45 mL/min/1.73 m^2, and had no other diseases except hypertension or dyslipidemia. All patients provided verbal consent before the examination. Written informed consent for data acquisition and analysis was obtained from 35 patients who visited our outpatient clinic after being referred by their primary physician, retrospectively. A public announcement was made from March to June 2016 on the homepage of our website (http://twmu-diabetes.jp) to inform the remaining 14 patients, whom we were unable to reach directly, about the purpose of the study and the possible application of its findings in research. Participants were informed about the voluntary nature of the study. Informed consent or substitute for it was obtained from all patients for being included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration. The study protocol complied with the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study design was approved by the Ethics Committee of the Tokyo Women's Medical University School of Medicine (9 May, 2017 No. 3829R).

Study design

Oxygen consumption and carbon dioxide production were measured using indirect calorimetry (Vmax29n; Sensor Medics, CA, USA) for 10 min at room temperature (25 °C). The participants had dinner, the composition,

and caloric content of which was ideal for diabetes management (58–63% carbohydrate, 16–18% protein, and 22–24% fat) at 6 pm. After fasting overnight, the subjects were tested in the decubitus position, the next morning. To measure using the indirect calorimetry, we used Vmax systems, which is one of the most valid instruments (in comparison with the Deltatrac Metabolic Monitor), and widely used in western countries [10, 11]. Mean values for oxygen consumption and carbon dioxide production on days 2–5 after hospitalization (day 2, $n=25$; day 3, $n=17$; day 4, $n=2$; day 5, $n=5$) were obtained. The gas results were converted into (a) the respiratory quotient (VCO_2/VO_2), which reflects the inner respiration and (b) the REE (kcal/day), using the Weir formula [12]. The heights of the participants were measured on the day of admission. Body composition analysis was performed using a bioelectrical impedance analyzer (DC-320; Tanita, Tokyo), right before the REE measurement, to provide data on body weight, body fat percentage, fat mass, fat-free mass, and muscle mass. Predicted REE (pREE) values were calculated using sex, age, body weight (KU equation), and height (HB and GN equations). The equation used for pREE calculation is shown in Table 1.

Clinical and laboratory data, including age, sex, duration of diabetes, medical history and medicaments, diabetic microangiopathy (neuropathy, retinopathy, and nephropathy), hypertension, dyslipidemia, and fatty liver (diagnosed using abdominal ultrasonography), were obtained from medical records. Diabetic neuropathy was diagnosed based on decreased or absent deep tendon reflex, or reduced sense of vibration in the lower extremities. Retinopathy was diagnosed by an ophthalmologist within 7 months of admission. Nephropathy was diagnosed based on the patient's urinary albumin–creatinine ratio, which was determined using a first-morning urine sample and estimated glomerular filtration rate, with the following three-variable equation, as proposed by the Japanese Society for Nephrology: eGFR (mL/min/1.73 m2) = 194 × serum creatinine (mg/dL) − 1.094 × age (years) − 0.287 × ([if women] × 0.739) [13].

Blood samples were obtained 1 or 2 days before the examination, for general evaluations. Glycated hemoglobin (HbA1c) levels within 1 month of each patient's admission were measured using high-performance liquid chromatography. The HbA1c values were standardized to the National Glycohaemoglobin Standardization Program (NGSP) units using the following formula: HbA1c (%) = 1.02 × Japan Diabetes Society (%) + 0.25% [14]. Blood samples were also tested to determine complete blood cell counts, and levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine, uric acid, calcium, and phosphate. Data on uric acid were not obtained for four patients who were receiving antihyperuricemic medication. Fasting plasma glucose (FPG) level was tested within 3 days of REE measurement using venous blood. Insulin secretion was evaluated based on C-peptide immunoreactivity (CPR) levels in samples from the 24 h urine collection (U-CPR).

Statistical analysis

The results were expressed as mean ± standard deviation. All analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). Intergroup comparisons were carried out using the Student's *t* test.

Linear regression analysis was used to evaluate correlations of mREE with each clinical feature and correlation of difference between each pREE and mREE values and clinical features. Correlation covariance value (β) and adjusted coefficient determination (r^2) were calculated. For further evaluation, multiple regression analysis was performed by the stepwise method. After categorizing the mREE values into tertiles, we performed intergroup comparisons using analysis of variance followed by Bonferroni correction, and statistically evaluated for Gaussian distribution using the Shapiro–Wilk test. Differences were considered statistically significant based on a two-tailed *P*-value of <0.05.

Results

The characteristics of the 49 participants (men/women: 32/17) are shown in Table 2. The mean age, height, body weight, and BMI of the participants were 56 ± 11 years, 165 ± 9 cm, 68.8 ± 13.4 kg, and 25.2 ± 3.6 kg/m^2, respectively. The mean HbA1c level was 9.6 ± 1.6% without ketosis. Thirty-eight participants used medicament to lower their blood glucose level (Table 2), of which 19 used plural oral hypoglycemic agents and 6 used combination of insulin and oral therapy (Supplement Table). The mean mREE was 1099 ± 212 kcal/day. Univariate analyses revealed that the mREE values were positively associated with height, body weight, BMI, fat mass, fat-free mass, presence of fatty liver, WBC count, hemoglobin level, and levels of U-CPR, γ-glutamyl transpeptidase (γ-GTP), serum creatinine, and uric acid (Table 3). The mean fasting plasma glucose (FPG) levels on admission were 157 ± 50 mg/dl and showed a positive association with mREE that was not statistically significance. Age and female sex were significantly and negatively correlated with mREE. Although height, body weight, age, and sex could affect mREE values, multiple regression analysis by the stepwise method of these four variables showed that body weight was the only variable significantly associated with mREE (Table 4).

Table 2 Clinical characteristics of the subjects

	Total	Tertile of measured resting energy expenditure (kcal/day)			*P*-value
		Tertile 1	Tertile 2	Tertile 3	
		772–1.011	1028–1173	1186–1594	
n	49	16	17	16	
Male/Female	32 /17	4/12	13/4	15/1	< 0.001
Age (years)	56.3 ± 11.0	62.7 ± 8.7	56.8 ± 9.9	49.5 ± 10.6	0.002
Female, > 50 years old (*n*)	15	12	2	1	< 0.001
Duration of diabetes (years)	10.9 ± 7.9	13.7 ± 9.1	10.2 ± 7.4	8.9 ± 6.7	n.s
Height (cm)	165 ± 9	158 ± 7	165 ± 7	172 ± 6	< 0.001
Body weight (kg)	68.8 ± 13.4	56.4 ± 8.6	69.2 ± 5.9	80.9 ± 11.7	< 0.001
BMI (kg/m^2)	25.2 ± 3.6	22.6 ± 2.8	25.6 ± 2.3	27.4 ± 3.9	< 0.001
Body Fat (%)	28.8 ± 5.9	29.6 ± 6.4	28.1 ± 6.2	28.9 ± 5.4	n.s.
Fat Mass (kg)	20.0 ± 6.1	16.8 ± 4.9	19.4 ± 4.4	23.7 ± 7.0	0.004
Fat-free Mass (kg)	48.9 ± 9.6	39.5 ± 6.2	49.8 ± 6.0	57.2 ± 6.9	< 0.001
Muscle Mass (kg)	46.3 ± 9.2	37.4 ± 5.9	47.2 ± 5.8	54.3 ± 6.6	< 0.001
mREE (kcal/day)	1099 ± 212	865 ± 72	1091 ± 47	1341 ± 126	< 0.001
KU pREE (kcal/day)	1351 ± 196	1157 ± 135	1367 ± 90	1528 ± 149	< 0.001
GN pREE (kcal/day)	1379 ± 260	1123 ± 183	1396 ± 125	1618 ± 186	< 0.001
HB pREE (kcal/day)	1450 ± 262	1210 ± 152	1438 ± 125	1700 ± 227	< 0.001
RQ	0.89 ± 0.12	0.87 ± 0.12	0.90 ± 0.12	0.91 ± 0.12	n.s.
Nephropathy (N:micro:macroalbuminuria)	32:11:6	10:5:1	13:3:1	9:3:4	n.s.
Retinopathy (N:S:PP:P)	21:16:6:6	4:9:1:2	9:5:2:1	8:2:3:3	n.s.
Neuropathy	42 (86%)	15 (94%)	12 (71%)	15 (94%)	n.s.
Hypertension	27 (55%)	8 (50%)	9 (53%)	10 (63%)	n.s.
Dyslipidemia	38 (78%)	12 (75%)	14 (82%)	12 (75%)	n.s.
Fatty liver	35 (71%)	8 (50%)	14 (83%)	13 (81%)	n.s.
Insulin / OHA / diet	14 / 30 / 11	5 / 9 / 3	5 / 8 / 5	4 / 13 / 3	n.s.
SU or Glin / Pio / Met / αGI	26 / 6 / 19 / 13	8 / 2 / 7 / 5	7 / 2 / 7 / 3	11 / 2 / 5 / 5	n.s.
HbA1c (%)	9.6 ± 1.6	10.0 ± 1.8	8.8 ± 1.1	10.1 ± 1.6	0.030
FPG (mg/dL)	157 ± 50	145 ± 46	148 ± 30	180 ± 68	n.s.
	n = 47	*n* = 16	*n* = 17	*n* = 14	
U-CPR (μg/day)	73.7	47.4	71.6	102.3	0.004
AST (U/L)	21.6 ± 9.8	20.4 ± 7.5	22.9 ± 12.5	21.5 ± 9.0	n.s.
ALT (U/L)	27.7 ± 17.5	24.1 ± 18.2	30.6 ± 20.0	28.1 ± 14.2	n.s.
γ-GTP (U/L)	37.0 ± 24.7	23.1 ± 9.0	41.5 ± 29.6	45.9 ± 24.8	0.017
Serum albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	4.2 ± 0.3	n.s.
Serum creatinine (mg/dL)	0.71 ± 0.16	0.63 ± 0.14	0.73 ± 0.13	0.77 ± 0.18	0.023
eGFR (mL/min/1.73 m^2)	84.5 ± 16.8	83.2 ± 19.1	83.0 ± 12.0	87.4 ± 19.4	n.s.
ACR (mg/g × Cr)	79.4 ± 149.5	52.4 ± 108.8	57.9 ± 92.5	129.1 ± 215.9	n.s.
Uric acid (mg/dL)	5.3 ± 1.4	4.3 ± 1.0	5.5 ± 1.3	6.2 ± 1.1	< 0.001
	n = 45	*n* = 15	*n* = 17	*n* = 13	
Calcium (mg/dL)	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.2	9.1 ± 0.3	n.s.
	n = 26	*n* = 9	*n* = 10	*n* = 7	
Phosphate (mg/dL)	3.2 ± 0.6	3.0 ± 0.4	3.2 ± 0.7	3.5 ± 0.5	n.s.
	n = 26	*n* = 9	*n* = 10	*n* = 7	
Total cholesterol (mg/dL)	192 ± 26	192 ± 28	185 ± 29	200 ± 20	n.s.
	n = 40	*n* = 12	*n* = 15	*n* = 13	
HDL-C (mg/dL)	46 ± 12	53 ± 13	43 ± 10	43 ± 10	0.041
	n = 44	*n* = 13	*n* = 16	*n* = 15	

Table 2 (continued)

	Total	Tertile of measured resting energy expenditure (kcal/day)			*P*-value
		Tertile 1	Tertile 2	Tertile 3	
		772–1.011	1028–1173	1186–1594	
LDL-C (mg/dL)	115 ± 26	112 ± 18	115 ± 32	118 ± 26	n.s.
	n = 37	*n* = 12	*n* = 13	*n* = 12	
triglyceride (mg/dL)	161 ± 72	108 ± 44	166 ± 56	206 ± 79	< 0.001
	n = 45	*n* = 14	*n* = 16	*n* = 15	
WBC (/μL × 10^3)	6.47 ± 1.56	5.60 ± 1.37	6.58 ± 1.32	7.23 ± 1.62	0.009
RBC (/μL × 10^6)	4.77 ± 0.56	4.32 ± 0.52	4.87 ± 0.32	5.13 ± 0.52	< 0.001
Hemoglobin (g/dL)	14.4 ± 1.8	13.0 ± 1.9	14.7 ± 1.1	15.4 ± 1.4	< 0.001
Hematocrit (%)	42.7 ± 4.3	38.8 ± 4.7	43.6 ± 3.0	45.6 ± 4.3	< 0.001
Platelet (/μL × 10^4)	23.8 ± 6.5	21.9 ± 6.7	23.7 ± 6.2	26.0 ± 6.3	

Data are reported as mean ± standard deviation

n.s. not significant, *BMI* body mass index, *mREE* measured resting energy expenditure, *KU* Kyoto University equation, *pREE* predicted resting energy expenditure, *GN* Ganpule equation, *HB* Harris-Benedict equation, *RQ* respiratory quotient, *N* none, *S* simple, *PP* pro-proliferative, *P* proliferative, *OHA* oral hypoglycemic agent, *SU* sulfonylurea, *Glin* glinide, *Pio* pioglitazone, *Met* metformin, *αGI* α glucosidase inhibitor, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin, *U-CPR* c-peptide in 24-h urine collection, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γGTP* γ-glutamyl transpeptidase, *ACR* urinary albumin creatinine ratio, *eGFR* estimated glomerular filtration rate, *WBC* white blood cell, *RBC* red blood cell

[a]Some of the participants received multiple combination therapy of OHAs and/or insulin. Details are shown in (Supplement Table)

Figure 1 shows the negative relationship between mREE and age. Sex was a significant factor for differences in mREE, with women over 50 years of age showing significantly low values. Although the relationship between mREE values and age in men and women was almost equally inclined, the average level of mREE values in men was 258 kcal higher than in women.

The mean pREE values were 1351 ± 196 kcal/day for the KU equation, 1379 ± 260 kcal/day for the GN equation, and 1450 ± 262 kcal/day for the HB equation. Although all pREE values were overestimated compared with mREE values, the one measured by the KU equation was the closest to mREE value. The relationship between pREE and mREE values, evaluated using the three major equations, is shown in Fig. 2. pREE values obtained from all three equations were positively correlated with mREE values. When we compared the overall pREE and mREE values, the KU equation had a significantly smaller difference (252 ± 109 kcal/day) than the GN equation (277 ± 90 kcal/day) and the HB equation (350 ± 137 kcal/day). When subjects were divided into tertiles based on the mREE values, differences between pREE and mREE values were noted for each equation in each tertile (Fig. 3). In the T2 and T3 groups, the KU equation provided a pREE value that was closest to the mREE value, whereas in the T1 group, the GN equation provided the closest pREE value.

The results of the univariate analysis of difference between pREE and mREE values with clinical factor in each equation are shown in Table 5. Different clinical factors were evaluated for their levels of significance at univariate analysis of differences in each equation. Therefore, multiple regression analysis was done for different models according to the significant factors at univariate analysis (Table 6). Finally, WBC was significant in the difference between KU equation, while height was significant in the difference between GN equation, and age and height were significant in the difference between HB equation.

For further evaluation, mREE values were divided into normally distributed tertiles: tertile 1 (T1: 772–1011 kcal/day), tertile 2 (T2: 1028–1173 kcal/day), and tertile 3 (T3: 1186–1594 kcal/day) (Table 2). Each tertile group followed the Gaussian distribution. Clinical characteristics of the subjects in each tertile group are shown in Table 2. Univariate analysis revealed significant tertile-specific differences by the stepwise pattern for height, body weight, fat-free mass, muscle mass (even after Bonferroni correction), BMI, uric acid levels, and hemoglobin levels in all subjects. Only HbA1c levels and the presence of fatty liver did not show significant tertile-specific differences. Significant differences between the T1 and T3 groups were observed for age, fat mass, γ-GTP levels, creatinine levels, and WBC count. mREE values for most women 50 years or older were in the lowest tertile (12 of 15 subjects). A total of 75% of the

Table 3 Results of the regression analysis for resting energy expenditure

	β	*P*-value	R^2
Male = 0/Female = 1	–0.585	< 0.001	0.328
Age (years)	–0.547	< 0.001	0.285
Female, > 50 years old (*n*)	–0.616	< 0.001	0.366
Duration (years)		n.s.	
Height (cm)	0.639	< 0.001	0.395
Body weight (kg)	0.863	< 0.001	0.74
BMI (kg/m^2)	0.7	< 0.001	0.479
Body fat (%)		n.s.	
Fat mass (kg)	0.608	< 0.001	0.356
Fat-free mass (kg)	0.818	< 0.001	0.662
Muscle Mass (kg)	0.817	< 0.001	0.66
KU (kcal/day)	0.86	< 0.001	0.735
GN (kcal/day)	0.851	< 0.001	0.718
HB (kcal/day)	0.855	< 0.001	0.725
RQ		n.s.	
Nephropathy (N:micro:macroalbuminuria)		n.s.	
Retinopathy (N:S:PP:P)		n.s.	
Neuropathy		n.s.	
Hypertension		n.s.	
Dyslipidemia		n.s.	
Fatty liver	0.376	0.008	0.123
Medication		n.s.	
Insulin		n.s.	
SU or glinide		n.s.	
Metformin		n.s.	
αGI		n.s.	
Pioglitazone		n.s.	
HbA1c (%)		n.s.	
FPG (mg/dL) *n* = 47	0.270	0.066	0.073
U-CPR (μg/day)	0.435	0.002	0.172
AST (U/L)		n.s.	
ALT (U/L)		n.s.	
γ-GTP(U/L)	0.332	0.02	0.091
Serum albumin (g/dL)		n.s.	
Serum creatinine (mg/dL)	0.356	0.012	0.108
eGFR (mL/min/1.73 m^2)		n.s.	
ACR (mg/g × Cr)		n.s.	
Uric acid (mg/dL) *n* = 45	0.655	< 0.001	0.415
Calcium (mg/dL) *n* = 26		n.s.	
Phosphate (mg/dL) *n* = 26		n.s.	
Total cholesterol (mg/dL) *n* = 40		n.s.	
HDL-C (mg/dL) *n* = 44	− 0.353	0.019	0.125
LDL-C (mg/dL) *n* = 37		n.s.	
triglyceride (mg/dl) *n* = 45	0.496	0.001	0.246
WBC (/μL × 10^3)	0.413	0.003	0.153
RBC (/μL × 10^6)	0.568	< 0.001	0.308
Hemoglobin (g/dL)	0.503	< 0.001	0.237
Hematocrit (%)	0.539	< 0.001	0.275
Platelet (/μL × 10^4)	0.246	n.s.	0.04

n.s. not significant, *BMI* body mass index, *mREE* measured resting energy expenditure, *KU* predicted values of resting energy expenditure by Kyoto Univ. equation, *GN* that of Ganpule equation, *HB* that of Harris-Benedict equation, *RQ* respiratory quotient, *N* none, *S* simple, *PP* pro-proliferative, *P* proliferative, *SU* sulfonylurea, *αGI* α glucosidase inhibitor, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin, *U-CPR* c-peptide in 24-h urine collection, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γGTP* γ-glutamyl transpeptidase, *ACR* urinary albumin creatinine ratio, *eGFR* estimated glomerular filtration rate, *WBC* white blood cell, *RBC* red blood cell

Table 4 Result of stepwise multiple regression analysis of measured REE

Variables	Measured REE		
	β	*P*	R^2
Body weight (kg)	0.863	< 0.001	0.78
Age (years)	− 0.160	0.09	
Sex (M = 0, F = 1)	− 0.129	0.17	
Height (cm)	0.07	0.53	
FPG (mg/dL)	0.130	0.09	
γ-GTP (U/L)	0.130	0.09	
Uric acid (mg/dL)	0.148	0.13	
WBC (/μL × 10^3)	0.106	0.19	
RBC (/μL × 10^6)	0.145	0.18	

REE resting energy expenditure (kcal/day), *M* male, *F* female, *FPG* fasting plasma glucose, *γGTP* γ-glutamyl transpeptidase, *WBC* white blood cell, *RBC* red blood cell

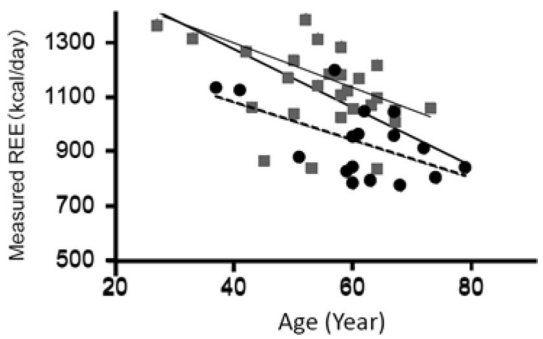

Fig. 1 Relationship between measured resting energy expenditure (mREE) and age of the participants. Thick solid line indicates regression line of the relation between mREE and age in all participants. Age shows negative correlation with mREE ($y = -10.6x + 1696$, $R^2 = 0.300$, $P < 0.001$). Closed square with thin dashed line indicates mREE and the regression line for male subjects. The closed circle with the thin dotted line indicates mREE and regression line for female subjects. Age shows negative correlation with mREE in both males and females ($y = -8.3x + 1632$, $R^2 = 0.202$, $P = 0.01$ and $y = -6.9x + 1354$, $R^2 = 0.307$, $P = 0.021$, respectively)

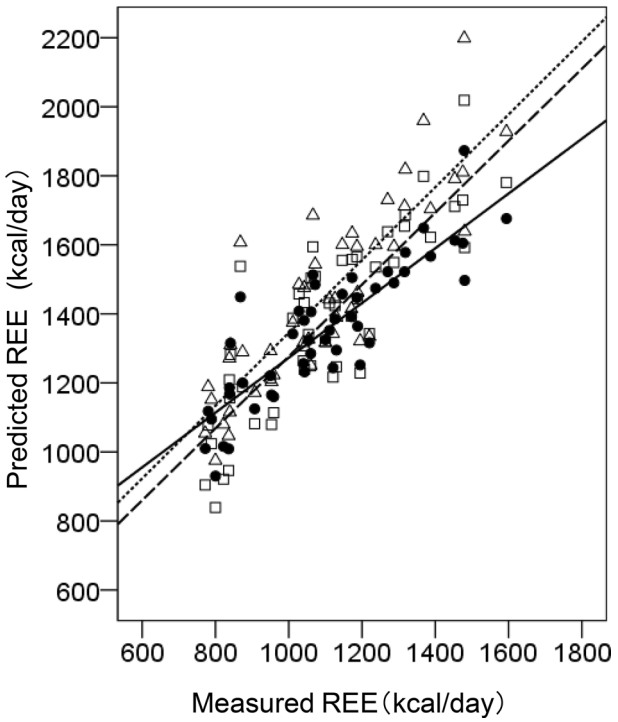

Fig. 2 Measured resting energy expenditure (mREE) and predicted resting energy expenditure REE (pREE). The closed circle indicates calculated pREE from the Kyoto University equation (KU) and the solid line indicates the regression line. The KU pREE shows positive correlation with mREE ($y=0.79x+479$, $R^2=0.74$, $P<0.001$). Open squares indicate estimated pREE using the Ganpule equation (GN) and the dashed line indicates the regression line. The GN pREE shows positive correlation with mREE ($y=1.04x+235$, $R^2=0.724$, $P<0.001$). Open triangles indicate pREE calculated using the Harris-Benedict equation (HB) and the dotted line indicates the regression line. The HB pREE shows positive correlation with mREE ($y=1.05x+290$, $R^2=0.731$, $P<0.001$). The distribution of mREE and pREE by each equation is shown to be divided into three groups at 976 kcal/day and 1250 kcal/day, respectively

patients in the T1 group were women over 50 years (12 of 16 subjects), short statured (158 ± 7 cm), and had low hemoglobin levels (13.0 ± 1.9 g/dL).

When we compared the overall pREE and mREE values, the KU equation had a significantly smaller difference (252 ± 109 kcal/day) than the GN equation (277 ± 90 kcal/day) and the HB equation (350 ± 137 kcal/day). When subjects were divided into tertiles based on the mREE values, differences between pREE and mREE values were noted for each equation in each tertile (Fig. 3). In the T2 and T3 groups, the KU equation provided a pREE value that was closest to the mREE value, whereas in the T1 group, the GN equation provided the closest pREE value.

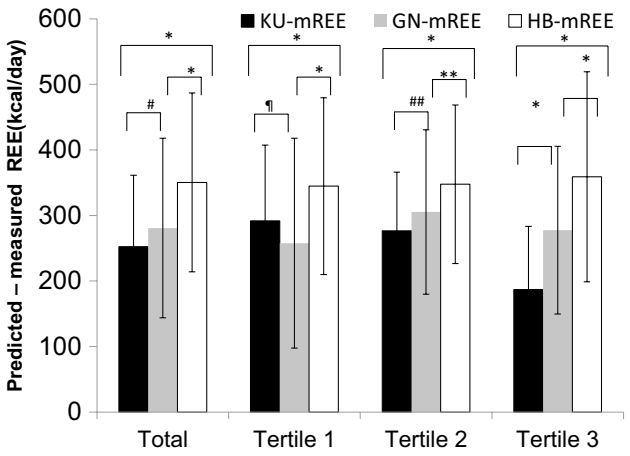

Fig. 3 Differences between predicted and measured resting energy expenditures (pREE—mREE) by each equation for each of the three tertiles. KU: Kyoto University equation, GN: Ganpule equation, HB: Harris-Benedict equation. *$P<0.001$, #$P=0.005$, ¶$P=0.023$, ##$P=0.018$, **$P=0.002$

Discussion

To evaluate for the factors, which made a difference between the pREE and mREE, basal metabolism was measured by indirect calorimetry under similar strict hospitalized conditions for diet and blood glucose control, and evaluated together with the data of the physiological backgrounds in the patients Japanese patients with T2DM without severe complications. mREE values were calculated by indirect calorimetry during admission, in the morning after an overnight fast, thereby excluding possible confounders, such as consumption of high amounts of alcohol, fat, and/or carbohydrates on the previous day.

The participants were of upper middle age (56 ± 11 years), slightly obese (BMI of 25.2 ± 3.6 kg/m^2), and had poorly controlled diabetes (HbA1c: 9.6 ± 1.6%) without ketosis.

Univariate analysis revealed that mREE was associated with factors previously reported (height, age, and body weight), and other factors that have not been previously reported (RBC count, hematocrit, and uric acid).

Consistent with the previous reports on non-diabetic [3, 5] and diabetic individuals [15], our data showed that various clinical factors such as height, body weight, age, and sex could affect mREE values in individuals with T2DM. However, multiple regression analysis by the stepwise method showed that body weight was the only variable significantly associated with mREE (Table 5).

The reported relationships between diabetic status and REE are inconsistent. Some studies have reported that diabetic status tends to decrease the accuracy in predicting REE [16], while other studies have shown that obese Japanese adults with T2DM have higher BMR than non-diabetic

Table 5 Results of regression analysis for the difference from predicted to measured resting energy expenditure ($n=49$)

Variables		KU -mREE			GN -mREE			HB -mREE		
		β	P	R^2	β	P	R^2	β	P	R^2
mREE		−0.40*	0.004	0.14	0.07	n.s.		0.09	n.s.	
Age (years)		−0.11	n.s.		−0.37*	0.010	0.13	−0.54*	<0.001	0.27
Sex (M=0, F=1)		−0.23	n.s.		−0.57*	<0.001	0.31	−0.36*	0.010	0.11
Height		0.15	n.s.		0.61*	<0.001	0.36	0.51*	<0.001	0.25
Body weight		0.04	n.s.		0.46*	0.001	0.20	0.50*	<0.001	0.23
Fat mass		−0.15	n.s.		0.08	n.s.		0.24	n.s.	
Fat-free Mass		0.15	n.s.		0.60*	<0.001	0.35	0.54*	<0.001	0.28
Muscle mass		0.15	n.s.		0.60*	<0.001	0.35	0.54*	<0.001	0.28
U-CPR		−0.17	n.s.		0.09	n.s.		0.06	n.s.	
γGTP		−0.29*	0.044	0.06	−0.17	n.s.		−0.14	n.s.	
Serum creatinine		0.15	n.s.		0.37*	0.01	0.114	0.22	n.s.	
Uric acid	$n=45$	−0.08	n.s.		0.23	n.s.		0.17	n.s.	
HDL-C	$n=44$	0.09	n.s.		−0.06	n.s.		−0.11	n.s.	
triglyceride	$n=45$	−0.24	n.s.		−0.01	n.s.		−0.01	n.s.	
WBC		−0.30*	0.037	0.07	−0.18	n.s.		−0.10	n.s.	
RBC		0.02	n.s.		0.29*	0.043	0.07	0.27	n.s.	
Hemoglobin		0.07	n.s.		0.32*	0.027	0.08	0.22	n.s.	
Hematocrit		0.08	n.s.		0.34*	0.016	0.10	0.25	n.s.	

KU predicted value of resting energy expenditure by Kyoto University equation, *GN* that of Ganpule equation, *HB* that of Harris-Benedict equation, *pREE* predicted resting energy expenditure, *mREE* measured resting energy expenditure, *M* male, *F* female, *U-CPR* c-peptide in 24-h urine collection, *γGTP* γ-glutamyl transpeptidase, *HDL-C* high density lipoprotein cholesterol, *WBC* white blood cell, *RBC* red blood cell

*$P<0.05$

adults [8]. However, the reported equations for pREE calculation among Japanese patients with T1DM and T2DM yielded low values when used for our patients in tertiles 2 and 3 (Figs. 2, 3) [6]. There are also conflicting results about the relationship between REE and glucose levels. In a previous outpatient study, REE was shown to correlate significantly with fasting plasma glucose levels (higher than 180 mg/dL) [17]. However, another report showed that fasting plasma glucose levels did not independently predict REE and that REE was unaffected by glucose levels and its control among mildly hyperglycemic obese hospitalized patients with diabetes (mean ± SD 171.0 ± 46.8 mg/dl) [18]. In this study, using single variate analysis, mild hyperglycemia (mean FPG level of 157 ± 50 mg/dl) tended to show a positive association with mREE. In our study, FPG was measured in a hospitalized condition and was, therefore, more controlled than previous reports [18]. Although factors such as consumption of high amounts of alcohol, fat, and/or carbohydrates on the previous day were excluded, body weight could disturb the relationship between FPG and mREE. Consistent with a previous report [19], HbA1c levels showed no correlation with REE. A long-term follow-up study of mREE changes in patients with T2DM is needed for further evaluation.

The findings on the relationship between insulin secretion levels and REE have also been inconsistent. For example, the CPR levels at 6 min after intravenous glucagon injection are negatively correlated with mREE [19], while fasting insulin levels, are positively correlated with energy expenditure [20]. The present study revealed a positive association between U-CPR and mREE, although this significant association disappeared after adjustment for body weight.

Uric acid levels also have been reported to be associated with total energy expenditure per unit of body weight in men [21]. Although sex-based differences in uric acid levels (1.6 mg/dl) have been reported previously [22], uric acid levels were associated with mREE among both men and women in our study. However, this association again disappeared after adjustment for body weight.

Hemoglobin showed an association with mREE, which disappeared after adjustment for body weight. However, RBC counts were found to be related to mREE independent of body weight only in men. Erythropoietin treatment and endurance training can both induce a significant increase in REE in humans [23], suggesting that bone-marrow functions can affect energy metabolism [24].

Our results indicate that the average mREE of 1099 ± 212 kcal/day was lower than pREE values calculated by the KU, GN, and HB equations. However, all pREE

Table 6 Results of multiple regression analysis for the difference from predicted to measured resting energy expenditure

A. KU -mREE			
Variables	β	P	R^2
Model 1			0.07
γGTP	−0.2	n.s.	
WBC	−0.2	0.037	
B. GN -mREE			
Variables	β	P	R^2
Model 2			0.36
Age	−0.12	n.s.	
Sex	0.26	n.s.	
Height	0.61	< 0.001	
Body weight	0.07	n.s.	
creatinine	0.11	n.s.	
RBC	0.01	n.s.	
C. HB -mREE			
Variables	β	P	R^2
Model 3			0.35
Age	−0.39	0.004	
Sex	0.02	n.s.	
Height	0.34	0.011	
Body weight	0.09	n.s.	

Model 1 was adjusted for γGTP, WBC which is significant in univariate analysis for the difference from KU pREE-mREE

Model 2 was adjusted for Age, Sex, Height, Body weight, creatinine, RBC which is significant in univariate analysis for the difference from GN pREE-mREE

Model 3 was adjusted for Age, Sex, Height, Body weight; which is significant in univariate analysis for the difference from HB -mREE

KU predicted value of resting energy expenditure by Kyoto University equation, *GN* that of Ganpule equation, *HB* that of Harris-Benedict equation, *mREE* measured resting energy expenditure, *γGTP* γ-glutamyl transpeptidase, *WBC* white blood cell, *RBC* red blood cell

values showed positive and significant correlation with mREE values ($r = 1.04$, 1.05 and 0.79, by GN, HB, and KU, respectively). The comparatively lower mREE values may be due to the clinical characteristics of the subjects, which were not factored into the model equations, and the therapeutic diet conditions as regularly controlled subjects.

The differences between calculated pREE and mREE were also distributed in tertiles that were statistically divided by mREE with a Gaussian distribution (Fig. 3). Therefore, the differences between tertiles were selected to evaluate the clinical manifestations that contribute to the differences between mREE and pREE values. When patients were divided into tertile groups based on mREE values, they showed different clinical characteristics according to the group (Table 2). Consistent with our hypothesis, mREE in participants within the lowest tertile showed the largest deviation from pREE. The most accurate equation for pREE previously reported also changed at the same lineage of the lowest tertile. The patients within the lowest tertile group (T1) of mREE were mostly (approximately 75%) women who were older than 50 years of age, short statured, and had low hemoglobin. Participants in the highest tertile group (T3) were tall and mostly men with high BMI.

In the T1 group, i.e., the lowest mREE group, pREE value derived using the GN equation was 44 kcal/day, which was the closest to the mREE value and was lower than the value derived using the KU equation, which was closest to mREE in other groups. This discrepancy may be because the KU equation does not consider height as a variable. Arciero et al. reported that height, weight, and menopausal status were associated with resting metabolic rate of healthy women > 50 years [25]. In the T1 group, 75% of the patients were women > 50 years. With only 9% of the subjects being women > 50 years in the T2 and T3 groups, the KU equation was the most appropriate option for subjects in these groups, which supports the findings from its development. However, the KU equation to examine older women or short patients with T2DM should be used with caution. Women over 50 years of age have significantly lower REE (904 ± 121 kcal/ day) than men over

Table 7 Clinical characteristics of the subjects aged over 50 years

	Male	Female	*P*-value
n =	24	15	
Age (years)	58.7 ± 5.8	64.0 ± 7.2	0.016
mREE (kcal/day)	1162 ± 174	904 ± 121	< 0.001
KU-mREE/mREE	0.23 ± 0.12	0.26 ± 0.10	0.449
GN-mREE/mREE	0.27 ± 0.11	0.21 ± 0.12	0.360
HB-mREE/mREE	0.29 ± 0.11	0.32 ± 0.11	0.101
height (cm)	168 ± 7	156 ± 5	< 0.001
BW (kg)	71.4 ± 9.9	57.4 ± 9.0	< 0.001
BMI	25.3 ± 3.1	23.5 ± 3.1	0.092
Body fat (%)	25.6 ± 4.3	33.3 ± 5.0	< 0.001
Fat mass (kg)	18.6 ± 5.3	19.5 ± 5.4	0.624
Fat-Free Mass (kg)	52.8 ± 5.2	37.9 ± 4.0	< 0.001
U-CPR (μg/day)	82.4 ± 52.0	56.5 ± 36.3	0.100
Uric acid (mg/dL) (Male 21/ Female 15)	5.8 ± 1.1	4.2 ± 0.9	< 0.001

mREE measured resting energy expenditure, *KU* predicted value of resting energy expenditure by Kyoto Univ. equation, *GN* that of Ganpule equation, *HB* that of Harris-Benedict equation, *BMI* body mass index, *U-CPR* c-peptide in 24-h urine collection, *BW* body weight

50 years of age (1162 ± 174 kcal/day) with 26% overestimation of pREE even by KU (Table 7). Figure 1 shows the age-dependent variations in mREE among the patients in this study. The mREE values in women were approximately 250 kcal lower than those in men. However, REE values derived using the KU equation showed a difference of only 125 kcal between the two sexes. For women in their late fifties, we must consider 250 kcal difference for the sex in the KU equation. The first possible reason for the sex-based difference in REE is the body composition. Men and women have similar resting energy expenditure when normalized to kilograms of lean body mass [26]. The second reason is the effect of menopause. Recently, follicle-stimulating hormone (FSH) was reported to be a key regulator of energy production [27]. Women in the postmenopausal age have increased FSH levels, which can lower their mREE values. Resting and total energy expenditure in postmenopausal compared with premenopausal women were reported to be approximately 80 kcal lower in healthy Caucasian subjects [28]. It is interesting that multiple regression analysis showed that the difference between pREE by KU and mREE negatively correlated with WBC, which is a factor that was not included in the equation for the prediction of REE. Height was significantly correlated with the difference between pREE by GN and mREE, and by HB and mREE. Postmenopausal women were reported to have low WBC [29], and at a high risk of osteoporosis, which causes short stature [30].

This finding also indicates that the mREE of postmenopausal women must be measured carefully especially in Japanese patients with diabetes.

When using the KU equation and factoring body weight and age to predict mREE, the results should be decreased by 125 kcal in postmenopausal women with T2DM. The result is in line with mREE from our previous study on postmenopausal women with T2DM without major complications ($n = 46$: 940.5 ± 155.7 kCal and 1130.1 ± 94.5 kCal, mREE and pREE, respectively, using KU) [31].

Although all enrolled patients were diagnosed with T2DM with no severe complications, our study has several limitations. First, this is a relatively small-sample cross-sectional study. Second, patients were in a single center.

In conclusion, mREE among Japanese patients with T2DM tends to be lower than calculated REE, especially in those with certain clinical characteristics. Particularly, women with T2DM, who were older than 50 years showed a lower REE. Factors associated with pREE-mREE were different among the predictions. Care must be taken when using predictive values to evaluate REE in patients with these clinical features.

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Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest to declare.

Human and animals rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration, and the study protocol complied with the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study design was approved by the Ethics Committee of the Tokyo Women's Medical University School of Medicine (9 May, 2017 No. 3829R).

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