ORIGINAL ARTICLE

Fat-free mass and calf circumference as body composition indices to determine non-exercise activity thermogenesis in patients with diabetes

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ABSTRACT

Aims/Introduction: To investigate the clinical and anthropometrical parameters that are associated with non-exercise activity thermogenesis that is composed of basal energy expenditure (BEE) and diet-induced thermogenesis (DIT) in patients with diabetes.

Materials and Methods: Body composition was assessed using bioelectrical impedance, and BEE and DIT were measured using indirect calorimetry in 40 Japanese patients with diabetes.

Results: BEE correlated positively with bodyweight, body mass index, fat mass, and fat-free mass, and correlated negatively with age in both men and women. In multivariate logistic regression analysis, BEE correlated positively with both fat mass and fat-free mass independently of sex and age. In addition, DIT correlated positively with bodyweight, body mass index, fat mass and fat-free mass, and correlated negatively with age in women, but not men. Fat-free mass contributed to DIT at least partly, and an aging-related decrease in DIT was observed. The best anthropometric parameter that reflected fat mass and fat-free mass was hip circumference (HC) and calf circumference (CC), respectively, in both men and women. Indeed, both HC (men $\beta = 0.600$, $P < 0.001$; women $\beta = 0.752$, $P < 0.001$) and CC (men $\beta = 0.810$, $P = 0.012$; women $\beta = 0.821$, $P = 0.002$) were correlated with BEE independently of age and sex. In addition, CC ($\beta = 0.653$, $P = 0.009$), but not HC was correlated with DIT significantly only in females, independently of age.

Conclusions: HC reflects fat mass and was positively associated with BEE, but not with DIT. In contrast, CC reflects fat-free mass, and was positively associated with BEE in both men and women, and with DIT in women.

INTRODUCTION

Obesity is caused by perturbations in the balance between energy intake and expenditure. Daily energy expenditure consists of three components: basal energy expenditure (BEE), diet-induced thermogenesis (DIT) and the energy expended by physical activity, which account for ~60, 15 and 25% of the total amount of energy ingested over 24 h, respectively[1]. Of these, BEE and DIT are responsible for unconscious non-exercise activity thermogenesis (NEAT), and therefore

could be therapeutic targets for obesity-associated diseases, such as type 2 diabetes. BEE can be calculated from age, height and bodyweight using the Harris–Benedict equation[2]. More specifically, it was reported that fat-free mass is the main body predictor of BEE, whereas fat mass is poorly related to BEE in healthy non-pregnant adults[3]. Age has an important effect on body composition, because the decrease in lean body mass with aging is the most relevant change that leads to a reduction in BEE. In contrast, DIT is influenced by nutritional components[4,5], and the DIT values reported for individual nutrients are 0–3% for fat, 5–10% for carbohydrates, 20–30% for protein[6]

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and 10–30% for alcohol[7]. However, whether and how body composition and insulin resistance status affect DIT remains unclear, particularly in patients with diabetes.

In the present study, we investigated the clinical and anthropometrical parameters associated with BEE and DIT in patients with diabetes.

MATERIALS AND METHODS

Participants

A total of 40 inpatients with diabetes (type 2 : type 1 = 37:3) who were admitted to the Kanazawa University Hospital, Kanazawa, Japan, between 2011 and 2013 were enrolled in the study. Of the 40 diabetic participants, 23 (58%) were treated with antidiabetic agents, and detailed information is shown in Table S1. Furthermore, all patients received dietary and exercise therapies. The current study was an integrated subanalysis of three separate trials approved by the ethics committee at Kanazawa University Hospital and registered with the University Hospital Medical Information Network Clinical Trials Registry (Nos. 000008369, 000010353 and 000010407). Informed consent was obtained from all participants. All patients were diagnosed according to criteria established by an expert committee on the diagnosis and classification of diabetes mellitus[8].

Patient eligibility

All eligible participants were inpatients aged 18–80 years with diabetes. The exclusion criteria were as follows: (i) treatment with the glucagon-like-peptide-1 (GLP-1) analogs within the 4 weeks before the study; (ii) treatment with glucocorticoids; (iii) uncontrolled hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg); (iv) a significant medical history and/or malignancy; (v) severe complications and problems not suitable for evaluating NEAT; and (vi) pregnant or lactating women.

Biochemical parameters

Blood samples were collected from all participants after an 8-h fast. Samples were centrifuged immediately, and plasma and serum samples were stored at −20°C until analysis. Glucose was measured using a standard glucose oxidase method. Total cholesterol, high-density lipoprotein cholesterol and triglycerides were measured enzymatically using a chemical analyzer (Hitachi 747, Daiichi, Tokyo, Japan). Fasting serum insulin levels were determined using chemiluminescence, and glycosylated hemoglobin was measured using immunoturbidimetry.

The quantitative insulin sensitivity check index (a measure of insulin sensitivity) was calculated by logarithmic transformation using the following formula[9]:

Table 1 | Correlations between basal energy expenditure or diet-induced thermogenesis, and body composition parameters

	Basal energy expenditure						Diet-induced thermogenesis					
	All		Men		Women		All		Men		Women	
	R	P	R	P	R	P	R	P	R	P	R	P
Age (years)	−0.669	<0.001	−0.643	0.002	−0.698	<0.001	−0.511	<0.001	−0.194	0.400	−0.690	0.001
Bodyweight (kg)	0.897	<0.001	0.822	<0.001	0.937	<0.001	0.421	0.007	−0.007	0.977	0.597	0.007
Body mass index (kg/m^2)	0.762	<0.001	0.695	<0.001	0.877	<0.001	0.343	0.030	−0.102	0.660	0.511	0.025
Fat mass (kg)	0.737	<0.001	0.727	<0.001	0.905	<0.001	0.340	0.032	−0.097	0.677	0.521	0.022
Fat-free mass (kg)	0.649	<0.001	0.846	<0.001	0.886	<0.001	0.314	0.049	0.083	0.722	0.726	<0.001
Total protein (g/dL)	0.310	0.051	0.152	0.510	0.472	0.041	0.009	0.956	−0.279	0.221	0.265	0.273
Total cholesterol (mg/dL)	0.004	0.979	0.094	0.686	−0.024	0.924	−0.152	0.350	0.080	0.729	−0.261	0.280
Triglyceride (mg/dL)	−0.169	0.296	0.137	0.554	−0.225	0.355	−0.105	0.519	−0.057	0.805	−0.130	0.597
HDL cholesterol (mg/dL)	−0.037	0.822	−0.265	0.246	0.067	0.787	−0.247	0.125	−0.024	0.917	−0.364	0.125
Aspartate aminotransferase (U/L)	0.339	0.032	−0.044	0.849	0.472	0.041	0.348	0.028	−0.249	0.277	0.572	0.010
Alanine aminotransferase (U/L)	0.225	0.163	−0.177	0.444	0.360	0.130	0.352	0.026	−0.418	0.060	0.637	0.003
Lactate dehydrogenase (U/L)	0.396	0.012	0.233	0.310	0.516	0.029	0.076	0.647	−0.281	0.216	0.327	0.185
γ-Glutamyl transpeptidase (IU/L)	−0.153	0.347	−0.262	0.252	−0.095	0.700	0.166	0.307	0.090	0.699	0.219	0.369
Creatinine (mg/dL)	0.146	0.368	0.314	0.165	−0.143	0.560	0.046	0.776	0.123	0.596	−0.149	0.544
Immunoreactive.insulin (mU/mL)	0.172	0.288	0.093	0.689	0.316	0.187	0.057	0.727	−0.222	0.334	0.430	0.066
Fasting plasma glucose (mg/dL)	−0.213	0.187	−0.114	0.622	−0.289	0.230	−0.150	0.355	−0.263	0.249	−0.115	0.640
Quantitative insulin sensitivity check index	−0.417	0.007	−0.444	0.044	−0.425	0.690	−0.113	0.488	0.071	0.760	−0.202	0.407
HbA1c (%)	−0.264	0.110	−0.136	0.567	−0.404	0.096	−0.440	0.006	−0.312	0.180	−0.631	0.005
Thyroid-stimulating hormone (mU/mL)	0.289	0.074	0.063	0.787	0.466	0.051	0.006	0.971	−0.134	0.562	0.118	0.641
Free triiodothyronine (pg/mL)	0.477	0.053	0.723	0.043	0.507	0.163	0.355	0.162	−0.084	0.843	0.785	0.012
Free thyroxine (ng/dL)	−0.048	0.773	−0.402	0.071	0.039	0.876	−0.106	0.520	−0.205	0.372	−0.085	0.736

HbA1c, glycated hemoglobin; HDL, high-density lipoprotein.

$$\frac{1}{(\log \text{fasting insulin } [\text{U/mL}] + \log \text{fasting glucose } [\text{mg/dL}])}$$

Anthropometric data

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at the umbilical level. Hip circumference (HC) was measured at the maximum protruding part of the buttocks at the level of the greater trochanter with the patient wearing minimal clothing and standing with feet together. Thigh circumference and calf circumference (CC) were measured on both sides at the root of the thigh, and at the maximum levels and at the greatest dimension of the calf, respectively, and the mean values were calculated. Measurements of the mid upper arm circumference and the tricep skinfold thickness at the same level were used to calculate arm muscle area[10].

Body composition was assessed by multifrequency bioelectrical impedance analysis using the Multi-Frequency Body Composition Analyzer BC-118D (Tanita Corporation, Tokyo, Japan), which has an eight-point footpad-style electrode arrangement. Participants stood in bare feet with the heel and toe of each foot in contact with the metal footpads, and with their arms hanging to each side holding the analyzer handgrips lightly. Multifrequency bioelectrical impedance analysis was reported to be more accurate than single-frequency bioelectrical impedance analysis using a dual energy X-ray absorptiometry, which is a gold standard method for assessing body composition[11].

Measurements of BEE and DIT

Energy expenditure was calculated by measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2) in recumbent participants at room temperature over a 10-min period by indirect calorimetry using a Minato AE-310s AERO-MONITOR (Minato Medical Science Company Ltd., Osaka, Japan). Energy expenditure was calculated from VO_2 and VCO_2 using the equation reported by de Weir[12].

After an overnight fast (12–14 h), the participants rested for 30 min in the supine position on a bed (07.00–07.30 h). Energy expenditure was measured for 10 min before (07.30–07.40 h), and 1 h (at 09.00 h) and 3 h (at 11.00 h) after the consumption of breakfast, and was presented as BEE, EE60 and EE180, respectively. The participants were given a meal providing 28 kcal/kg (standard bodyweight) derived from protein (20%), fat (20%) and carbohydrates (60%). Breakfast accounted for 30% of the energy provided per day. It was served at 08.00 h, and was consumed within 30 min in a sitting position. The participants refrained from exercise, and rested as much as possible during the 3-h energy expenditure measurement period. Generally, DIT peaks 1 h after the ingestion of food[13]. Indeed, EE180 was lower than EE60 in all study participants. Therefore, DIT was measured as the difference between EE60 and BEE.

Table 2 | The independent explanatory variables for basal energy expenditure or diet-induced thermogenesis

| Independent variables | Basal energy expenditure | | | | | | | | | Diet-induced thermogenesis | | | | | | | | |
| | All | | | Men | | | Women | | | All | | | Men | | | Women | | |
	β	t	P	β	t	P	β	t	P	β	t	P	β	t	P	β	t	P
Model 1																		
Sex	-0.444	-5.405	<0.001							-0.106	-0.635	0.529						
Age	-0.334	-4.180	<0.001	-0.461	-3.508	0.003	-0.267	-2.392	0.029	-0.428	-2.645	0.012	-0.248	-1.035	0.315	-0.583	-2.701	0.016
Fat mass	0.808	8.782	<0.001	0.583	4.438	<0.001	0.751	6.730	<0.001	0.199	1.067	0.293	-0.174	-0.726	0.477	0.186	0.861	0.402
Model 2																		
Sex	0.499	3.727	<0.001							0.126	0.597	0.554						
Age	-0.250	-2.139	0.039	-0.258	-1.876	0.077	-0.074	-0.425	0.677	-0.407	-2.204	0.034	-0.212	-0.768	0.452	-0.332	-1.345	0.197
Fat-free mass	0.849	5.667	<0.001	0.705	5.134	<0.001	0.831	4.763	<0.001	0.209	0.883	0.383	-0.033	-0.120	0.905	0.476	1.928	0.072

Model 1, adjusted for sex, age and fat mass; model 2, adjusted for sex, age and fat-free mass. Multiple regression was used for the analysis.

Statistical analysis

Normally distributed data were presented as means ± standard deviations, and the differences between the two groups were analyzed using Student's *t*-tests. Data that had an irregular distribution were presented as medians and ranges, and differences between the two groups were analyzed using Mann–Whitney *U*-tests. Relationships were determined using regression analyses, and *P*-values <0.05 were considered to show significance. Then, multivariate logistic regression analysis (forced entry method) was carried out using independent variables that achieved significance at $P < 0.05$ in univariate analysis. All statistical analyses were carried out using SPSS software, version 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Body composition parameters associated with BEE

The clinical anthropometry and biochemical characteristics of the study participants are shown in Table S1. There were no significant differences in BEE and DIT between the sexes. Because significant differences were observed regarding BMI, fat mass, fat-free mass and some blood parameters (such as creatinine, fasting plasma glucose, glycated hemoglobin [HbA1c] and free triiodothyronine), factors associated with BEE and DIT were analyzed separately in men and women.

Table 1 shows the results of the single correlation analyses between BEE or DIT and clinical/body composition parameters. No blood biochemical parameters were associated with BEE commonly in either men or women. BEE correlated positively with bodyweight, BMI, fat mass and fat-free mass, and correlated negatively with age in both men and women. As shown in Table 2, BEE correlated positively with both fat mass and fat-free mass independently of sex and age.

Body composition parameters associated with DIT

As shown in Table 1, no biochemical or anthropometrical parameters were associated with DIT in either men or women. In women, but not men, DIT correlated positively with bodyweight, BMI, fat mass and fat-free mass, and correlated negatively with age. In addition, age was correlated with DIT independently of fat mass in women: the higher the age the lower the DIT (Table 2). In contrast, fat-free mass tended to correlate with DIT ($P = 0.072$) after adjusting for age in women, whereas age did not correlate significantly with DIT after adjusting for fat-free mass in women.

Anthropometric parameters reflecting fat mass or fat-free mass

Because the body composition parameters, fat mass and fat-free mass, reflected BEE independently, we next screened for the anthropometric parameters that best reflected the body composition parameters in each sex. In single correlation analyses (Table 3), all the anthropometric parameters evaluated (waist circumference, HC, thigh circumference, CC and arm muscle area) correlated positively with fat mass and fat-free mass in both men and women. The anthropometric parameters that best reflected fat mass and fat-free mass were HC and CC in men and women, respectively (Table 3).

Association of HC and CC with BEE and DIT

Next, we investigated the significance of HC and CC for estimating BEE and DIT (Table 4). Similarly to fat mass and fat-free mass, both HC and CC were correlated with BEE independently of age and sex. In men, neither HC nor CC correlated with DIT. In women CC, but not HC, was correlated significantly with DIT independently of age.

Association of HbA1c with DIT

HbA1c was significantly higher in women than in men (Table S1). Univariate analysis showed that HbA1c negatively correlated with DIT, especially in women (Table 1). Therefore, we carried out multiple regression analyses as shown in Table S2. CC was still positively associated with DIT independently of HbA1c at least in women (Table S2). In contrast, HbA1c was negatively associated with DIT independently of sex, age (model 1–3), fat-free mass (model 2) and CC (model 3).

DISCUSSION

The factors associated with BEE have mainly been investigated in non-diabetic humans. Age[14], fat mass[15,16], fat-free mass/ muscle mass[17–19], sympathetic nerve activity[20] and thyroid hormone[21] are all associated with BEE. However, these

Table 3 | Correlations between anthropometric parameters and fat mass or fat-free mass

	Fat mass						Fat-free mass					
	All		Men		Women		All		Men		Women	
	R	P	R	P	R	P	R	P	R	P	R	P
Waist circumference (cm)	0.954	<0.001	0.959	<0.001	0.975	<0.001	0.343	0.030	0.869	<0.001	0.780	<0.001
Hip circumference (cm)	0.966	<0.001	0.961	<0.001	0.983	<0.001	0.364	0.021	0.917	<0.001	0.847	<0.001
Thigh circumference (cm)	0.921	<0.001	0.838	<0.001	0.927	<0.001	0.298	0.062	0.833	<0.001	0.845	<0.001
Calf circumference (cm)	0.908	<0.001	0.917	<0.001	0.982	<0.001	0.600	0.004	0.950	<0.001	0.935	<0.001
Arm muscle area (cm^2)	0.797	<0.001	0.759	<0.001	0.897	<0.001	0.465	0.002	0.794	<0.001	0.702	<0.001

Table 4 | The independent explanatory variables for basal energy expenditure or diet-induced thermogenesis

		Basal energy expenditure									Diet-induced thermogenesis								
	Independent variables	All			Men			Women			All			Men			Women		
		β	t	P	β	t	P	β	t	P	β	t	P	β	t	P	β	t	P
Model 1	Sex	−0.281	−3.474	0.001							−0.080	−0.536	0.595						
	Age	−0.256	−3.280	0.002	−0.434	−3.318	0.004	−0.217	−1.576	0.135	−0.393	−2.356	0.024	−0.217	−0.883	0.389	−0.559	−2.430	0.027
	HC	0.737	7.722	<0.001	0.600	4.595	<0.001	0.752	5.462	<0.001	0.231	1.307	0.200	−0.068	−0.275	0.787	0.205	0.893	0.385
Model 2	Sex	−0.132	−1.134	0.273							−0.095	−0.541	0.595						
	Age	−0.144	−1.009	0.327	−0.048	−0.198	0.810	−0.138	−0.754	0.473	−0.423	−1.962	0.066	−0.365	−0.882	0.407	−0.343	−1.798	0.110
	CC	0.809	5.734	<0.001	0.810	3.371	0.012	0.821	4.486	0.002	0.391	1.835	0.084	−0.163	−0.395	0.705	0.653	3.425	0.009

Model 1, adjusted for sex, age and HC; model 2, adjusted for sex, age and CC Multiple regression was used for the analysis. HC, hip circumference; CC, calf circumference.

relationships remain unclear in humans with diabetes, who are associated with an increased risk of sarcopenia[22]. In the present study, we observed that BEE was strongly associated with age, BMI, fat mass and fat-free mass in both men and women with diabetes. Notably, BEE was associated with both fat mass and fat-free mass independently of age and sex. Fat-free mass represents the weights of muscle, bone and internal organs. In patients with type 2 diabetes, bone mass is either normal or increased, whereas bone quality is impaired. Thus, the differences in skeletal muscle mass could be attributed mainly to variations in fat-free mass among the study participants. Indeed, BEE results from oxygen consumption largely in skeletal muscle[23]. Recently, it was recognized that skeletal muscle might play a role in preventing obesity-associated metabolic abnormalities, in part by secreting bioactive peptides named myokines[24]. For example, the myokines interleukin-6 and irisin upregulate uncoupling protein-1 in adipose tissue, and thereby increase heat production[24].

Although the exact source of DIT remains unclear, postprandial metabolism mainly involves oxygen consumption in the gastrointestinal tract and liver[23]. Studies in non-diabetic humans suggested that meal size[25–27] and nutritional components[4–7] were associated with DIT. In the present study, DIT was significantly associated with age, BMI, fat mass and fat-free mass in women, but not men. In women, DIT was significantly associated with age independently of fat mass. In addition, DIT tended to be associated with fat-free mass after adjustment for age, but was not associated with age after adjustment for fat-free mass. This suggests that fat-free mass at least partly contributes to DIT and the aging-related decrease in DIT.

Based on these findings, we conclude that fat-free mass, possibly skeletal muscle mass, is closely associated with BEE and partly with DIT, and therefore, might protect against obesity. Unfortunately, estimating fat-free mass requires an electrical impedance method or dual energy X-ray absorptiometry.

Here, we investigated the relationship between anthropometric parameters and fat-free mass. The best anthropometric parameter reflecting fat-free mass was CC in both men and women. Recently, CC was used as an index of the nutritional state and weight of bedridden elderly[28]. Furthermore, an association between CC and insulin resistance or carotid atherosclerosis was reported[29].

The limitations of the present study were as follows. First, BEE and DIT were not measured in a respiration chamber, because one was not available in our hospital, unfortunately. Second, because there was a significant difference in BMI between women and men, the present results should be confirmed in subjects with various BMI ranges in the future. Third, HbA1c was significantly higher in women than in men. However, CC was still positively associated with DIT independently of HbA1c at least in women. Of note, HbA1c was negatively correlated with DIT, independently of sex, age, fat-free mass and CC. It could be possible that poor glycemic control meaningfully impairs DIT, which might lead to further obesity. A

large-scale study including both non-diabetic and diabetic people will test this hypothesis.

The present study has shed light on the significance of fat-free mass as a determinant of NEAT. In future health examinations, in addition to waist circumference and/or HC measurements, CC might be used as an index of skeletal muscle mass contributing to obesity resistance through increases in NEAT. A prospective intervention study is required to confirm the possibility that increased fat-free mass and CC protect against obesity and related metabolic abnormalities.

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Clinical anthropometry and biochemical characteristics of the study participants.

Table S2 | The independent explanatory variables for diet-induced thermogenesis.