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Correlation of body muscle/fat ratio with insulin sensitivity using hyperinsulinemic-euglycemic clamp in treatment-naïve type 2 diabetes mellitus

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ABSTRACT

Aims: Fat deposition and obesity are crucial pathological components of diabetes mellitus (DM). In clinical practice, assessment of insulin resistance is important. We hypothesized that body muscle and fat composition might be a key factor for insulin resistance in patients with type 2 DM.

Methods: Subjects included 61 untreated DM patients. Hyperinsulinemic-euglycemic clamp examination was performed to calculate the M/I value as the insulin resistance reference indicator. Elementary body composition was measured by impedance analysis using InBody770.

Results: Simple regression analysis showed that total muscle quantity/total fat quantity ratio (muscle/fat) was significantly correlated with M/I value ($B = 0.806$, $P < 0.001$). The regression equation was M/I value = 3.6934 × (muscle/fat ratio) + 0.0347 ($R^2 = 0.6503$, $P < 0.001$). Multivariate logistic regression analysis showed that muscle/fat ratio was independently and significantly associated with insulin resistance, defined by M/I value <9 (odds ratio, 0.89; 95% confidence interval, 0.80–0.99, $P = 0.04$). With receiver operating curve analysis, the cutoff value of muscle/fat ratio for insulin resistance was 2.40 and area under the curve was 0.87 (sensitivity 91% and specificity 76%, $P < 0.001$), indicating that muscle/fat ratio was significantly effective for predicting insulin resistance in treatment-naïve DM. The result could provide a possible estimation of the M/I value using the regression equation M/I value = 2.5438 × (muscle/fat ratio) + 48.6194 × QUICKI − 13.6522 ($R^2 = 0.7012$).

Conclusion: In treatment-naïve DM, the muscle/fat ratio, assessed by InBody770 is clinically useful for evaluating the presence of insulin resistance in daily clinical practice.

1. Introduction

Obesity and fat deposition are closely involved in the pathogenesis of diabetes mellitus (DM) because of their effect on insulin resistance. Insulin resistance is a concept derived from blood glucose levels that cannot be decreased even after large doses of insulin are administered [1]. Insulin resistance is affected by adipocytokines secreted from fat tissue,

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systemic cytokines, and many other factors, and it is generally accepted that it is associated with obesity [2]. The number of obese people is increasing globally [3], and considerable attention is being paid to the metabolic syndrome, the main condition of insulin resistance. It has been reported that metabolic syndrome increases the risk of cardiovascular disease by 1.53–2.18-fold, and overall mortality risk increases by 1.27–1.60-fold [4–6]. Thus, the assessment of insulin resistance is important in daily clinical practice.

The three principal methods for assessing insulin resistance are the glucose clamp technique [7], the 1985 published method of homeostatic model assessment (HOMA) [8], and the minimal model method [9]. Since then, numerous other methods and clinical indicators have been proposed. Today, the euglycemic clamp technique is considered the gold standard [10–12] for assessing insulin resistance, and the precision of other techniques should be evaluated against this method. Body composition analyzers using bioelectrical impedance analysis with InBody770 are used in clinical practice and research facilities in over 70 countries, and they are highly portable, non-invasive, and simple to use. In this study, we tested our hypothesis that body composition determines insulin resistance in patients with treatment-naïve type 2 DM.

2. Materials and methods

2.1. Subjects and protocol

Patients with untreated type 2 DM who visited the Diabetes Care Center of Jinnouchi Hospital between June 2014 and December 2015 were enrolled. Those with already treated diabetes, severe uncontrolled diabetes, diabetic ketoacidosis that needed immediate treatment, uncontrolled severe hypertension, and those who could not remain standing to have an elementary body composition tests were excluded. Hyperinsulinemic-euglycemic clamp examinations were performed to calculate insulin sensitivity index (M/I) values as the reference indicator of insulin resistance. The M/I value was compared with elementary body composition and various clinical parameters. All tests were conducted within 1 week. Written informed consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki and the study protocol was approved by the Human Ethics Review Committee of Jinnouchi Hospital.

2.2. Hyperinsulinemic-euglycemic clamp examination

Insulin sensitivity was evaluated by a hyperinsulinemic-euglycemic clamp examination using an artificial pancreas (Nikkiso STG-55, Tokyo, Japan), as reported previously [13]. Insulin was given as intravenous loading doses (starting from 4.77 mU/kg/min and were gradually decreased to 1.67 mU/kg/min; under these conditions, the estimated plasma insulin concentration would be about 100 mU/L) over 10 min followed by a continuous infusion at 1.5 mU/kg/min for 120 min. Plasma glucose concentrations were maintained at 5.5 mmol/L by a variable infusion of 10% glucose. Blood insulin concentration at steady state was measured at the time of termination of the hyperinsulinemic-euglycemic clamp examination (I value). Because of variations in the insulin clearance rate for each patient, it has been reported that the actual blood insulin concentrations during hyperinsulinemic -euglycemic clamp test are different from the calculated insulin levels [14]. To correct for the effect of the variability in insulin concentrations among individual patients, we adopted an M/I value as an index of insulin sensitivity, which was a value calculated by dividing the M value by the steady-state serum insulin value (I) in this study. This value indicates the glucose utilization per 1 unit of blood insulin and is a good index representing tissue insulin sensitivity [7].

2.3. Measurement of body fat and muscle composition

Elementary body composition was measured using a direct segmental multi-frequency bioelectrical impedance analyzer (InBody770; Biospace, Seoul, Korea). This analyzer processes 30 impedance measurements by using six different frequencies (1, 5, 50, 250, 500, 1000 kHz) at each of five segments of the body (right arm, left arm, trunk, right leg, left leg) and 15 reactance measurements using tetrapolar 8-point tactile electrodes at three different frequencies (5, 50, 250 kHz) at each of five segments of the body (right arm, left arm, trunk, right leg, left leg) [15,16]. The body composition analysis was conducted within 1 week of the hyperinsulinemic-euglycemic clamp examination.

2.4. Blood sampling and measurement of clinical parameters

Fasting blood samples were collected from the antecubital vein in the morning. Blood analyses were conducted in the hospital laboratory for the measurement of blood glucose, glycated hemoglobin (HbA1c), lipids, creatinine and insulin. The quantitative insulin sensitivity check index (QUICKI) was calculated using the formula: QUICKI = 1/(log(fasting insulin μU/mL) + log(fasting glucose mg/dL)). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR = fasting plasma glucose (mmol/L) × fasting plasma insulin (μIU/ml)/22.5.

2.5. Statistical analysis

The Shapiro–Wilk test was used to assess the normal distribution of continuous data. Data were expressed as mean ± SD, whereas those with skewed distributions were expressed as the median value with interquartile range. Categorical data were presented as frequencies and percentages. Differences between two groups were tested with Fisher's exact test for categorical variables. Differences in continuous variables were analyzed by the unpaired *t*-test or Mann–Whitney U test, as appropriate. Calculation of Spearman's partial correlation coefficient was used to examine the relationships between the independent variables used in the multivariate analysis. As this time, parameters other than the two pairs for calculating the correlation coefficient were used for all control variables. Simple regression analysis was used to eval-

uate the association between the M/I value and the parameters of InBody770 and laboratory data. Multivariate regression analysis was conducted using the significant outcomes by simple regression analysis. In this study, simple regression analysis showed that the M value was strongly and significantly correlated with the M/I value ($R = 0.884$, $P < 0.001$). A previous report demonstrated an M value of 5.7 as the cutoff for the presence of insulin resistance [17]; the M/I value of 9.0 was equivalent to the M value of 5.7 in the present study. Thus, we defined the cutoff value of M/I < 9.0 for the existence of insulin resistance in the logistic regression and receiver-operating characteristic (ROC) analyses.

Simple regression analysis was used to evaluate the association between the M/I value and the parameters of InBody770 and laboratory data. We performed single logistic regression analysis to consider the influence of parameters of InBody770 and laboratory data on insulin resistance (M/I value < 9.0). Next, for performing linear regression analysis and logistic regression analysis, we constructed multivariate models, QUICKI and HOMA-IR, consisting of fasting plasma glucose and fasting blood insulin factors. QUICKI showed the strongest correlation by simple linear regression analysis for the factors, fasting plasma glucose and fasting blood insulin. Therefore, to prevent multicollinearity, we excepted HOMA-IR, fasting plasma glucose, and fasting blood insulin. Furthermore, regarding BMI calculated from body measurements, weight, waist circumference, and muscle/fat ratio, to avoid multicollinearity, goodness of fit was extracted from the highest factor to M/I value. For the fit index, we used Nagelkerke R^2. Nagelkerke R^2 is a method for checking the most suitable equation for logistical regression analysis. We selected a significant factor of simple linear regression and multiple linear regression, avoiding multicollinearity. After multiple linear regression analysis was performed, we calculated VIF and checked actual multicollinearity. Regarding multivariate logistic regression analysis, we built models according to the following methods. Model 1: muscle/fat for which goodness of fit to M/I value was optimal for the body measurement index, and adjusted for age and sex. Model 2: muscle/fat, and HDL and TG lipid items, which were significant factors at simple logistic regression. Model 3: muscle/fat, and HbA1c and QUICKI as carbohydrate metabolism indices. Model 4: comprehensive metabolism model comprising muscle/fat, and QUICKI as carbohydrate metabolism indices and, HDL and TG as lipid items.

We used ROC curve analysis to calculate the area under the curve (AUC) and the cutoff value. A $P < 0.05$ denoted statistical significance. Statistical analyses were performed using SPSS version 23 (SPSS Inc, Tokyo, Japan).

3. Results

3.1. Subjects

A total of 64 patients with untreated type 2 DM were enrolled and three patients (one each ketoacidosis, impossible to perform InBody770, and uncontrolled hypertension) were excluded. Table 1 shows the baseline characteristics of the enrolled patients. Subjects included 15 (24.6%) with hypertension and 25 (41.0%) with dyslipidemia. Patients were treated with angiotensin II receptor blockers (ARBs) ($n = 5$, 8.2%), and statins ($n = 5$, 8.2%). Overall, 45 subjects (73.8%) were not taking any medication. All subjects had untreated type 2 DM and 31 patients (50.8%) demonstrated high levels of HbA1c > 8.4% (68 mmol/mol).

3.2. Results of body composition analysis

Analysis of elementary body composition by InBody770 showed that total muscle quantity was 45.3 ± 9.7 kg, total fat quantity was 17.6 (12.1 to 26.2) kg, body fat percentage was 27.7 ± 9.7%, and muscle/fat ratio was 2.72 (1.77 to 3.34).

3.3. Results of hyperinsulinemic-euglycemic clamp examination

Hyperinsulinemic-euglycemic clamp examination showed that the M-value was 6.29 (4.26 to 8.76) (mg m^{-2} min^{-1}) and the M/I value was 9.28 (6.22 to 13.98) (mg m^{-2} min^{-2} μIU^{-1} · mL). Patients in the insulin resistance group (M/I value <9.0) had significantly higher levels of weight, BMI, waist circumference, body muscle quantity, body fat quantity, body fat percentage, fasting blood insulin, HOMA-IR, total cholesterol, and triglycerides compared with the non-insulin resistance group (M/I value $\geqslant$9.0). Patients in the insulin resistance group showed significantly lower levels of muscle/fat ratio, QUICKI, and HDL-cholesterol compared with the non-insulin resistance group.

3.4. Simple and multivariable regression analysis for M/I value

First, we showed partial correlation coefficients of all possible combinations for multiple independent variables in supplementary tables (Supplemental Tables 1 and 2). Table 2 shows the results of simple regression analysis for M/I value. Weight, BMI, waist circumference, body muscle quantity, body fat quantity, body fat percentage, fasting plasma glucose, fasting plasma insulin, HOMA-IR, and triglycerides showed a significant negative relationship with M/I value. Muscle/fat ratio and QUICKI, HDL-cholesterol showed a significant positive relationship with M/I value. Height, HbA1c and triglyceride were not significant factors. The standardized partial regression coefficient of the muscle/fat ratio was 0.806 ($P < 0.001$), which was the largest value among the anthropometric factors and InBody770 parameters. The regression equation was M/I value = 3.6934 × (muscle/fat ratio) + 0.0347 ($R^2 = 0.6503$, $P < 0.001$). We analyzed multivariable regression analysis using entries that displayed more significant results in a simple regression analysis as independent factors, avoiding multicollinearity. Table 3 shows the results of multivariable regression analysis for the M/I value. The muscle/fat ratio and QUICKI were significant and independent factors (muscle/fat ratio: $\beta = 0.5291$, $P < 0.0001$; QUICKI: $\beta = 0.2964$, $P = 0.0071$). The regression equation was M/I value = 2.5438 × (muscle/fat ratio) + 48.6194 × QUICKI – 13.6522 ($R^2 = 0.7012$).

Table 1 – Baseline clinical characteristics of the study subjects (N = 61).

		M/I value <9 (n = 29)	9 ⩽ M/I value (n = 32)	P value
Male (%)	39 (63.9)	16 (55.2)	23 (78.6%)	P = 0.19
Age (years)	55.2 ± 12.7	52.2 ± 12.9	57.9 ± 12.2	P = 0.08
Height (cm)	163.0 ± 8.26	163.5 ± 9.5	162.5 ± 7.1	P = 0.65
Weight (kg)	67.4 ± 16.3	76.7 ± 14.4	59.0 ± 13.1	P < 0.001
BMI (kg/m^2)	25.1 ± 5.0	28.3 ± 4.2	22.1 ± 6.8	P < 0.001
Waist circumference (cm)	88.7 ± 13.4	97.4 ± 10.6	80.8 ± 10.7	P < 0.001
Muscle quantity (kg)	45.3 ± 9.7	47.6 ± 10.4	43.2 ± 8.6	P = 0.07
Body fat quantity (kg)	17.6 (12.1–26.2)	26.2 (19.3–30.9)	12.7 (8.1–16.8)	P < 0.001
Body fat percentage (%)	27.7 ± 9.7	34.2 ± 8.2	21.9 ± 6.8	P < 0.001
Muscle quantity/body fat quantity	2.72 (1.77–3.34)	1.83 (1.41–2.37)	3.12 (2.75–4.94)	P < 0.001
Hypertension, n (%)	15 (24.6)	7 (21.9)	8 (27.6)	P = 1.00
Dyslipidemia, n (%)	25 (41.0)	12 (16.4)	13 (21.3)	P = 1.00
Angiotensin II receptor blocker	5 (8.2)	3 (10.3)	2 (6.25)	P = 0.56
Statin	5 (8.2)	1 (3.4)	4 (12.5)	P = 0.20
Hemoglobin A1c (mmol/mol)	69 (52–89)	70 (56–88)	61 (50–91)	P = 0.53
Fasting plasma glucose (mmol/L)	8.4 (6.8–10.1)	8.4 (7.4–10.7)	8.0 (6.6–9.3)	P = 0.18
Fasting blood insulin (pmol/L)	38.0 (20.9–56.9)	56.2 (46.8–94.3)	25.9 (15.1–35.3)	P < 0.001
QUICKI	0.35 ± 0.05	0.32 ± 0.03	0.38 ± 0.28	P < 0.001
HOMA-IR	2.07 (0.92–3.34)	3.06 (2.30–4.73)	1.12 (0.68–2.01)	P < 0.001
Total cholesterol (mmol/L)	5.3 (4.6–5.8)	5.7 (5.1–6.2)	5.0 (4.4–5.5)	P = 0.042
HDL-cholesterol (mmol/L)	1.24 (1.11–1.55)	1.14 (1.06–1.24)	1.42 (1.25–1.66)	P = 0.0056
LDL-cholesterol (mmol/L)	3.4 ± 0.8	3.5 ± 0.8	3.2 ± 0.8	P = 0.13
Triglycerides (mmol/L)	1.25 (0.80–1.95)	1.93 (1.25–2.35)	0.91 (0.68–1.26)	P = 0.0004
eGFR (mL/min/1.73 m^2)	75.8 ± 16.3	73.2 ± 17.3	78.1 ± 15.3	P = 0.25

Data are represented as the mean ± SD, median [25th to 75th percentile range], or number (%). BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low-density lipoprotein; QUICKI, Quantitative insulin sensitivity check index.

Table 2 – Results of simple regression analysis for the M/I ratio.

	B	R^2	P value	F test
Weight (kg)	−0.633	0.3900	P < 0.0000	P < 0.0000
Height (cm)	−0.156	0.0244	P = 0.2295	P = 0.2295
BMI (kg/m^2)	−0.699	0.4890	P < 0.0000	P < 0.0000
Waist circumference (cm)	−0.744	0.5534	P < 0.0000	P < 0.0000
Muscle quantity (kg)	−0.297	0.0885	P < 0.0199	P = 0.0199
Body fat quantity (kg)	−0.754	0.5692	P < 0.0000	P < 0.0000
Body fat percentage (%)	−0.744	0.5535	P < 0.0000	P < 0.0000
Muscle quantity/Body fat quantity	0.806	0.6503	P < 0.0000	P < 0.0000
HbA1c (%)	−0.218	0.0474	P = 0.0920	P = 0.0920
Fasting plasma glucose (mmol/L)	−0.263	0.0690	P = 0.0409	P = 0.0409
Fasting blood insulin (pmol/L)	−0.476	0.2261	P = 0.0001	P = 0.0001
QUICKI	0.751	0.563	P < 0.0001	P < 0.0001
HOMA-IR	−0.511	0.2613	P < 0.0000	p < 0.0000
HDL-cholesterol (mmol/L)	0.332	0.1100	P = 0.009	P = 0.009
Triglycerides (mmol/L)	−0.402	0.1612	P = 0.0013	P = 0.0013
eGFR (mL/min/1.73 m^2)	0.155	0.0241	P = 0.2325	P = 0.2325

BMI, body mass index; GFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, Quantitative insulin sensitivity check index.

3.5. *Logistic regression analysis for insulin resistance*

Simple logistic regression analysis was performed for insulin resistance (M/I value <9.0), as shown in Table 4. Weight, BMI, waist circumference, body fat quantity, body fat percentage, muscle/fat ratio, fasting plasma glucose, fasting blood insulin, QUICKI, HOMA-IR, total cholesterol, HDL-cholesterol, and triglycerides were significantly associated factors for insulin resistance in the logistic regression analysis. Because the standard regression coefficient of muscle/fat ratio was highest in simple regression (Table 2), muscle/fat ratio was included in the multivariate logistic regression analysis from the parameters of InBody770. Additionally, the NagelkerkeR^2 model fit test was performed to prevent multicollinearity (NagelkerkeR^2: weight 0.406, BMI 0.506, waist circumference 0.512, muscle/fat ratio 0.533). The NagelkerkeR^2 measurement of the muscle/fat ratio was the highest among these parameters. Four forced inclusion multivariate models were

Table 3 – Results of multivariable regression analysis for the M/I ratio.

	Unstandardized coefficients		Standardized coefficients			
	B	SEM	β	Lower bound	Upper bound	P value
Constant	−12.6422	4.7709		−22.1996	−3.0849	0.0104
Muscle quantity/body fat quantity	2.4236	0.4811	0.5291	1.4598	3.3873	0.0000
QUICKI	42.6959	15.2648	0.2964	12.1169	73.2750	0.0071
HDL-cholesterol	0.0456	0.0415	0.0839	−0.0375	0.1288	0.2763
Triglycerides	−0.0062	0.0037	−0.1290	−0.0136	0.0012	0.0997

HDL, high-density lipoprotein; QUICKI, Quantitative insulin sensitivity check index. $R^2 = 0.7288$. F test for a linear relationship was 37.6263 with $P < 0.001$. Variance inflation factors (VIF is an index used to detect the multicollinearity between exploratory variables): muscle quantity/body fat quantity 2.28; QUICKI 2.32; HDL-cholesterol 1.20; triglycerides 1.23.

constructed to determine the significance of the muscle/fat ratio as a continuous variable. Table 4 shows the results of multivariable logistic analysis for insulin resistance. The muscle/fat ratio was independently and significantly associated with insulin resistance: Model 1 [odds ratio (OR) 0.79, 95% confidence interval (CI) 0.68–0.91; $P < 0.01$], Model 2 (OR 0.86, 95% CI 0.78–0.94, $P < 0.01$), Model 3 (OR 0.87, 95% CI 0.77–0.98, $P < 0.01$), Model 4 (OR 0.89, 95% CI 0.80–0.99, $P = 0.04$).

In a sub-analysis of non-obese research subjects (BMI < 25 kg/m^2), muscle/fat ratio showed a significant positive relationship in simple regression analysis for M/I value ($B = 0.7526$, $P < 0.0001$). Moreover, in logistic regression analysis for insulin resistance in this non-obese group, the muscle/fat ratio was significantly associated with insulin resistance (OR 0.3309, 95% CI 0.1192–0.9188, $P = 0.03$).

3.6. *ROC analysis*

Insulin resistance was analyzed by ROC. Fig. 1 shows the result of the muscle/fat ratio. The cutoff value for muscle/fat ratio for insulin resistance was 2.40 and the AUC was 0.87 (sensitivity 91% and specificity 76%, $P < 0.001$), indicating that the muscle/fat ratio was significantly useful for making a clinical prediction for the presence of insulin resistance in treatment-naïve DM.

4. Discussion

This study demonstrated that body composition is significantly associated with insulin resistance, particularly muscle/fat ratio. The M/I values, assessed by the hyperinsulinemic-euglycemic clamp, and the muscle/fat ratio, assessed by body composition analysis with InBody770, are significantly and independently correlated. The regression equation was M/I value = 3.6934 × (muscle/fat ratio) + 0.0347. Multivariate regression analysis showed that the muscle/fat ratio was a significant factor and that the regression equation had an M/I value = 2.5438 × (muscle/fat ratio) + 48.61 94 × QUICKI – 13.6522. The muscle/fat ratio was independent and a significant factor for the presence of insulin resistance.

Obesity is a state of excessive accumulation of fat tissue, and it has been revealed that some of the biologically active substances (adipocytokines) secreted from the accumulated fat tissue, can cause insulin resistance in skeletal muscle tissue. Previous reports have shown that insulin resistance assessed by euglycemic clamp was significantly correlated with the abdominal fat area measured in a CT scan [18]. Results of the present study also showed that total body fat mass is strongly associated with M/I values. Therefore, it would be reasonable to assume that waist circumference, BMI, and body weight could reflect increased total body fat and could exhibit a correlation with the M/I value. It has been shown that insulin resistance contributes more to increased risk of cardiovascular diseases than simple obesity [19]. However, using BMI from a clinical point of view to assess cardiovascular risk, is not enough. Quantitative assessment of total body fat is fundamentally important in clinical practice. Skeletal muscle is known to be one of the insulin-sensitive tissues in the body [20], and the results of the present study showed that total muscle mass was significantly correlated with the M/I value. We showed that the body composition indicators, muscle-fat balance, and muscle/fat ratio, demonstrated a significant and independent correlation with the M/I value as a measurement of insulin resistance.

In the present study, we investigated the correlation between the ratio of total fat quantity and total muscle quantity that could be measured simply by a bioelectrical impedance method while insulin resistance was assessed by hyperinsulinemic-euglycemic clamp examination in clinical practice. Regarding body fat distribution, the significant association between ectopic fat accumulation and insulin resistance has been attracting much clinical attention [21]. Unfortunately, we did not perform quantitative assessment of ectopic fat volume in the present study. Ectopic fat accumulation in the liver and skeletal muscle has been shown to be involved in the pathogenesis of insulin resistance [22]; hence, surely it is important to examine the association between ectopic fat mass and insulin resistance in clinical practice. Since ectopic fat accumulation in the liver was significantly correlated with the percent body fat measured by bioelectrical impedance method in a previous study [23], it is possible that total fat quantity can reflect ectopic fat mass. However, currently there is no conclusive evidence that ectopic fat accumulation in muscle and/or liver might affect the total fat mass measured by bioelectrical impedance method using inBody-770. If a method that can simply and non-invasively measure ectopic fat mass in an outpatient department is developed for clinical practice, the ectopic fat quantity and total muscle quantity ratio could become the more

Table 4 – Results of logistic regression analysis for insulin resistance.

Variable	Simple regression			Multivariate regression using forced inclusion model 1			Multivariate regression using forced inclusion model 2			Multivariate regression using forced inclusion model 3			Multivariate regression using forced inclusion model 4		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Sex	2.08	0.72–6.01	0.18	0.18	0.02–1.34	0.09									
Age	0.96	0.92–1.01	0.09	1.00	0.95–1.34	0.92									
Height	1.01	0.95–1.01	0.64												
Weight	1.11	1.05–1.17	<0.01												
BMI	1.49	1.23–1.81	<0.01												
Waist circumference	1.17	1.08–1.26	<0.01												
Muscle quantity	1.05	0.99–1.11	0.08												
Body fat quantity	1.32	1.15–1.51	<0.01												
Body fat percentage	1.24	1.12–1.39	<0.01												
Muscle quantity /body fat quantity per (0.1)	0.84	0.77–0.92	<0.01	0.79	0.68–0.91	<0.01	0.86	0.78–0.94	<0.01	0.87	0.77–0.98	0.02	0.89	0.80–0.99	0.04
Hypertension,n	0.95	0.30–3.07	0.94												
Dyslipidemia,n	1.03	0.37–2.87	0.95												
Angiotensin II Receptor Blocker	1.73	0.27–11.2	0.56												
Statin	0.25	0.03–2.38	0.23												
HbA1c	1.05	0.82–1.34	0.68							0.78	0.52–1.15	0.21			
Fasting plasma glucose	1.01	1.00–1.02	0.19												
Fasting blood insulin	1.52	1.19–1.94	<0.01												
QUICKI per 0.1	0.009	0.0007–0.1184	<0.01							0.04	0.003–0.644	0.02	0.07	0.005–1.082	0.06
HOMA-IR	2.15	1.33–3.49	<0.01												
Total cholesterol	1.02	1.01–1.04	0.01												
HDL-cholesterol	0.95	0.91–0.99	0.02				0.98	0.93–1.04	0.58				0.98	0.93–1.04	0.54
LDL-cholesterol	1.01	1.00–1.03	0.13												
Triglycerides	1.02	1.01–1.03	<0.01				1.01	1.00–1.02	1.02				1.00	1.00–1.01	0.31
eGFR	0.98	0.95–1.01	0.25												

BMI, body mass index; eGFR; estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low-density lipoprotein; QUICKI, Quantitative insulin sensitivity check index.

Model 1: Hosmer–Lemeshow goodness-of-fit χ^2 was 13.95 with *P* value of 0.08.

Model 2: Hosmer–Lemeshow goodness-of-fit χ^2 was 7.97 with *P* value of 0.44.

Model 3: Hosmer–Lemeshow goodness-of-fit χ^2 was 2.767 with *P* value of 0.948.

Model 4: Hosmer–Lemeshow goodness-of-fit χ^2 was 8.534 with *P* value of 0.383.

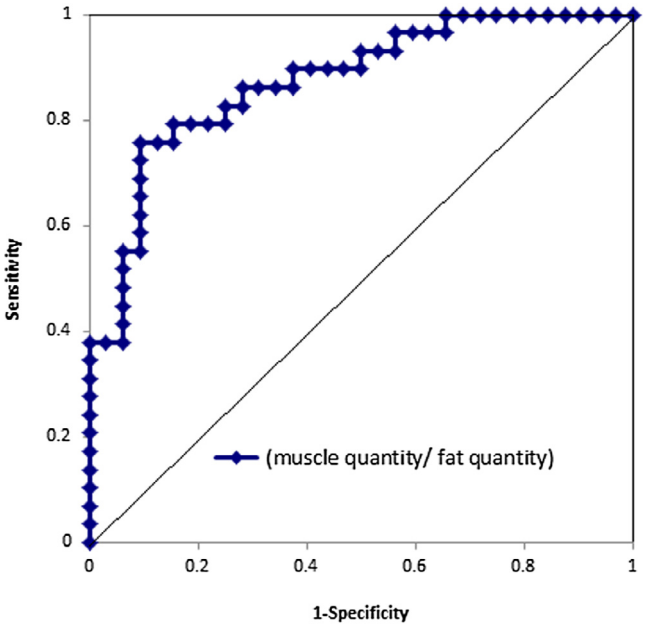

Fig. 1 – Receiver-operating characteristic curve analysis of muscle/fat ratio for insulin resistance. The cutoff value of the muscle/fat ratio for insulin resistance, defined as the optimal sensitivity and specificity of the ROC curve, was 2.40 (AUC 0.87, SE 0.04, 95% confidence interval 0.7866–0.9612; $P < 0.0001$). AUC, area under the curve; ROC, receiver-operating characteristic analysis; muscle/fat ratio, muscle quantity/fat quantity.

useful and significant indicator of insulin resistance. Further clinical investigation is needed in future studies.

The clinical causes of insulin resistance can be broadly divided into genetic factors, obesity, and glucotoxicity. There have also been reports indicating that a high blood glucose level itself could be an important contributing factor to insulin resistance, although the details are still unclear [24]. In our study, fasting blood glucose levels, but not HbA1c, were found to have a significant correlation with the M/I value. This study included 17 patients with HbA1c values >10% (>86 mmol/mol) and 12 patients with fasting blood glucose levels >200 mg/dL. However, we could not confirm a significant influence of glucotoxicity on insulin resistance in this study population, which may be due to an inclusion bias that only examined untreated subjects with diabetes and not those with normoglycemia or patients with prediabetes. In the present study, we were unable to confirm the influence of glucotoxicity on insulin resistance as our study was not designed to make this observation. To clarify the potential effects of glucotoxicity on insulin resistance, in future studies, two clamp examinations should be made under high-glucose ambient levels and after reducing glucose levels.

Some medicines have been reported to improve insulin resistance. In this study, statins and ARBs corresponded to those medicines, but statins and ARBs were not a significant factor for insulin resistance in the multivariate logistic regression analysis.

At present, the composite index (ISI composite) [25], which correlates well with the euglycemic clamp studies at $r \geqslant 0.73$ and is calculated from oral glucose tolerance tests, is the most widely used indicator in actual clinical situations. In our study, we did not conduct oral glucose tolerance tests, and could not make a direct comparison with the ISI composite. QUICKI was a significant and independent factor for M/I value in the multivariable regression analysis, but was not a significant factor for insulin resistance in the multivariate logistic regression analysis. Reportedly, the blood insulin value itself in the fasting state can be used as an indicator of insulin resistance [26], and the results of this study confirmed its influence on the M/I value.

The body composition analyzer that we used in this study was non-invasive and reproducible, and it only took 60 s to measure. Based on these beneficial points, it is suggested that body composition assessment may be superior to the preexisting methods regarding its significant correlation with the euglycemic–clamp technique.

This study has several limitations. Only DM patients who were not taking any treatments for DM were enrolled. Also, we did not conduct oral glucose tolerance tests on all patients, and therefore, we were unable to make a direct comparison with the ISI composite. Moreover, to confirm the present results, we need to validate our observations in another independent population in a future study.

In conclusion, muscle/fat ratio derived from InBody770 is useful as a clinical surrogate indicator of insulin resistance in type 2 DM. Insulin resistance diagnosed by hyperinsulinemic-euglycemic clamp examination can be estimated with the regression equation, M/I value = 2.5438 × (-muscle/fat ratio) + 48.6194 × QUICKI – 13.6522. When only a body composition analysis result is used, the regression equation, M/I value = 3.6934 × (muscle/fat ratio) + 0.0347 is valid. We propose that a muscle/fat ratio of 2.40 can be the cutoff value for the presence of insulin resistance in patients with untreated DM.

Conflict of interest

H.J. has received honoraria from Novo Nordisk, Sanofi, AstraZeneca Pharmaceuticals, Astellas Pharma, Boehringer Ingelheim, Daiichi-Sankyo, Eli Lilly, Takeda, and Novartis Pharmaceuticals. S.S. has received honoraria from MSD, AstraZeneca Pharmaceuticals, Itamar Medical, Ono Pharmaceutical, and Novo Nordisk. There are no other potential conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabres.2016.07.018.

REFERENCES

[1] Himsworth HP. Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types. Diabet Med 2011;28(12):1440–4. http://dx.doi.org/10.1111/j.1464-5491.2011.3508.x.

[2] World Fact Organization: Obesity and overweight: WHO Fact Sheet, No. 311, 2013.
[3] Rabe K, Lehrke M, Parhofer KG, Broedl CU. Adipokines and insulin resistance. Mol Med 2008;14(11–12):741–51. http://dx.doi.org/10.2119/2008-00058.Rabe. Epub 2008 Sep 17.
[4] Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the US. Diabetes Care 2005;28(11):2745–9.
[5] Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med 2006;119 (10):812–9.
[6] Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 2007;49(4):403–14.
[7] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;273(3):E214–23.
[8] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.
[9] Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. Am J Physiol 1979;236(6): E667–77.
[10] Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, et al. Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. J Clin Endocrinol Metab 1989;68(2):374–8.
[11] Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G. Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. Diabetes 1981;30 (5):387–92.
[12] Bergman RN, Prager R, Volund A, Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest 1987;79(3):790–800.
[13] Jinnouchi H, Sugiyama S, Hieshima K, Kurinami N, Kajiwara K, Jinnouchi T, et al. Liraglutide, a glucagon-like peptide-1 analog, increased insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp examination in patients with uncontrolled type 2 diabetes mellitus. J Diabetes Res 2015;2015:706416. http://dx.doi.org/10.1155/2015/706416. Epub 2015 Apr 2.
[14] Yki-Järvinen H, Koivisto VA. Insulin sensitivity in newly diagnosed type 1 diabetics after ketoacidosis and after three months of insulin therapy. J Clin Endocrinol Metab 1984;59 (3):371–8.
[15] Cha K, Chertow GM, Gonzalez J, Lazarus JM, Wilmore DW. Multifrequency bioelectrical impedance estimates the distribution of body water. J Appl Physiol 1995;79(4):1316–9.
[16] Cha K, Shin S, Shon C, Choi S, Wilmore DW. Evaluation of segmental bioelectrical impedance analysis (SIBA) for measuring muscle distribution. J ICHPER SD-ASIA 1997:11–4.
[17] Cobb J, Gall W, Adam KP, Nakhle P, Button E, Hathorn J, et al. A novel fasting blood test for insulin resistance and prediabetes. J Diabetes Sci Technol 2013;7(1):100–10.
[18] Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2002;283(6):E1135–43.
[19] Meigs JB, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, et al. Body mass index, metabolic syndrome, and risk of type2 diabetes or cardiovascular disease. J Clin Endocrinol Metab 2006;91(8):2906–12.
[20] DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37(6):667–87.
[21] Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med 2014;371 (12):1131–41.
[22] Gastaldelli A. Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. Diabetes Res Clin Pract 2011;93 (Suppl 1):S60–5. http://dx.doi.org/10.1016/S0168-8227(11) 70015-8.
[23] Yatsuya H, Nihashi T, Li Y, Hotta Y, Matsushita K, Muramatsu T, et al. Independent association of liver fat accumulation with insulin resistance. Obes Res Clin Pract 2014;8(4):e350–5.
[24] Yki-Järvinen H, Helve E, Koivisto VA. Hyperglycemia decreases glucose uptake in type 1 diabetes. Diabetes 1987;36 (8):892–6.
[25] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22(9):1462–70.
[26] DeFronzo RA. Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview. Diabetologia 1992;35 (4):389–97.