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ORIGINAL ARTICLE

Association between fat mass index, fat-free mass index and hemoglobin A1c in a Japanese population: The Tohoku Medical Megabank Community-based Cohort Study

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ABSTRACT

Aims/Introduction: Fat mass and fat-free mass affect glycated hemoglobin A1c (HbA1c) levels and blood glucose levels, respectively. The aim of the present study was to examine the association between the fat mass index and fat-free mass index with HbA1c.

Materials and Methods: We carried out a cross-sectional study that included 3,731 men and 9,191 women aged ≥20 years, living in Miyagi Prefecture, Japan, who were not treated for diabetes. The fat mass index and fat-free mass index were calculated as fat mass and fat-free mass divided by the height squared, respectively. The indices were classified into sex-specific quartiles and combined into 16 groups. An analysis of covariance was used to assess associations between the combined fat mass index and fat-free mass index with HbA1c adjusted for potential confounders. The linear trend test was carried out by stratifying the fat mass index and fat-free mass index, entering the number as a continuous term in the regression model.

Results: In multivariable models, a higher fat mass index was related to higher HbA1c levels in men and women in all fat-free mass index subgroups ($P < 0.001$ for linear trend). When we excluded the participants who had been identified as having diabetes, the fat-free mass index was also related to higher HbA1c levels in most fat mass index subgroups ($P < 0.05$ for linear trend).

Conclusions: Fat mass index was positively related to HbA1c levels. The fat-free mass index was also related to HbA1c levels when we excluded participants who had been identified as having have diabetes.

INTRODUCTION

Body mass index (BMI) is widely used to measure obesity. Previous studies have shown that BMI is related to the incidence of type 2 diabetes[1–4]. However, recent studies have shown that a high body fat percentage (BF%) is associated with hyperglycemia and the incidence of type 2 diabetes, even in non-obese individuals[5,6]. Many studies reported that fat-free mass (FFM) and FFM percentage (FFM%) were inversely associated with the incidence of type 2 diabetes; however, others have shown no association[7–15]. These findings suggest that adipose tissue and FFM have different roles in blood glucose levels and glycated hemoglobin A1c (HbA1c).

The fat mass (FM) index (FMI) and FFM index (FFMI) have been proposed as indicators of body composition[16]. These

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indices are calculated as FM and FFM divided by height squared, respectively[16], and are useful for comparing individuals with different height measurements[16–19].

Because the FMI and FFMI calculated using height are not independent of each other, the estimated results might become unstable if they are used in the same statistical model. Therefore, they cannot be used to understand the different roles of FMI and FFMI in HbA1c levels. However, several approaches can be considered. In one such method of investigating the associations between FMI and HbA1c levels in consideration of FFMI, it is necessary to stratify for FFMI. Similarly, to investigate the associations between the FFMI and HbA1c in consideration of FMI, it is necessary to stratify and examine the FMI. By combining the FMI and FFMI, it is possible to identify individuals with low FM and high FFM, and those with high FM and low FFM. Therefore, when combined, the FMI and FFMI can be used to examine the associations between FMI and HbA1c in each FFMI subgroup, and between FFMI and HbA1c in each FMI subgroup. To the best of our knowledge, no study has investigated associations between the combined FMI and FFMI with blood glucose and HbA1c. Therefore, we aimed to examine the associations between the combined FMI and FFMI with HbA1c, which is an indicator of glycemic control and risk marker for diabetes[20].

MATERIALS AND METHODS

Study design and population

We carried out a cross-sectional study using data from the Tohoku Medical Megabank Community-Based Cohort Study (TMM CommCohort Study)[21], which was a population-based prospective cohort study of individuals aged ≥20 years living in Miyagi or Iwate Prefecture in northeastern Japan. In the present study, participants were recruited only from Miyagi using three approaches. A type 1 survey was carried out at specific municipal health examination sites. We visited the sites and asked the participants to join our cohort (n = 40,433). A type 1 additional survey was carried out on dates different from those of the specific health examinations in the municipality (n = 664). A type 2 survey was carried out at an assessment center (the Community Support Center in the Tohoku Medical Megabank Organization; n = 13,855). Type 2 survey participants underwent several detailed measurements. All three surveys collected basic information on blood and urine composition, a questionnaire, and municipal health checkups. Additionally, only in the type 2 survey, several physiological measurements (carotid echography, calcaneal ultrasound bone mineral density, body composition etc.) were carried out. Informed consent was obtained from all the 54,952 individuals. All participants were recruited from May 2013 to March 2016. This study was approved by the institutional review board of the Tohoku Medical Megabank Organization (approval number: 2021-4-028, approval date: 31 May 2021).

To be included in the analysis, participants were required to undergo several physiological measurements. Furthermore, we considered that it might be better to use simultaneously collected blood data. Therefore, we only used data from 13,855 participants included in the type 2 survey. We excluded those who withdrew from the study by 27 July 2020, failed to return the self-reported questionnaire, did not undergo physiological measurements, and had missing data regarding BF%, height, weight, HbA1c and treatment for diabetes (n = 933). Therefore, only data from 12,922 participants were analyzed.

Anthropometry

Height was measured to the nearest 0.1 cm using a stadiometer (AD-6400; A&D Co., Ltd., Tokyo, Japan). Information regarding diabetes treatment was obtained using a self-reported questionnaire. Weight and BF% were measured using a body composition analyzer (InBody720; Biospace Co., Ltd., Seoul, Korea). Weight was measured in increments of 0.1, and 1.0 kg was subtracted to account for the weight of the participant's clothing. BMI was calculated as weight (kg) divided by height in meters squared (m^2). FM was calculated by multiplying weight (kg) by the BF%. FMI was calculated as FM (kg) divided by height in m^2. To calculate the FFMI, FFM% was calculated by subtracting the BF% from 100%. The FFM was calculated by multiplying the weight by the FFM%. The FFMI was calculated as FFM (kg) divided by height in m^2.

HbA1c and diabetes status

Blood samples were collected from non-fasting participants. Plasma glucose concentrations and HbA1c levels were measured using an enzymatic method. Information regarding diabetes treatment was obtained using a self-reported questionnaire. Participants chose one of the following options: (a) undergoing treatment for diabetes; (b) discontinued diabetes treatment; (c) undertaking lifestyle modifications without medication; (d) undergoing observation without medication; or (e) never been diagnosed with diabetes. Participants who chose answer (a) were classified as 'undergoing treatment,' whereas those who chose answers (b) through (e) were classified as 'without treatment.' Diabetes was defined as plasma glucose ≥200 mg/dL, HbA1c ≥6.5% and receiving treatment for diabetes.

Potential confounders

We administered a self-reported questionnaire to assess the participant demographic characteristics, smoking status and drinking status. The questionnaire was the same for all survey types. Age was determined at the time of visiting the community support center. Smoking status was classified as: never-smoker; past-smoker; and current smoker. Never-smokers were defined as participants who had smoked <100 cigarettes during their lifetime. Past-smokers were participants who had smoked ≥100 cigarettes during their lifetime, but indicated on the questionnaire that they no longer smoked. Current smokers were participants who had smoked ≥100 cigarettes during their lifetime and indicated on the questionnaire that they currently smoke. Drinking status was classified as: never-drinker; past-drinker;

and <23 and ≥23 g/day. Never-drinkers were those who indicated that they consumed little or no alcohol or were constitutionally incapable of alcohol consumption. Past-drinkers were those who indicated that they stopped drinking alcohol. To differentiate between <23 and ≥23 g/day, alcohol types were classified into six categories: sake; distilled spirits; shochu-based beverages; beer; whiskey; and wine. The alcohol intake frequency was also classified into six categories: almost never; 1–3 days/month; 1–2 days/week; 3–4 days/week; 5–6 days/week; and daily. Participants indicated how much of each type of alcohol they drank. Each type of alcohol was multiplied by the frequency and amount, and then converted to the amount of ethanol. In the TMM CommCohort Study, one drink was estimated to contain 23 g ethanol for 180 mL of sake, 36 g ethanol for 180 mL of shochu-based beverages, 12.96 g ethanol for 350 mL of distilled spirits, 23 g ethanol for 633 mL of beer, 10 g ethanol for 30 mL of whiskey, 6 g ethanol for 60 mL of wine. We considered the alcohol intake cut-off value to be 23 g, because it is the traditional Japanese unit of sake.

Statistical analysis

Data are presented as the mean (standard deviation) or median (interquartile range [IQR]) for continuous variables and as the number (%) for categorical variables. All analyses were carried out separately for men and women, because the distributions of the FMI and FFMI differed between them. FMI was categorized into the following quartile groups using the whole population: Q1 (lowest group); Q2; Q3; and Q4 (highest group). FFMI was also categorized into the following quartile groups using the whole population: Q1 (lowest group); Q2; Q3; and Q4 (highest group).

In terms of baseline characteristics of FMI, we tested for trends in FMI based on characteristics, including age, height, BMI, BF%, FM, FFM and HbA1c, to evaluate the linear relationship between the FMI and these variables. We used a general linear model for age, height, BMI, BF%, FM, FFM and HbA1c as continuous variables. To compare the smoking status and drinking status among the quartile groups for the FMI, we used the χ^2-test. Similarly, trend tests in FFMI were carried out based on baseline characteristics. Smoking status and drinking status were compared using the χ^2-test.

To consider potential confounders and calculate the least squares (LS) means of HbA1c, we used analysis of covariance (ANCOVA) to assess the associations between the FMI and HbA1c. The LS means and corresponding 95% confidence intervals (CIs) were presented. The multivariable-adjusted models included age (years), smoking status (never-smoker, past-smoker and current smoker) and drinking status (never-drinker, past-drinker, those consuming <23 g/day and those consuming ≥23 g/day). *P*-values for linear trends were calculated using the quartiles of the FMI. Similarly, we analyzed the associations between the FFMI and HbA1c.

To consider the FMI and FFMI, we combined both indices and categorized them into 16 groups. In terms of baseline characteristics among FMI subgroups, *P*-values for the analysis of linear trends of FFMI and HbA1c were calculated by stratifying for the FMI. We also used ANCOVA to assess the associations between the combined FMI and FFMI with HbA1c. *P*-values for linear trends of FMI were calculated using the quartiles of FMI by stratifying the quartiles in FFMI groups. Similarly, *P*-values for linear trends of FFMI were calculated using the quartiles of FFMI by stratifying the quartiles in FMI groups. To eliminate the possible effect of participants who were identified as having diabetes, we carried out an analysis by excluding individuals who had diabetes, discontinued diabetes treatment and those who were undertaking lifestyle modification without medication.

$P < 0.05$ was considered significant. All analyses were carried out using SAS version 9.4 for Windows (SAS Inc., Cary, NC, USA).

RESULTS

Figure 1 presents the characteristics of the study participants. A total of 3,731 men and 9,191 women fulfilled all inclusion criteria, and their data were included in the analyses. The mean age (±standard deviation) was 59.6 years (±14.3 years) for men and 55.9 years (±13.5 years) for women (Table S1). The median FMI was higher for women (6.7 kg/m^2 [IQR 5.1–8.5 kg/m^2]) than for men (5.5 kg/m^2 [IQR 4.3–7.0 kg/m^2]); however, the median FFMI was higher for men (17.9 kg/m^2 [IQR 17.0–18.9 kg/m^2]) than for women (15.2 kg/m^2 [IQR 14.4–16.0 kg/m^2]; Tables S1 and S2, respectively). The percentages of current smokers and current alcohol drinkers were higher in men than in women. The correlations of the FMI and FFMI were $r = 0.39$ for men and $r = 0.51$ for women (data not shown).

In the present study, the FMI quartiles for men were as follows: Q1, <4.3 kg/m^2; Q2, 4.3–5.5 kg/m^2; Q3, 5.6–7.0 kg/m^2; and Q4, ≥7.1 kg/m^2. For women, they were as follows: Q1, <5.1 kg/m^2; Q2, 5.1–6.7 kg/m^2; Q3, 6.8–8.5 kg/m^2; and Q4, ≥8.6 kg/m^2. The characteristics of the participants according to the FMI are shown in Table S1. In both men and women, age, BMI, and HbA1c were positively associated with FMI (*P* for linear trend <0.001). For men, the smoking status and drinking status were not significantly different among the quartile groups. For women, the smoking status and drinking status were statistically different among the quartile groups (*P* for difference <0.05).

The FFMI was categorized into the following sex-specific quartiles for men: Q1, <17.0 kg/m^2; Q2, 17.0–17.9 kg/m^2; Q3, 18.0–18.9 kg/m^2; and Q4, ≥19.0 kg/m^2. For women, they were as follows: Q1, <14.4 kg/m^2; Q2, 14.4–15.2 kg/m^2; Q3, 15.3–16.0 kg/m^2; and Q4, ≥16.1 kg/m^2. The characteristics of the participants according to the FFMI are shown in Table S2. For both men and women, BMI and HbA1c were positively associated with the FFMI (*P* for linear trend <0.01). The age of men, but not of women, was inversely associated with the FFMI (*P* for linear trend <0.001). In both men and women, the smoking and drinking statuses showed statistically significant differences among the quartile groups (*P* for difference <0.01).

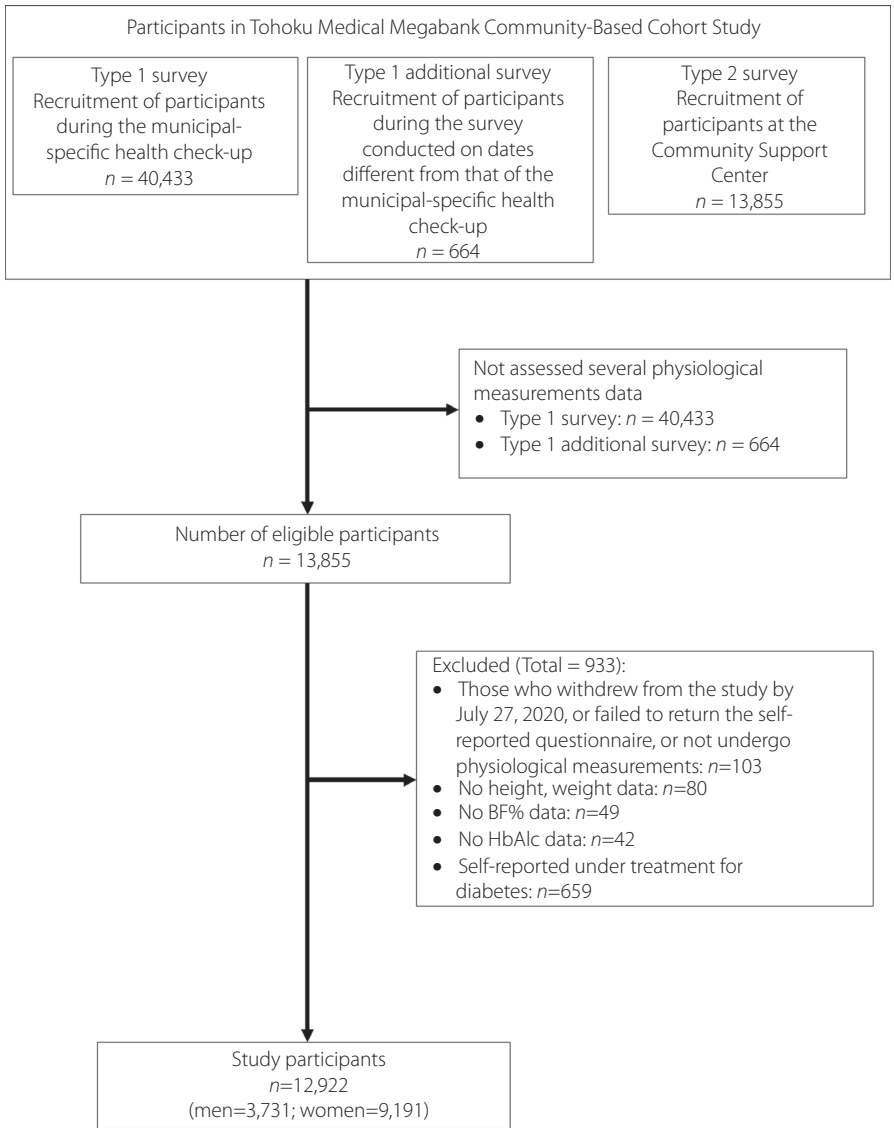

Figure 1 | Participant recruitment flowchart. BF%, body fat percentage; HbA1c, glycated hemoglobin A1c.

Tables 1 and 2 present the participant characteristics according to the combined FMI and FFMI. Both men and women with a higher FMI and higher FFMI were more likely to have a higher BMI. Men categorized under FMI Q1 and FFMI Q4 (the lowest FMI quartile and the highest FFMI quartile) had a younger age, were taller and were current alcohol drinkers. Men categorized under FMI Q4 and FMI Q1 (the highest FMI quartile and the highest FFMI quartile) had an older age, higher HbA1c, were shorter, current smokers and current alcohol drinkers. Women categorized under FMI Q1 and FFMI Q4 were taller and were current alcohol drinkers. Women categorized under FMIQ4 and FMIQ1 had an older age, were shorter, current smokers and current drinkers.

In both men and women, the FMI was associated with HbA1c, even after adjusting for potential confounders (P for ANCOVA <0.001). In men, the HbA1c LS mean values were 5.46 (95% CI 5.37–5.55) for Q1, 5.51 (95% CI 5.42–5.61) for Q2, 5.59 (95% CI 5.50–5.68) for Q3,and 5.67 (95% CI 5.57–5.76) for Q4 (P for linear trend <0.001). In women, the HbA1c LS mean values were 5.40 (95% CI 5.37–5.55) for Q1, 5.42 (95% CI 5.50–5.68) for Q2, 5.47 (95% CI 5.50–5.68) for Q3 and 5.57 (95% CI 5.57–5.76) for Q4 (P for linear trend <0.001) .

The multivariable analysis showed an association between FFMI and HbA1c in both men and women. In men, the HbA1c LS means were 5.48 (95% CI 5.39–5.58) for Q1, 5.52 (95% CI 5.43–5.61) for Q2, 5.55 (95% CI 5.45–5.64) for Q3 and 5.63 (95% CI 5.54–5.72) for Q4 (P for linear trend<0.001). In women, the HbA1c LS means were 5.41 (95% CI 5.35–5.47) for Q1, 5.43 (95% CI 5.37–5.48) for Q2, 5.46 (95% CI 5.40–5.52) for Q3 and 5.54 (95% CI 5.54–5.60) for Q4 (P for linear trend <0.001).

Table 1 | Characteristics of men among fat mass index subgroups

FMI	Q1 (<4.3)					Q2 (4.3–5.5)					Q3 (5.6–7.0)					Q4 (>7.1)					All male participants
FFMI	Q1 (<17.0)	Q2 (17.0–17.9)	Q3 (18.0–18.9)	Q4 (>19.0)	$P^{\dagger}$	Q1 (<17.0)	Q2 (17.0–17.9)	Q3 (18.0–18.9)	Q4 (>19.0)	$P^{\dagger}$	Q1 (<17.0)	Q2 (17.0–17.9)	Q3 (18.0–18.9)	Q4 (>19.0)	$P^{\dagger}$	Q1 (<17.0)	Q2 (17.0–17.9)	Q3 (18.0–18.9)	Q4 (>19.0)	$P^{\dagger}$	
n	373	240	190	130		242	294	237	160		199	213	278	243		118	186	228	400		3731
Age (years)	58.6 (16.0)	56.7 (15.3)	54.2 (14.8)	49.0 (14.7)		65.0 (13.5)	61.5 (12.9)	58.4 (13.5)	54.3 (13.5)		66.9 (11.3)	62.8 (12.6)	60.7 (12.8)	56.3 (12.7)		70.0 (11.4)	66.2 (10.9)	62.9 (12.8)	54.7 (13.8)		59.6 (14.3)
Height (cm)	168.0 (6.8)	168.3 (6.3)	169.9 (6.6)	171.3 (6.6)		165.9 (6.3)	168.0 (6.4)	167.7 (6.0)	170.0 (5.7)		165.6 (5.9)	166.1 (5.8)	167.6 (5.9)	169.1 (5.9)		163.4 (5.8)	164.9 (6.0)	166.0 (5.8)	169.4 (5.9)		167.7 (6.4)
BMI	19.1 (1.2)	20.8 (0.8)	21.8 (0.7)	23.0 (1.1)		21.1 (0.8)	22.4 (0.5)	23.3 (0.5)	24.6 (0.7)		22.4 (0.8)	23.6 (0.5)	24.6 (0.5)	26.1 (0.9)		24.5 (1.2)	25.7 (1.1)	26.7 (1.3)	29.3 (2.8)		23.7 (3.1)
FMI	3.2 (2.6–3.8)	3.5 (2.8–3.9)	3.5 (3.0–3.9)	3.6 (3.0–3.9)		4.9 (4.6–5.2)	4.9 (4.6–5.2)	4.9 (4.6–5.2)	4.9 (4.6–5.2)		6.1 (5.8–6.5)	6.1 (5.8–6.5)	6.2 (5.8–6.6)	6.2 (5.9–6.6)		8.1 (7.5–8.9)	7.8 (7.4–8.6)	7.9 (7.4–8.8)	8.5 (7.6–9.9)		5.5 (4.3–7.0)
FFMI	16.1 (15.6–16.6)	17.5 (17.2–17.7)	18.3 (18.1–18.6)	19.4 (19.1–19.9)		16.4 (15.9–16.7)	17.5 (17.2–17.7)	18.4 (18.2–18.6)	19.5 (19.2–20.0)		16.4 (15.9–16.7)	17.5 (17.2–17.7)	18.4 (18.2–18.7)	19.6 (19.3–20.2)		16.4 (15.9–16.8)	17.5 (17.3–17.7)	18.4 (18.2–18.6)	20.0 (19.4–20.8)		17.9 (17.0–18.9)
HbA1c (%)	5.4 (0.3)	5.4 (0.3)	5.4 (0.3)	5.4 (0.3)	0.458	5.5 (0.5)	5.4 (0.4)	5.5 (0.5)	5.5 (0.6)	0.773	5.5 (0.4)	5.6 (0.7)	5.5 (0.4)	5.5 (0.5)	0.786	5.7 (1.0)	5.6 (0.4)	5.6 (0.4)	5.6 (0.6)	0.821	5.5 (0.5)
Never-smoker	110 (29.5)	74 (30.8)	58 (30.5)	37 (28.5)		82 (23.9)	90 (30.6)	77 (32.5)	44 (27.5)		61 (30.7)	67 (31.5)	72 (25.9)	62 (25.5)		44 (37.3)	54 (29.0)	61 (26.8)	96 (24.0)		1,089 (29.2)
Past-smoker	178 (47.7)	110 (45.8)	87 (45.8)	52 (40.0)		111 (45.9)	145 (79.3)	118 (49.8)	80 (50.0)		99 (49.8)	107 (50.2)	152 (54.7)	114 (46.9)		61 (51.7)	103 (55.4)	132 (57.9)	193 (48.3)		1,842 (49.4)
Current smoker	85 (22.8)	56 (23.3)	45 (23.7)	39 (30.0)		48 (19.8)	58 (19.7)	42 (17.7)	35 (21.9)		36 (18.1)	39 (18.3)	53 (19.1)	66 (27.2)		12 (10.2)	28 (15.1)	35 (15.4)	107 (26.8)		784 (21.0)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)		1 (0.4)	1 (0.3)	0 (0.0)	1 (0.6)		3 (1.5)	0 (0.0)	1 (0.4)	1 (0.4)		1 (0.9)	1 (0.5)	0 (0.0)	4 (1.0)		16 (0.4)
Never-drinker	66 (17.7)	38 (15.8)	26 (13.7)	25 (19.2)		55 (22.7)	44 (15.0)	39 (16.5)	20 (12.5)		48 (24.1)	35 (16.4)	45 (16.2)	36 (14.8)		31 (26.3)	32 (17.2)	40 (17.5)	91 (22.8)		671 (18.0)
Past-drinker	13 (3.5)	14 (5.8)	4 (2.1)	4 (3.1)		11 (4.6)	6 (2.0)	8 (3.4)	8 (5.0)		5 (2.5)	10 (4.7)	7 (2.5)	6 (2.5)		5 (4.2)	8 (4.3)	10 (4.4)	14 (3.5)		133 (3.6)
<23 g/day	157 (42.1)	103 (42.9)	87 (45.8)	42 (32.3)		93 (38.4)	121 (41.2)	93 (39.2)	53 (33.1)		65 (32.7)	81 (38.0)	108 (38.9)	90 (37.0)		43 (36.4)	72 (38.7)	75 (32.9)	150 (37.5)		1,433 (38.4)
≥23 g/day	137 (36.7)	85 (35.4)	73 (38.4)	57 (43.9)		83 (34.3)	123 (41.8)	97 (40.9)	79 (49.4)		80 (40.2)	87 (40.9)	118 (42.5)	110 (45.3)		39 (33.1)	74 (39.8)	103 (45.2)	144 (36.0)		1,489 (39.9)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		1 (0.5)	0 (0.0)	0 (0.0)	1 (0.4)		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)		5 (0.1)

Values are expressed as the mean (standard deviation) or median (interquartile range) for continuous variables, or number (%) for categorical variables. BF%, body fat percentage; BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; HbA1c, glycated hemoglobin A1c; Q, quartile. † The *P*-value for the analysis of linear trends was calculated by stratifying fat mass index (FMI), scoring the FMI categories, from 1 for the lowest category to 4 for the highest, entering the number as a continuous term in the regression model.

Table 2 | Characteristics of women among fat mass index subgroups

FMI	Q1 (<5.1)					Q2 (5.1–6.7)					Q3 (6.8–8.5)					Q4 (>8.6)					All women participants
FFMI	Q1 (<14.4)	Q2 (14.4–15.2)	Q3 (15.3–16.0)	Q4 (>16.1)	$P^{\dagger}$	Q1 (<14.4)	Q2 (14.4–15.2)	Q3 (15.3–16.0)	Q4 (>16.1)	$P^{\dagger}$	Q1 (<14.4)	Q2 (14.4–15.2)	Q3 (15.3–16.0)	Q4 (>16.1)	$P^{\dagger}$	Q1 (<14.4)	Q2 (14.4–15.2)	Q3 (15.3–16.0)	Q4 (>16.1)	$P^{\dagger}$	
n	928	675	453	242		696	671	570	361		526	599	663	509		148	353	611	1,186		9,191
Age (years)	52.3 (14.1)	50.8 (13.7)	50.7 (13.7)	51.1 (12.1)		55.7 (14.4)	55.7 (13.6)	53.7 (13.7)	54.6 (13.9)		58.9 (12.2)	59.6 (11.8)	58.7 (12.6)	56.3 (13.1)		63.8 (12.0)	62.0 (11.5)	59.9 (11.8)	56.1 (13.1)		55.9 (13.5)
Height (cm)	157.4 (5.4)	157.6 (5.5)	157.4 (5.5)	158.5 (5.4)		155.9 (5.5)	155.9 (5.6)	156.5 (5.7)	156.6 (5.9)		154.6 (5.5)	154.7 (5.7)	155.4 (5.6)	156.0 (5.9)		152.6 (5.5)	153.3 (5.8)	153.7 (5.3)	155.2 (5.9)		155.8 (5.8)
BMI	17.7 (1.0)	18.9 (0.8)	19.6 (0.8)	20.7 (0.9)		19.7 (0.7)	20.6 (0.5)	21.4 (0.5)	22.4 (0.7)		21.3 (0.7)	22.3 (0.6)	23.1 (0.6)	24.2 (0.8)		23.4 (1.1)	24.6 (1.2)	25.7 (1.5)	28.3 (2.9)		22.3 (3.4)
FMI	4.1 (3.3–4.6)	4.2 (3.6–4.7)	4.2 (3.7–4.7)	4.3 (3.8–4.8)		5.8 (5.5–6.3)	5.8 (5.5–6.2)	5.9 (5.5–6.3)	5.9 (5.5–6.3)		7.4 (7.0–7.8)	7.5 (7.0–7.9)	7.6 (7.1–8.0)	7.6 (7.2–8.0)		9.1 (8.8–9.8)	9.4 (8.9–10.4)	9.8 (9.0–10.8)	10.7 (9.5–12.3)		6.7 (5.1–8.5)
FFMI	13.8 (13.5–14.2)	14.8 (14.6–15.0)	15.5 (15.3–15.7)	16.3 (16.1–16.7)		14.0 (13.6–14.2)	14.8 (14.6–15.0)	15.5 (15.3–15.7)	16.4 (16.1–16.8)		14.0 (13.6–14.2)	14.8 (14.6–15.0)	15.5 (153–15.7)	16.4 (16.2–16.8)		14.0 (13.8–14.2)	14.9 (14.7–15.0)	15.6 (15.4–15.8)	16.8 (16.4–17.5)		15.2 (14.4–16.0)
HbA1c (%)	5.3 (0.3)	5.3 (0.3)	5.3 (0.3)	5.3 (0.3)	0.703	5.4 (0.5)	5.4 (0.3)	5.4 (0.3)	5.4 (0.4)	0.451	5.5 (0.4)	5.5 (0.5)	5.5 (0.3)	5.5 (0.4)	0.873	5.5 (0.4)	5.5 (0.4)	5.6 (0.4)	5.6 (0.5)	<0.001	5.5 (0.4)
Never-smoker	724 (78.0)	517 (76.6)	335 (74.0)	174 (71.9)		553 (79.5)	544 (81.1)	457 (80.2)	270 (74.8)		422 (80.2)	485 (81.0)	538 (81.2)	392 (77.0)		125 (84.5)	294 (83.3)	496 (81.2)	890 (75.0)		7,216 (78.5)
Past-smoker	122 (13.2)	94 (13.9)	71 (15.7)	43 (17.8)		91 (13.1)	84 (12.5)	79 (13.9)	58 (16.1)		66 (12.6)	76 (12.7)	85 (12.8)	73 (14.3)		18 (12.2)	43 (12.2)	75 (12.3)	182 (15.4)		1,260 (13.7)
Current smoker	79 (8.5)	60 (8.9)	45 (9.9)	23 (9.5)		51 (7.3)	39 (5.8)	33 (5.8)	33 (9.1)		37 (7.0)	36 (6.0)	39 (5.9)	39 (7.7)		5 (3.4)	16 (4.5)	37 (6.1)	107 (9.0)		679 (7.4)
Unknown	3 (0.3)	4 (0.6)	2 (0.4)	2 (0.8)		1 (0.1)	4 (0.6)	0 (0.0)	0 (0.0)		1 (0.2)	2 (0.3)	1 (0.2)	5 (1.0)		0 (0.0)	0 (0.0)	3 (0.5)	7 (0.6)		36 (0.4)
Never-drinker	470 (50.7)	302 (44.7)	208 (45.9)	94 (38.8)		395 (56.8)	352 (52.5)	259 (45.4)	160 (44.3)		304 (57.8)	339 (56.6)	334 (50.4)	249 (48.9)		102 (68.9)	210 (59.5)	339 (55.5)	630 (53.1)		4,747 (51.7)
Past-drinker	21 (2.3)	17 (2.5)	8 (1.8)	6 (2.5)		9 (1.3)	15 (2.2)	14 (2.5)	10 (2.8)		9 (1.7)	10 (1.7)	9 (1.4)	8 (1.6)		3 (2.0)	4 (1.1)	6 (1.0)	24 (2.0)		173 (1.9)
<23 g/day	356 (38.4)	298 (44.2)	180 (39.7)	105 (43.4)		238 (34.2)	261 (38.9)	243 (42.6)	151 (41.8)		174 (33.1)	215 (35.9)	261 (39.4)	208 (40.9)		39 (26.4)	113 (32.0)	221 (36.2)	425 (35.8)		3,488 (38.0)
≥23 g/day	81 (8.7)	58 (8.6)	56 (12.4)	37 (15.3)		54 (7.8)	42 (6.3)	53 (9.3)	40 (11.1)		39 (7.4)	34 (5.7)	58 (8.8)	43 (8.5)		4 (2.7)	26 (7.4)	44 (7.2)	106 (8.9)		775 (8.4)
Unknown	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)		0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)		0 (0.0)	1 (0.2)	1 (0.2)	1 (0.2)		0 (0.0)	0 (0.0)	1 (0.2)	1 (0.1)		8 (0.1)

Values are expressed as the mean (standard deviation) or median (interquartile range) for continuous variables, or number (%) for categorical variables. BF%, body fat percentage; BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; HbA1c, glycated hemoglobin A1c; Q, quartile. † The *P*-value for the analysis of linear trends was calculated by stratifying fat mass index (FMI), scoring the FMI categories, from 1 for the lowest category to 4 for the highest, entering the number as a continuous term in the regression model.

In both men and women, the combined FMI and FFMI with HbA1c levels were related, even after adjusting for potential confounders (*P* for ANCOVA <0.001; Table S3). In both men and women, a higher FMI was associated with higher HbA1c levels in all FFMI subgroups (*P* for linear trend <0.001). Conversely, except for men categorized under FMI Q2 and women categorized under FMI Q4, a higher FFMI did not tend to be associated with higher HbA1c levels in most FMI subgroups.

To eliminate the influence of participants who were identified as having diabetes, we excluded individuals who had plasma glucose levels ≥200 mg/dL, HbA1c ≥6.5%, discontinued diabetes treatment or undertook lifestyle modification without medication. As a result, a higher FMI was associated with higher HbA1c levels in all FFMI subgroups, and a higher FFMI was also associated with higher HbA1c levels in most FMI subgroups (Table 3).

DISCUSSION

The present study showed that when FFMI was not considered, a higher FMI tended to be associated with higher HbA1c levels in both men and women. Furthermore, when the FMI was not considered, a higher FFMI tended to be associated with higher HbA1c levels in both men and women. When the FMI and FFMI were combined and participants who had been identified as having diabetes were excluded, FMI was related to higher HbA1c levels in all FFMI subgroups, and FFMI was also related to higher HbA1c levels in most FMI subgroups.

Previous studies used FM and BF% as an index of body fat[4,6,10]. However, the absolute FM is affected by height, and the BF% is affected by FFM, because the weight is equal to the sum of FM and FFM[19]. Therefore, we used the FMI, which was not affected by FFM and allowed for comparisons of individuals with different height measurements. The present findings were consistent with those from previous studies using FM and BF% as an index of body fat[4,6,10]. Potentially harmful effects of body fat have been considered. Adipose tissue secretes adipocytokines, including tumor necrosis factor-α and resistin, which lead to insulin resistance[22–24]. Furthermore, non-esterified fatty acids released from adipose tissue can lead to insulin resistance in the liver and muscles, and impair pancreatic β-cells, which can lead to hyperglycemia[23–25].

In the present study, when the FMI was not considered, a higher FFMI tended to accompany a higher HbA1c level. Many previous studies have shown that FFM is inversely associated with incident type 2 diabetes, but others have shown no association[7–15]. However, the present findings were inconsistent with those from previous studies. The findings of a Danish cohort study showed that although FFM was associated with type 2 diabetes in both sexes, when adjusting for FM, an inverse relationship with type 2 diabetes was seen for men, but not for women[10]. Furthermore, the Health, Aging and Body Composition Study showed that FFM was associated with the incidence of diabetes only in the unadjusted model, that the association disappeared after adjusting for the FM of both men and women[13]. Therefore, not considering FMI might be the reason why the present results were inconsistent with the previous studies. However, because a higher BMI resulted in not only a higher FMI, but also a higher FFMI[19], there is a correlation between FMI and FFMI ($r = 0.39$ for men; $r = 0.51$ for women). It might be inappropriate to use FMI and FFMI in

Table 3 | Adjusted least square means of glycated hemoglobin A1c associated with fat mass index and fat-free mass index excluding plasma glucose ≥200 mg/dL, glycated hemoglobin A1c ≥6.5%, discontinued diabetes treatment, undertaking lifestyle modifications without medication and undergoing observation without medication

Men	FMI Q1	FMI Q2	FMI Q3	FMI Q4	*P* for trend among FFMI subgroups†
LS means HbA1c, % (95% CI)					
FFMI Q1	5.36 (5.29–5.43)	5.37 (5.29–5.43)	5.41 (5.34–5.48)	5.43 (5.35–5.51)	0.007
FFMI Q2	5.37 (5.30–5.45)	5.41 (5.34–5.48)	5.41 (5.34–5.49)	5.49 (5.41–5.56)	<0.001
FFMI Q3	5.39 (5.31–5.46)	5.4 (5.33–5.47)	5.46 (5.39–5.53)	5.5 (5.43–5.57)	<0.001
FFMI Q4	5.41 (5.33–5.48)	5.42 (5.34–5.49)	5.44 (5.37–5.51)	5.54 (5.47–5.61)	<0.001
P for trend among FMI subgroups‡	0.036	0.048	0.188	0.023	
Women					
FFMI Q1	5.33 (5.28–5.37)	5.33 (5.28–5.38)	5.36 (5.31–5.41)	5.36 (5.30–5.42)	0.047
FFMI Q2	5.33 (5.29–5.38)	5.35 (5.30–5.40)	5.37 (5.32–5.42)	5.41 (5.36–5.46)	<0.001
FFMI Q3	5.34 (5.30–5.39)	5.37 (5.33–5.42)	5.39 (5.35–5.44)	5.45 (5.40–5.49)	<0.001
FFMI Q4	5.35 (5.30–5.41)	5.35 (5.30–5.40)	5.41 (5.36–5.46)	5.5 (5.46–5.55)	<0.001
P for trend among FMI subgroups‡	0.083	0.042	0.003	<0.001	

Adjusted for age, smoking status (never-smoker, past-smoker, current smoker and unknown) and drinking status (never-drinker, past-drinker, <23 and ≥23 g/day). ANCOVA, analysis of covariance; CI, confidence interval; HbA1c, glycated hemoglobin A1c; LS, least squares; Q, quartile. † The *P*-values for the analysis of linear trends were calculated by stratifying fat-free mass index (FFMI), scoring the fat mass index (FMI) categories, from 1 for the lowest category to 4 for the highest, entering the number as a continuous term in the regression model. ‡ The *P*-values for the analysis of linear trends were calculated by stratifying FMI, scoring the FMI categories, from 1 for the lowest category to 4 for the highest, entering the number as a continuous term in the regression model.

the same statistical model to consider FMI and FFMI, respectively. Therefore, in the present study, we investigated the associations between the combination of FMI and FFMI with HbA1c. As a result, we observed that a higher FMI tended to accompany higher HbA1c levels in all FFMI subgroups.

The present findings were consistent with those from previous studies, even when considering FFMI[4,11]. Conversely, the FFMI did not tend to accompany higher HbA1c levels in most FMI subgroups. We investigated the associations with HbA1c by combining the FMI and FFMI to avoid collinearity of FMI and FFMI, which was consistent with previous studies that adjusted for FM[13]. In women classified under FMI Q4, higher FFMI tended to be associated with higher HbA1c levels. Sex hormones and menopause in women could affect body composition, and lead to different metabolic effects between men and women. Indeed, women had higher FMI and lower FFMI than men. Additionally, women classified under FMI Q4 in the present study had a greater increase in FMI with increasing FFMI than in other FMI subgroups. Therefore, this result might reflect the effect of FMI on HbA1c in women classified under FMI Q4. However, when we excluded participants who were identified as having diabetes, the FFMI was associated with higher HbA1c levels in most FMI subgroups. This result suggests that the association between FFMI and HbA1c level was influenced by higher HbA1c levels in participants with diabetes. Skeletal muscle is the main component of FFM, and contributes to glucose metabolism under insulin-stimulated conditions by disposing of glucose and storing glycogen[26,27]. Therefore, greater FFM might lead to better glucose homeostasis and glucose control. However, the present findings showed that the protective effect of FFM was not observed, even when the FMI was considered.

The present study had several strengths. It is the first to examine the relationship between the combination of FMI and FFMI with HbA1c. Because this study involved a large population of approximately 13,000 participants, we were able to classify 16 groups of combined FMI and FFMI quartiles based on sex. Therefore, we were able to show respective relationships with HbA1c due to differences in body composition.

However, the present study also had limitations. First, BF% was measured using the bioelectrical impedance method, which might have caused measurement errors. However, a high correlation between the whole-body FM measured using BIA and the whole-body FM measured using the dual-energy X-ray absorptiometry method has been verified (men $r = 0.95$, women $r = 0.92$)[28]. Therefore, we considered that this method was suitable for large epidemiological studies. Second, the present study included a Japanese population only. Compared with Western populations, Asians populations are more likely to develop diabetes, despite a low rate of overweight individuals and obesity[29,30]. Furthermore, body composition varies according to race. The FFMI differed among the four ethnic groups (white, African American, Hispanic and Asian), with African American individuals having the highest FFMI and Asian individuals having the lowest FFMI[31]. South Asian individuals have a higher body fat level and lower skeletal muscle mass than white individuals do[32]. Therefore, similar investigations in other populations are required. Finally, the present study had a cross-sectional design, and there was a causal relationship between the combined FMI and FFMI with HbA1c that could not be definitively established. Therefore, prospective cohort studies are required to clarify this causal relationship.

In conclusion, when the FFMI was not considered, the FMI tended to accompany higher HbA1c levels. When the FMI was not considered, the FFMI tended to accompany higher HbA1c levels. The FMI was also associated with higher HbA1c levels in all the FFMI subgroups. Although, the FFMI was not associated with higher HbA1c levels in most FMI subgroups among the whole population, when we excluded participants who were identified as having diabetes, the FFMI was still associated with higher HbA1c levels in most FMI subgroups.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: This study was approved by the institutional review board of the Tohoku Medical Megabank Organization (approval number: 2021-4-028, approval date: 31 May 2021).

Informed consent: Informed consent was obtained from all individuals.

Registry and the registration no. of the study/trials: N/A.

Animal studies: N/A.

DATA AVAILABILITY STATEMENT

All data used to support the findings of this study may be released upon application to the Tohoku Medical Megabank Organization (Sendai, Japan), which can be contacted through Prof. Atsushi Hozawa (email: hozawa@megabank.tohoku.ac.jp).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Participant characteristics according to the fat mass index.

Table S2 | Participant characteristics according to the fat-free mass index.

Table S3 | Adjusted least square means of glycated hemoglobin A1c associated with fat mass index and fat-free mass index excluding participants receiving treatment for diabetes.