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ORIGINAL RESEARCH

Relationships between fat deposition in the liver and skeletal muscle and insulin sensitivity in Japanese individuals: a pilot study

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Purpose: To evaluate the relationships between insulin sensitivity (IS), body fat accumulation, and aerobic capacity in middle- to older-aged Japanese participants with visceral adiposity.

Participants and methods: Aerobic capacity was measured during an incremental ramp exercise test. Computed tomography was used to measure visceral (VFA) and subcutaneous (SFA) fat area, the fat in liver-to-spleen ratio (L/S), and low-density skeletal muscle area (LDMA). IS was assessed using euglycemic-hyperinsulinemic clamps.

Results: A total of 11 males and 9 females, age 58 ± 9 years (mean ± standard deviation), body mass index 29 ± 4.1 kg/m^2, and VFA 190 ± 53 cm^2 participated in this study. In unadjusted models, VFA, LDMA, and L/S were significantly correlated with IS, which remained in adjusted models for LDMA and L/S, but not for VFA. In multiple stepwise regression analysis including sex, age, body fat, VFA, SFA, alcohol consumption, and aerobic capacity (oxygen uptake at the lactate threshold), L/S, and LDMA accounted for 70% of the total variance in IS. Percentage body fat and SFA, but not VFA, were significantly correlated with high molecular-weight adiponectin levels ($r = 0.58$, $P < 0.01$ and $r = 0.54$, $P < 0.05$, respectively). IS and L/S were significantly and negatively correlated with tumor necrosis factor-α ($r = -0.67$ and -0.63, respectively; both $P < 0.01$) and plasminogen activator inhibitor-1 ($r = -0.58$, $P < 0.01$ and -0.52, $P < 0.05$, respectively), whereas LDMA was not.

Conclusion: These findings indicate that ectopic fat deposition in the liver and skeletal muscle may be associated with peripheral IS independently of body fat accumulation and aerobic capacity in middle- to older-aged Japanese individuals with visceral adiposity. Because of the small sample size, additional larger studies are needed to provide further insight into these preliminary findings.

Keywords: aerobic capacity, fat in liver, lipid-rich skeletal muscle, visceral fat, subcutaneous fat, peripheral insulin sensitivity

Introduction

Recent studies have shown that ectopic fat deposition in the liver[1–3] and skeletal muscle[4,5] is negatively correlated with insulin sensitivity (IS), even in relatively lean individuals. Obese patients often exhibit fat accumulation in the liver and skeletal muscle, regardless of visceral fat (VF) accumulation.[1,6] This suggests that ectopic fat in either organ may be involved in the pathogenesis of declining IS.

Decreased aerobic capacity is correlated with metabolic risk[7] and declining IS,[8] independently of VF and subcutaneous fat (SF). Similarly, a correlation between aerobic capacity and liver fat has also been reported.[6,9] These findings suggest that VF, fat accumulation in the liver and skeletal muscle, and aerobic capacity each exert specific effects on the decline in IS.

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To evaluate the strength of the correlation between these parameters and IS, it is necessary to measure all of the parameters in the same participants. To our knowledge, only one study has measured IS in relation to VF, SF, liver fat, intramyocellular fat, and aerobic capacity.[10] That study of Finnish subjects showed that liver fat was strongly correlated with IS rather than with intramyocellular fat. Furthermore, patients with metabolic syndrome had significantly higher liver fat content than did those without metabolic syndrome, whereas there was no significant difference in intramyocellular fat between these groups. However, such information has been lacking with regard to the Japanese population.

Such information is needed because Japanese individuals show a higher risk for type 2 diabetes at lower levels of obesity as compared with Caucasian individuals. For example, Kadowaki et al reported that Japanese individuals had greater VF area (VFA) than did Caucasians.[11] Furthermore, the incidence of fatty liver was higher, despite the lower body mass index (BMI), in Japanese individuals than in Caucasians.[12] Thus, these data suggest there are ethnic differences in fat distribution between Asians (Japanese) and Caucasians. Despite these findings, the relationship between ectopic fat deposition in the liver or skeletal muscle and IS in Japanese individuals is still unclear.

Therefore, in an attempt to address this limited knowledge, we performed this preliminary study to explore the relationship between peripheral IS, body fat accumulation, and aerobic capacity in middle- to older-aged Japanese participants with visceral adiposity.

Participants and methods

Participants

Participants were Japanese individuals >40 years old with a waist circumference >85 cm (males) or >90 cm (females) according to the Japanese diagnostic criteria for metabolic syndrome in adults, and VFA > 100 cm^2 according to the Japanese clinical criteria for visceral adiposity. The subjects who were enrolled in this study were not taking any medications affecting IS. Furthermore, individuals who were taking medications likely to affect glucose or lipid metabolism were excluded from this study. We confirmed that none of the subjects had liver disease, other than fatty liver, based on their past medical histories. Whereas, diabetes mellitus was diagnosed by demonstrating both of the following: glycosylated hemoglobin (HbA_{1c}) ≥ 6.5% and fasting plasma glucose ≥ 126 mg/dL. This study was advertized in a local newspaper.

All of the participants completed a questionnaire related to their lifestyle, including their own and their families' medical history, alcohol consumption, cigarette smoking, and physical activity. Moderate alcohol intake was defined as consumption of >20 g/day, based on the questionnaire's evaluation of the quantity and frequency of alcohol consumption. Daily alcohol consumption was estimated as a continuous variable (g/day) based on the assumption that the concentrations of alcohol were 5% for beer, 12% for wine, 40% for liquor, and 25% for shochu (distilled spirit).

All of the participants gave informed consent, and the study was approved by the Ethics Committee of Fukuoka University and Kyushu Clinical Pharmacology Research Clinic, Fukuoka, Japan.

Outcome parameters

The subjects were studied on four separate occasions for the purposes of 1) screening, 2) a body composition and exercise test, 3) computed tomography (CT) (Toshiba Multi-CT Aquilion TSX-101A Scanner; Toshiba Medical Systems, Tokyo, Japan) examination, and 4) serum biochemical analysis and a euglycemic-hyperinsulinemic clamp. All measurements were performed within 4–8 weeks. The subjects were instructed to maintain their habitual lifestyle (exercise and food intake) during the study period. The subjects were asked to avoid strenuous physical activity for 2 days before each visit. The clamp and exercise tests were performed at least 1 week apart.

Anthropometry and body composition

All anthropometric measurements were made with participants wearing only light undergarments. Height was measured using a stadiometer to the nearest 0.1 cm. Bodyweight was measured using a calibrated balance beam scale (Shinko Denshi Vibra Co. Ltd, Tokyo, Japan) to the nearest 0.01 kg and was rounded to one decimal place. Body fat percentage (%FAT) was estimated using the Brozek formula with body density, which was estimated by hydrostatic weighing with correction for the residual lung volume measured by simultaneous O_2 re-breathing.[13] Fat-free mass was calculated using the formula: fat-free mass = bodyweight − (bodyweight × %FAT/100). Body surface area (BSA) was calculated using a formula reported by Fujimoto et al,[14] as BSA = height $(cm)^{0.66}$ × bodyweight $(kg)^{0.444}$ × 88.83. All anthropometric and body composition data were determined in the morning after a 12-hour overnight fast.

Computed tomography

CT was performed at a peak voltage of 135 kVp, 400 mA, and a scan time of 0.5 seconds with a 512 × 512 matrix.

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The tested scan field of view was set at 400 mm for six participants and 500 mm for 14 participants according to the anthropometry of each subject. VFA and SF area (SFA) were assessed at the L4–L5 intervertebral disc space. Six 2-mm-thick scans were obtained to reconstruct a 10-mm-thick volume dataset, which was used to calculate VFA and SFA using image analysis software (M900PRIMAL; Ziosoft Inc, Tokyo, Japan).

CT was also used to measure the mid-thigh muscle area and quantify low-density skeletal muscle area (LDMA), a marker of lipid-rich skeletal muscle. Six 2-mm-thick scans were obtained at the midpoint between the anterior iliac crest and the superior border of the patella of the thigh to reconstruct a 10-mm-thick volume dataset.

The fat in liver-to-spleen ratio (L/S) was determined as an index of liver fat, as previously described.[15] Briefly, six contiguous abdominal CT scans (2 mm thick) from T12 to L1 were obtained to reconstruct a 10-mm-thick volume dataset. LDMA and L/S were determined from the calculation of the Hounsfield units (HU) values using CT image analysis software on a Macintosh computer (OsiriX ver 3.3; OsiriX Foundation, Geneva, Switzerland). LDMA was quantified within a 0–30 HU attenuation window.[16] The mean HU values of the liver and spleen were also obtained to determine L/S.[15] All participants fasted for at least 3 hours, but were allowed water, before the CT examination.

Euglycemic-hyperinsulinemic clamp

After an overnight fast, each participant underwent a 2-hour euglycemic-hyperinsulinemic clamp. To maintain a plasma insulin level of 100 μU/mL and to suppress endogenous hepatic glucose output, insulin was infused at 40 mU/m^2/min. To maintain the plasma glucose level at 100 mg/dL, a continuous, adjustable infusion of 20% glucose solution was given and adjusted according to plasma glucose concentrations, which were measured every 5 minutes. IS was assessed as the metabolic clearance rate (MCR_G/I_C), which was expressed as the ratio of the glucose disposal rate to the plasma glucose and insulin concentrations,[17] at 2 hours after starting the clamp.

Serum biochemical analysis

Blood samples were obtained from an antecubital vein in the morning after a 12-hour overnight fast. Biochemical parameters included: glucose (hexokinase ultraviolet assay kit; Shino-Test, Tokyo, Japan), insulin (chemiluminescent enzyme immunoassay [CLEIA] kit; Fujirebio, Tokyo, Japan), HbA_{1c} (latex agglutination assay kit; Fujirebio), triglyceride (enzymatic kit; Sekisui Medical, Tokyo, Japan), HDL and LDL cholesterol (direct assay; Sekisui Medical), free fatty acids (enzymatic kit; Eiken Chemical, Tokyo, Japan), interleukin (IL)-6 (CLEIA kit; Fujirebio), high-sensitivity C-reactive protein (hsCRP) (nephelometry kit; Siemens Healthcare Diagnostics, Berlin, Germany), high molecular weight (HMW)-adiponectin (enzyme-linked immunosorbent assay [ELISA]; Fujirebio), tumor necrosis factor (TNF)-α (ELISA, R&D Systems, Minneapolis, MN), and plasminogen activator inhibitor (PAI)-1 (latex photometric immunoassay; Mitsubishi Chemical Medicine, Tokyo, Japan). All serum biochemical analyses were conducted by an independent company (SRL, Inc., Tokyo, Japan). HbA_{1c} level (%), represented as the National Glycohemoglobin Standardization Program value, was calculated from the level of HbA_{1c} derived by the Japan Diabetes Society value plus 0.4%.

Aerobic capacity

Aerobic capacity was assessed by the oxygen uptake at the lactate threshold (LT), determined using an incremental exercise test on a bicycle ergometer (Rehcor; Lode BV, Groningen, The Netherlands). The work rate was initially set at 10 watts for 4 minutes at a frequency of 60 rpm and was then continuously increased at 15, 10, or 8 watts/min during the ramp test. The increment was determined from the sex, age (men $<$65 years old, 15 watts; men $\geq$65 years old, 10 watts; women $<$65 years old, 10 watts; women $\geq$65 years old, 8 watts), or medical supervision. The test was continued until subjective exhaustion was achieved. Respiratory gas analysis was conducted using the mixing chamber method to evaluate the volume of expired air, and the O_2 and CO_2 fractions were analyzed by mass spectrometry (ARCO-1000; Arco Systems, Chiba, Japan). The LT was divided by fat-free mass. Earlobe blood samples were obtained every 30 seconds to measure lactate levels. Blood samples (20 μL) were collected in blood collection tubes and analyzed using a Biosen 5040-lactate analyzer (EKF Diagnostik, Barleban, Germany).

Physical activity

The subject's daily step counts were measured by a validated piezoelectric uniaxial accelerometer (Lifecorder EX; Suzuken Co, Ltd, Nagoya, Japan).[18] The number of steps taken per day was determined by accelerometry signals due to body movements. The subjects wore the accelerometer on their waist throughout the day, except while sleeping or bathing, every day for at least 2 weeks. All measured variables were averaged over the 7 consecutive days of the

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measurement period to assess physical activity in free-living conditions.

Statistical analysis

Data are expressed as means ± standard deviation. Pearson's correlation coefficients among MCR_G/I_C, %FAT, VFA, SFA, LDMA, L/S, and biochemical markers were calculated after applying Fisher's r-to-z transformation. MCR_G/I_C and biochemical markers with nonnormally distributed values were logarithmically transformed to approximate normal distributions. We believe that normalization of these factors is necessary for comparisons among all of the parameters, considering dimensional inference and units. For example, blood samples are usually normalized by the volume of blood, while anthropometric measures are normalized by body size. Therefore, VFA and SFA were normalized by BSA, and LDMA was normalized for the mid-thigh area. Partial correlation coefficient analysis was performed to determine independent relationships among VFA, L/S, LDMA, and alcohol consumption. Forward stepwise linear regression analysis was used to determine the effects of age, sex, fat accumulation (%FAT, VFA, SFA, LDMA, and L/S), estimated alcohol consumption (g/day), and LT as independent variables on IS. Sex was coded as male = 1 and female = 2. Values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed using SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA).

Results

Participants

A total of 56 participants responded to the advertisements. Of these, 20 middle- to older-aged individuals (age 43–73 years; BMI 22.9–38.5 kg/m^2; 11 males and 9 females) met the prespecified inclusion criteria (waist circumference >85 cm [males] or >90 cm [females]; VFA > 100 cm^2; not taking medications likely to affect IS) and underwent a euglycemic–hyperinsulinemic clamp, and were thus included in this study. None of the subjects had diabetes mellitus (5.7% ± 0.4% and 111 ± 14 mg/dL of HbA_{1c} and fasting plasma glucose on average) or liver disease. The characteristics of the participants are summarized in Table 1. The average daily step count was 6486 ± 2637 steps/day, indicating that these participants were mostly sedentary. Two men consumed moderate amounts of alcohol (>20 g/day) for at least the preceding year. Although the mean time between the first and last visit was 40.1 ± 6.8 days, there was no change in bodyweight between the first and last visits (75.8 ± 14.7 versus 75.7 ± 14.3 kg, respectively; $P = 0.78$).

Table 1 Participant characteristics

	All participants	Males	Females
Sex (males/females)	11/9		
Age (y)	58 ± 9	53 ± 8	64 ± 8[a]
Height (cm)	161.1 ± 10.5	169.0 ± 6.1	151.3 ± 4.4[b]
BSA (m^2)	1.76 ± 0.21	1.89 ± 0.14	1.59 ± 0.16[b]
Weight (kg)	75.7 ± 14.7	83.0 ± 12.2	66.9 ± 12.9[c]
BMI (kg/m^2)	29.0 ± 4.1	29.0 ± 3.8	29.1 ± 4.7
%FAT (%)	33.2 ± 7.7	27.7 ± 5.2	40.0 ± 3.5[b]
VFA (cm^2)	190 ± 53	205 ± 52	172 ± 52
VFA/BSA (cm^2/m^2)	108 ± 24	108 ± 23	107 ± 27
SFA (cm^2)	264 ± 99	216 ± 84	323 ± 85[c]
SFA/BSA (cm^2/m^2)	152 ± 57	113 ± 35	200 ± 37[b]
LDMA (cm^2)	33.7 ± 15.7	32.5 ± 15.3	35.0 ± 17.0
LDMA/mid-thigh area (cm^2/cm^2)	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
L/S ratio	1.11 ± 0.23	1.05 ± 0.25	1.18 ± 0.19
Glucose (mg/dL)	111 ± 14	115 ± 17	107 ± 10
Insulin (μIU/mL)	11.4 ± 3.7	12.3 ± 3.2	10.4 ± 4.2
HbA_{1c} (%)	5.7 ± 0.4	5.5 ± 0.3	5.8 ± 0.4
Triglyceride (mg/dL)	120 ± 73	153 ± 84	85 ± 35[c]
HDL (mg/dL)	45 ± 12	42 ± 10	48 ± 13
LDL (mg/dL)	116 ± 30	120 ± 24	111 ± 36
FFA (μEq/L)	1.5 ± 1.5	1.7 ± 2.1	1.3 ± 0.3
hsCRP (ng/mL)	820 ± 776	1062 ± 937	523 ± 392
IL-6 (pg/mL)[d]	1.7 ± 0.8	1.5 ± 0.7	1.9 ± 0.8
TNFα (pg/mL)	1.4 ± 0.7	1.3 ± 0.8	1.4 ± 0.5
HMW adiponectin (μg/mL)	3.9 ± 2.3	2.7 ± 1.2	5.5 ± 2.5[a]
PAI-1 (ng/mL)	30.8 ± 12.9	35.1 ± 14.8	25.4 ± 7.8
MCR (mL/kg/min)	4.1 ± 1.2	4.1 ± 1.4	4.2 ± 1.0
MCR_G/I_C (mL/kg/min per mU/L × 100)	3.1 ± 1.3	2.9 ± 1.2	3.4 ± 1.4
LT (mL/min/kg per FFM)[e]	16.9 ± 3.9	16.6 ± 3.7	17.5 ± 4.5
Step counts (steps/day)	6486 ± 2637	7485 ± 2686	5264 ± 2108
Alcohol consumption (g/day)	7.3 ± 9.2	11.8 ± 9.6	1.8 ± 5.0[c]

Notes: Data are expressed as mean ± standard deviation; [a]$P < 0.01$, significantly different versus men; [b]$P < 0.001$, significantly different versus men; [c]$P < 0.05$, significantly different versus men; [d]n = 18 (9 females); [e]n = 19 (8 females).

Abbreviations: BSA, body surface area; BMI, body mass index; %FAT, body fat percentage; VFA, visceral fat area; SFA, subcutaneous fat area; LDMA, low density muscle area; L/S, liver to spleen ratio; FFA, free fatty acids; hsCRP, high sensitivity C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; HMW, high molecular weight; PAI, plasminogen activator inhibitor; MCR, metabolic clearance rate; LT, oxygen uptake at the lactate threshold; FFM, fat-free mass.

Correlations between indices of fat accumulation

The correlations between indices of body fat accumulation (%FAT, VFA, SFA, LDMA, and L/S) are shown in Table 2. Both %FAT and SFA were significantly correlated with

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Table 2 Correlations among indices of body fat accumulation

	VFA	SFA	LDMA	L/S
%FAT	ns	0.92[a]	0.65[b]	ns
VFA		ns	ns	ns
SFA			0.57[b]	ns
LDMA				ns

Notes: [a]$P < 0.001$; [b]$P < 0.01$.
Abbreviations: VFA, visceral fat area/body surface area; SFA, subcutaneous fat area/body surface area; LDMA, low density muscle area/mid-thigh area; L/S, liver to spleen ratio; %FAT, body fat percentage; ns, not significant.

LDMA, whereas VFA and L/S were not significantly correlated with other indices of fat accumulation.

Correlations with IS

The correlations between indices of body fat accumulation, aerobic capacity and IS are shown in Table 3. Partial correlation coefficient analysis was performed to adjust the effects of all fat accumulation and aerobic capacity parameters. In unadjusted models, VFA, LDMA, and L/S were significantly correlated with MCR_G/I_C, which remained in adjusted models for LDMA and L/S, but not for VFA. In contrast, %FAT, SFA, and LT were not significantly correlated with MCR_G/I_C, even after adjusting for other factors such as VFA, LDMA, or L/S. In addition, L/S was significantly correlated with MCR_G/I_C, even after adjusting for alcohol consumption ($r = 0.70$, $P < 0.01$; data not shown) and all parameters ($r = 0.74$, $P < 0.01$; data not shown).

As shown in Table 4, multiple stepwise regression analysis was performed to investigate the relative contribution of nine variables, namely sex, age, %FAT, VFA, SFA, LDMA, L/S, LT, and alcohol consumption, to MCR_G/I_C. This analysis showed that L/S was the main determinant of MCR_G/I_C, explaining 47% of the variance. A further 23% of the variance in MCR_G/I_C was attributed to LDMA after accounting for L/S; thus, L/S and LDMA explained 70% of the total variance in this model.

Table 3 Correlations among indices of fat accumulation, aerobic capacity, and insulin sensitivity

Index	MCR_G/I_C			
	Unadjusted	Model 1	Model 2	Model 3
%FAT	ns	ns	ns	ns
VFA	–0.46[a]	–	ns	ns
SFA	ns	ns	ns	ns
LDMA	–0.57[b]	–0.48[a]	–	–0.69[b]
L/S	0.70[b]	0.66[b]	0.78[c]	–
LT	ns	ns	ns	ns

Notes: Model 1 is adjusted for VFA; Model 2 is adjusted for LDMA; Model 3 is adjusted for L/S; [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.
Abbreviations: %FAT, body fat percentage; VFA, visceral fat area/body surface area; SFA, subcutaneous fat area/body surface area; LDMA, low density muscle area/mid-thigh area; L/S, liver to spleen ratio; LT, oxygen uptake at lactate threshold; MCR_G/I_C, metabolic clearance rate; ns, not significant.

Table 4 Multiple stepwise regression analysis for independent determinants of insulin sensitivity

		R^2	SEE	F value	*P* value
Step 1	L/S	0.47	0.13	17.2	<0.001
Step 2	L/S + LDMA	0.70	0.10	21.7	<0.001

Notes: Outcome variable: MCR_G/I_C; Independent variables: sex, age, %FAT, VFA, SFA, LDMA, L/S, LT, and alcohol consumption.
Abbreviations: %FAT, body fat percentage; VFA, visceral fat area/body surface area; SFA, subcutaneous fat area/body surface area; LDMA, low density muscle area/mid-thigh area; L/S, liver to spleen ratio; LT, oxygen uptake at the lactate threshold; MCR_G/I_C, metabolic clearance rate.

Correlations between indices of fat accumulation and plasma parameters

As shown in Table 5, HMW adiponectin was significantly associated with %FAT and SFA, while IL-6 was significantly correlated with %FAT. TNF-α and PAI-1 levels were significantly and negatively correlated with L/S as well as MCR_G/I_C, while hsCRP was negatively correlated with MCR_G/I_C. Surprisingly, none of the plasma parameters were associated with VFA or LDMA.

Discussion

This is the first study to evaluate associations among ectopic fat deposition in the liver and muscle, VF and SF accumulation, aerobic capacity, and peripheral IS in a cohort of Japanese subjects using insulin clamp techniques. The findings of the study indicate that L/S and LDMA are significantly associated with peripheral IS independently of other indices of body fat accumulation and aerobic capacity in middle- to older-aged Japanese individuals with visceral adiposity.

Table 5 Correlations among cytokines, indices of body fat accumulation, and insulin sensitivity

	%FAT	VFA	SFA	LDMA	L/S	MCR_G/I_C
FFA	ns	ns	ns	ns	ns	ns
HMW adiponectin	0.58[a]	ns	0.54[b]	ns	ns	ns
IL-6	0.56[b]	ns	ns	ns	ns	ns
hsCRP	ns	ns	ns	ns	ns	–0.57[a]
TNFα	ns	ns	ns	ns	–0.67[a]	–0.63[a]
PAI-1	ns	ns	ns	ns	–0.52[b]	–0.58[a]

Notes: MCR_G/I_C and cytokine concentrations were logarithmically transformed to approximate normal distributions; [a]$P < 0.01$; [b]$P < 0.05$.
Abbreviations: %FAT, percentage body fat; VFA, visceral fat area/body surface area; L/S, liver to spleen ratio; LDMA, low density muscle area/mid-thigh area; MCR_G/I_C, metabolic clearance rate; FFA, free fatty acid; HMW adiponectin, high molecular weight adiponectin; IL, interleukin; hsCRP, high sensitivity C-reactive protein; TNF, tumor necrosis factor; PAI-1, plasminogen activator inhibitor-1; ns, not significant.

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A previous study revealed that the prevalence of insulin resistance and nonalcoholic fatty liver disease in a similar ethnic group of Asian-Indians was higher than that in age- and weight-matched White, Black, and Hispanic men.[19] However, the characteristics of participants in that study were very different to those of the present study (for example, age 28.7 ± 8.3 versus 58.2 ± 9.3 years and BMI 22.4 ± 2.3 versus 29.0 ± 4.1 kg/m^2), limiting our ability to directly compare the two studies. Furthermore, differences in the risk of developing hepatic/skeletal muscle fat accumulation and its association with IS are not only due to race/ethnicity but also lifestyle (for example, physical activity and/or diet) and social factors. Indeed, the incidence rate of fatal coronary heart disease, which is strongly associated with glucose intolerance, in Japanese individuals living in Hawaii is approximately twice that in Japanese individuals living in Japan.[20]

Our results are consistent with those of earlier studies,[1,3] which indicate that L/S is associated with peripheral IS independently of other parameters of body fat accumulation in Japanese individuals with visceral adiposity. In addition, our study revealed that of various fat indices, liver fat is the most important determinant of IS. These results are consistent with the previous studies.[21–23] However, in contrast to the present study, Kirchhoff et al[21] reported that IS was associated with liver fat and VF, as well as SF. These different results might be due to the smaller range of values for parameters in the present study compared with those in the study by Kirchhoff et al.

The present study also showed that liver fat and cytokines PAI-1 and TNF-α were associated with IS. PAI-1 and TNF-α are associated with fat accumulation, particularly nonalcoholic fatty liver disease.[24–26] Furthermore, several studies have revealed that PAI-1 and TNF-α may directly contribute to insulin resistance.[27,28] Our results are consistent with several studies showing that insulin resistance is associated with an increase in circulating TNF-α and PAI-1 concentration.[29,30] Unexpectedly, the present study revealed that ectopic fat and IS were not associated with HMW adiponectin levels, whereas other studies have reported that HMW adiponectin was strongly associated with ectopic fat, IS, and metabolic syndrome.[31,32] On the other hand, Petersen et al reported that muscle insulin resistance can arise independently of changes in circulating adipocytokine concentrations, particularly adiponectin, TNF-α, IL-6, and resistin.[33] The relationships among cytokine levels, ectopic fat, and insulin resistance are still being debated, and more studies are clearly needed in this field.

Previous studies have proposed a relationship between VF and liver fat accumulation, as well as their associations with metabolic disease and insulin resistance.[34,35] In this study, L/S was not significantly associated with VFA adjusted for BSA, although it was significantly associated with VFA calculated as an absolute value (that is, cm^2; $r = -0.45$, $P < 0.05$). Similarly, Seppala-Lindroos et al reported no correlation between L/S and VFA.[1] Although the reasons for these apparent discrepancies are unclear, it is possible that differences in the characteristics of the study populations, such as ethnicity, presence of diabetes or metabolic diseases, and general metabolic status will be potential factors. It is also possible that the methods used to determine VFA, and whether VFA is corrected for body size, are responsible for the differences between studies. We believe that it is important to normalize VFA for BSA to provide more accurate comparisons between people of different sizes.

In terms of markers for metabolic dysfunction, Fabbrini et al reported that intrahepatic triglyceride content, but not visceral adipose tissue volume, is a marker for obesity-related metabolic dysfunction,[36] while Petersen et al found no difference in the mean volume of intra-abdominal fat between lean insulin-resistant and BMI-matched insulin-sensitive subjects.[37] However, considering that all the participants of the present study were viscerally obese, our findings may not apply for lean subjects.

Skeletal muscle is the primary site for the regulation of glucose metabolism in the human body.[38] Intramyocellular fat causes a decline in IS, thus impairing glucose and lipid metabolism.[4,5] Furthermore, several studies have suggested that decreases in IS are more strongly correlated with skeletal muscle fat deposition than with other indices of body fat accumulation.[4,39,40] Interestingly, chronic exercise increases muscle lipid content and muscle IS, while reducing diacylglycerol content.[41] In fact, improvements in IS can be achieved by a single bout of exercise, with increases in the muscle concentrations of triglycerides and reduced concentrations of diacylglycerol.[42] Similarly, another study revealed a significant relationship between skeletal muscle fat deposition and an increase in physical activity.[43] On the basis of these findings, one may hypothesize that aerobic capacity and physical activity would be closely associated with IS. Here, we found that LDMA was negatively correlated with IS, %FAT, and SFA, but not with VFA. Surprisingly, LT was not significantly correlated with any of the indices of fat accumulation. Thus, the magnitude and type of associations between LDMA as a marker for skeletal muscle fat, aerobic capacity, and IS may

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be affected by the type of fat and the extent of exercise; subjects in our study were relatively sedentary according to their LT and daily step count. However, this differs from the findings reported by Kotronen et al, who reported that liver fat and aerobic capacity were related to muscle IS in normal to obese Finnish subjects aged 18–60 years.[44] These differences might be explained as follows. First, we used LT as the index of aerobic capacity because the assessment of the maximum oxygen uptake ($VO_{2\,max}$) is often difficult in subjects with low aerobic capacity, particularly in elderly and obese subjects. Therefore, we used LT because it can be measured during a submaximal exercise test, and it is a reliable marker for aerobic capacity.[45] Second, the aerobic capacity (LT) of our subjects was spread over a relatively narrow range, limiting the extent of associations with other parameters.

This study has several limitations. First, the first and last measurements were taken a mean of 40.1 ± 6.8 days apart, and it is possible that fat deposition may change over this time. However, the subjects' bodyweights did not change during this period, and any changes in liver fat and related metabolic parameters would be very small. Previous studies showed that neither liver fat nor bodyweight changed in a control group of subjects over a 4–6-week observation period.[46,47] Second, two men consumed moderate amounts of alcohol (>20 g/day) for at least the preceding year, although alcohol intake was not excessively high (23 and 27 g/day). Even if we excluded these participants, the associations between IS and ectopic fat (LDMA, L/S) determined by correlation analysis (nonadjusted model and adjusted model) and multiple stepwise regression analysis were not affected. Third, fat in the liver and skeletal muscle were not assessed using magnetic resonance imaging or magnetic resonance spectrometry, two noninvasive methods that are more accurate than the methods used in this study. LDMA, a marker of lipid-rich skeletal muscle, was quantified using CT and cannot distinguish between extra- and intramyocellular lipids. Nevertheless, CT assessment of fat in liver and skeletal muscle shows high correlations with needle biopsy-based assessment of tissue fat content.[4,48,49] Fourth, this was a cross-sectional study of middle- to older-aged Japanese subjects with VF accumulation but without marked metabolic disorders. Thus, these results may not be applicable to other groups of subjects, particularly younger subjects or those without visceral obesity. This design also limited our analysis to correlations, preventing assessment of causality or changes over time. Fifth, the sample size of this preliminary study was small (20 subjects) and included males and females, although we found no significant relationship between IS and sex. On the other hand, we used a gold-standard technique – the euglycemic–hyperinsulinemic clamp technique – to determine IS in a quantitative manner, whereas other studies used less rigorous tests, such as tolerance tests and median values as cutoff values to define insulin resistance or IS. Furthermore, we attempted to minimize the influence of confounding variables such as sex and alcohol intake by performing multiple regression analysis, with the inclusion of these and other factors as possible influencing/confounding factors. Clearly, further studies in a larger cohort of subjects are needed to confirm our findings. Such studies should compare associations with IS in lean and obese subjects, physically active and inactive subjects, and among different ethnic groups. Similarly, longitudinal cohort and interventional (for example, with weight loss interventions) studies would provide further insight into the associations between liver and skeletal muscle fat and IS, and the effects of changes in lifestyle factors on these associations.

In conclusion, the findings of this study indicate that ectopic fat deposition in the liver and skeletal muscle is associated with peripheral IS independently of indices of body fat accumulation and aerobic capacity in middle- to older-aged Japanese individuals with visceral adiposity. Although our study cannot reveal the underlying mechanisms, our findings provide new insight as liver fat and cytokine levels (PAI-1 and TNF-α) were significantly associated with IS determined by euglycemic–hyperinsulinemic clamps in a cohort of Japanese subjects.

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Disclosure

The authors report no conflicts of interest in this work.

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