

Fatty Liver Has Stronger Association With Insulin Resistance Than Visceral Fat Accumulation in Nonobese Japanese Men

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Context: Asians have a high prevalence of insulin resistance, even in the nonobese state. Whereas both visceral fat accumulation (VFA) and fatty liver (FL) have been shown to be associated with insulin resistance, it is still unclear which is a better marker to predict insulin resistance in nonobese Asians.

Objective: The aim of this study was to investigate the relation between VFA or FL and insulin resistance in nondiabetic nonobese Japanese men who do not have diabetes.

Design and Participants: We studied 87 nonobese (body mass index <25 kg/m²) Japanese men without diabetes. Using a two-step hyperinsulinemic euglycemic clamp, we evaluated insulin sensitivity in adipose tissue, muscle, and liver. Intrahepatic lipid and abdominal visceral fat area were measured by ¹H-magnetic resonance spectroscopy and MRI, respectively. Subjects were divided into four groups based on the presence or absence of VFA (visceral fat area ≥100 cm²) and FL (intrahepatic lipid ≥ 5%): control (non-VFA, non-FL; n = 54), VFA only (n = 18), FL only (n = 7), and VFA plus FL (n = 8).

Results: Subjects in the FL only and VFA plus FL groups had insulin resistance in adipose tissue and muscle, as well as relatively lower hepatic insulin sensitivity. The specific insulin sensitivities in these organs were comparable in the VFA only and control groups.

Conclusions: In nonobese Japanese men without diabetes, subjects with FL only or VFA plus FL but not VFA only had insulin resistance, suggesting that FL may be a more useful clinical marker than VFA to predict insulin resistance in nonobese Japanese men without diabetes.

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Freeform/Key Word: insulin resistance, fatty liver, visceral fat, ectopic fat, nonobese

Abbreviations: BMI, body mass index; FFA, free fatty acid; FL, fatty liver; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; MCRI, metabolic clearance rate of serum insulin; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; VFA, visceral fat accumulation.

The number of individuals with type 2 diabetes mellitus has been increasing worldwide, particularly in Asia [1]. Insulin resistance is recognized as an important contributor to the development of type 2 diabetes and obesity exacerbates its onset [2], however, several studies suggested that insulin resistance in nonobese Asians is also an important contributor to metabolic abnormalities [3–6]. For example, we performed a two-step hyperinsulinemic euglycemic clamp study in 90 nonobese [body mass index (BMI) <25 kg/m²] Japanese men who did not have diabetes and found that even those with only one cardiometabolic risk factor had insulin resistance in muscle but not in liver [4], and the metabolic abnormalities in these subjects were closely associated with insulin resistance [4]. However, the mechanisms of insulin resistance in nonobese Asians have not yet been fully elucidated.

Insulin resistance in nonobese Asians may be caused by at least in part by dysregulated lipid storage [7]. Several studies have revealed that Asians, especially East Asians [8], have a low fat-storage capacity in their subcutaneous adipose tissue [8–10]. Even moderate weight gain in East Asians may easily exceed the lipid storage capacity of subcutaneous adipose tissue [8–10], and thus lipid may accumulate in visceral adipose tissue, muscle, and liver, the so-called lipid spillover hypothesis. Accumulation of visceral and ectopic fat may therefore somehow elicit insulin resistance in muscle and liver [7, 11]. Indeed, visceral fat accumulation (VFA) is closely associated with insulin resistance in nonobese East Asians [3–7]. Also, Asians were found to easily develop nonalcoholic fatty liver disease (NAFLD) [10, 12, 13], and intrahepatic lipid (IHL) accumulation measured by ¹H-magnetic resonance spectroscopy (MRS) has been closely associated with insulin resistance in nonobese Asians [6] and nonobese Japanese men with [14] and without diabetes [4].

Against this background, it is unsurprising that VFA has been shown to correlate with fatty liver (FL) [15], and that both VFA and FL predict insulin resistance in nonobese Asians [4, 14]. However, although FL is reported to be a better marker of obesity-related metabolic derangements than VFA in obese patients [16], it is still unclear which parameter better predicts insulin resistance in nonobese Asians. To address this issue, we compared the degree of insulin sensitivity among four groups defined by the presence and absence of VFA and FL, based on our previous data on tissue-specific insulin sensitivity derived using a two-step euglycemic hyperinsulinemic clamp in 87 nonobese (BMI < 25 kg/m²), middle-aged Japanese men without diabetes [4].

1. Research Design and Methods

A. Subjects

The Sportology Center Core Study was a prospective observational study involving hypothesis-driven, hypothesis-generating research on the mechanisms underlying metabolic abnormalities in nonobese Japanese subjects without diabetes [4]. In that study, we recruited Japanese men without diabetes with a BMI of 21 to 27.5 kg/m² (≥ 21.0 to <27.5 kg/m²) and between 30 and 50 years of age. In the current study, we selected those with a BMI of 21 to 25 kg/m² (≥ 21.0 to <25.0 kg/m²).

All participants gave written informed consent to participate in the study. This study was approved by the ethics committee of Juntendo University and carried out in accordance with the principles outlined in the Declaration of Helsinki.

B. Study Design and Measurements

All subjects were recruited at a screening session and were examined at three subsequent visits for baseline evaluation. We performed a two-step euglycemic hyperinsulinemic clamp combined with stable glucose tracer methodology, each for a duration of 180 minutes, with a constant insulin infusion of 10 and 20 mU/m² per minute (0 to 180 and 180 to 360 minutes, respectively). The metabolic clearance rate of serum insulin (MCRI) during glucose clamp at the second step was calculated as previously described [17]. The IHL and intramyocellular

lipid (IMCL) were measured with ^1H -MRS and percent fat was measured bioimpedance method; biochemical tests were measured by standard methods and hormonal levels were measure by radioimmunoassay or enzyme-linked immunosorbent assay. These and other methods have been previously reported in detail [4].

C. Statistical Analysis

Data are presented as mean \pm SD. The relation between IHL and VFA was assessed by Spearman rank correlation coefficient. Data were compared by one-way ANOVA or Kruskal-Wallis analysis for continuous variables, and groups were compared using the Tukey-Kramer or Games-Howell *post hoc* tests. All statistical tests were two-sided with a 5% level of significance.

2. Results

A. Comparison of Body Fat Distribution and Fat Derived Factors Among Groups

The anthropometric data of the study subjects are shown in Table 1. All subjects were middle-aged men, and the mean values of BMI, IHL, abdominal visceral fat area, and cardiometabolic risk factors were within the normal ranges. The VFA was correlated with IHL, but correlation coefficient was low ($r_s = 0.32$, $P = 0.003$) (Fig. 1). According to the generally

Table 1. Clinical Characteristics of the Study Subjects

	Total	Control	VFA Only	FL Only	VFA Plus FL	P^a
Number of subjects	87	54	18	7	8	
IHL, %	3.2 \pm 4.3	1.3 \pm 1.5	2.0 \pm 1.6	12.5 \pm 3.0 ^{b,c}	10.4 \pm 4.6 ^{b,c}	<0.001
Abdominal visceral fat area, cm ²	87.3 \pm 31.2	69.7 \pm 16.9	123.2 \pm 20.5 ^b	76.9 \pm 16.6 ^c	134.3 \pm 13.7 ^{b,d}	<0.001
Age, y	42.1 \pm 5.1	41.4 \pm 5.1	43.2 \pm 4.4	40.6 \pm 5.4	46.3 \pm 4.5	0.046
BMI, kg/m ²	23.5 \pm 1.0	23.3 \pm 1.1	23.8 \pm 0.7	23.6 \pm 0.7	24.1 \pm 0.6 ^b	0.033
Waist circumference, cm	83.6 \pm 5.2	81.6 \pm 4.6	87.0 \pm 3.5 ^b	84.8 \pm 6.2	88.2 \pm 4.7 ^b	<0.001
Total body fat content, %	21.5 \pm 4.1	20.7 \pm 4.4	22.1 \pm 2.7	20.9 \pm 3.4	26.1 \pm 2.2 ^{b,c,d}	<0.001
Abdominal subcutaneous fat area, cm ²	120.9 \pm 37.3	113.2 \pm 38.8	130.5 \pm 32.5	123.7 \pm 32.8	148.6 \pm 25.7	0.044
Fasting plasma glucose, mg/dL	96.1 \pm 7.5	95.0 \pm 7.7	96.1 \pm 5.7	101.9 \pm 7.1	98.9 \pm 8.1	0.090
Fasting serum insulin, $\mu\text{U/mL}$	5.8 \pm 2.5	5.0 \pm 1.7	6.2 \pm 2.4	8.9 \pm 2.9 ^b	8.2 \pm 3.2	<0.001
Triglyceride, mg/dL	141.0 \pm 76.9	120.6 \pm 67.0	170.9 \pm 93.4	193.1 \pm 82.4	166.0 \pm 53.2	0.005
Free fatty acid, $\mu\text{Eq/L}$	372.6 \pm 107.9	342.8 \pm 108.1	412.2 \pm 87.6	435.7 \pm 78.6	437.6 \pm 103.9	0.007
Aspartate aminotransferase, IU/L	22.2 \pm 6.8	20.6 \pm 5.9	23.3 \pm 6.4	29.1 \pm 10.0 ^b	24.6 \pm 6.4	0.007
Alanine aminotransferase, IU/L	25.7 \pm 15.3	22.3 \pm 9.7	23.1 \pm 6.9	45.1 \pm 36.4	37.3 \pm 16.1	0.005
γ -glutamyl transferase, IU/L	45.1 \pm 40.9	40.1 \pm 43.7	51.5 \pm 42.1	57.0 \pm 22.0	54.6 \pm 27.8	0.021
High molecular weight adiponectin, $\mu\text{g/mL}$	1.5 \pm 1.1	1.8 \pm 1.2	1.0 \pm 0.7 ^b	0.6 \pm 0.3 ^b	1.0 \pm 0.8	0.001
VO _{2peak} , mL/kg/min	32.3 \pm 6.6	33.8 \pm 6.4	31.6 \pm 6.0	29.6 \pm 3.5	25.7 \pm 7.4 ^b	0.006

Data are expressed as mean \pm SD.

Abbreviation: VO_{2peak}, peak oxygen consumption.

^a P value for one-way ANOVA or Kruskal-Wallis analysis.

^b P < 0.05 for Tukey-Kramer or Games-Howel; vs control.

^c P < 0.05 for Tukey-Kramer or Games-Howel; vs VFA Only.

^d P < 0.05 for Tukey-Kramer or Games-Howel; vs FL Only.

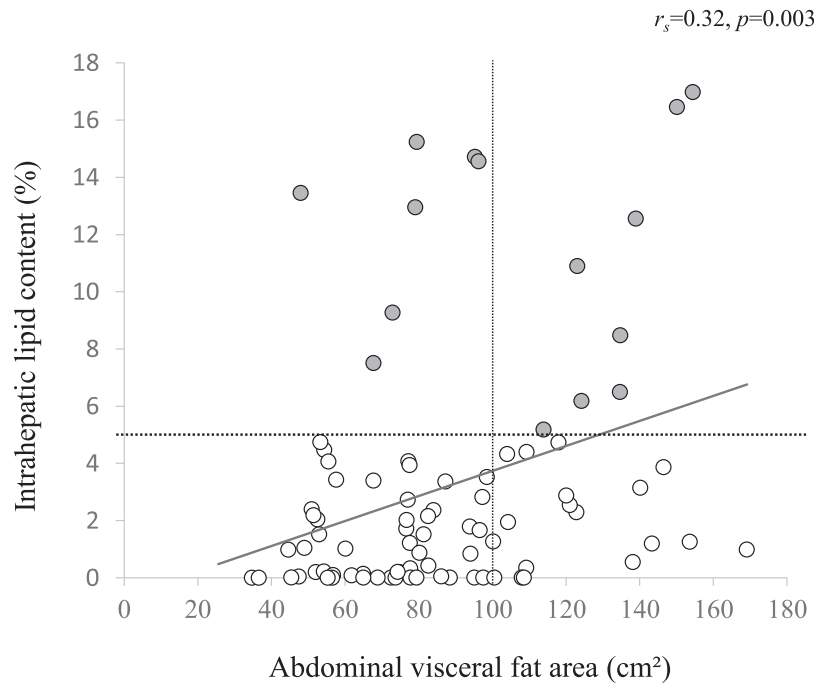


Figure 1. Relation between abdominal visceral fat area and IHL content in all subject. Dashed lines indicate the cutoffs for the grouping of VFA (visceral fat area ≥ 100 cm²) and FL (IHL $\geq 5\%$) in the current study. Open circles, subjects without FL; gray circles, subjects with FL.

accepted definition, subjects were divided into the following four groups based on the presence or absence of VFA (visceral fat area ≥ 100 cm²) [18] and FL (IHL $\geq 5\%$) [15]: control group (non-VFA, non-FL; n = 54), VFA only group (n = 18), FL only group (n = 7), and VFA plus FL group (n = 8) (Fig. 1). The anthropometric data in each group are shown in Table 1. Trends of BMI, waist circumference, total body fat content, and abdominal subcutaneous fat area in all four groups resembled the trends of VFA rather than FL. On the other hand, IMCL levels were comparable among the groups (data not shown). In terms of adipose tissue-derived factors, free fatty acid (FFA) was elevated and total and high molecular weight adiponectin were reduced in the VFA only, FL only and VFA plus FL groups relative to the control group.

Table 2. Euglycemic Hyperinsulinemic Clamp Data

	Control	VFA Only	FL Only	VFA Plus FL	<i>P</i> ^a
%Reduction of EGP/SS _{SI} at first step, %/ $\mu\text{U}\cdot\text{mL}^{-1}$	3.6 \pm 1.0	3.7 \pm 0.8	2.6 \pm 0.7	2.7 \pm 1.2	0.006
Rd/SS _{SI} at second step, mg/kg FFM $\cdot\text{min}^{-1}/\mu\text{U}\cdot\text{mL}^{-1}$	0.23 \pm 0.07	0.21 \pm 0.09	0.12 \pm 0.04 ^b	0.13 \pm 0.04 ^b	<0.001
%FFA suppression/insulin at first step, %/ $\mu\text{U}\cdot\text{mL}^{-1}$	4.4 \pm 1.0	3.9 \pm 0.9	2.7 \pm 0.7 ^{b,c}	2.8 \pm 1.0 ^b	<0.001
MCRI at second step, mL/min per m ²	551.7 \pm 78.7	536.7 \pm 125.4	478.9 \pm 52.9	488.1 \pm 77.4	0.080

Data are expressed as mean \pm SD.

Abbreviations: SS_{SI}, steady-state serum insulin; EGP, endogenous glucose production; Rd, rate of disappearance.

^a*P* value for one-way ANOVA or Kruskal-Wallis analysis.

^b*P* < 0.05 for Tukey-Kramer or Games-Howel; vs control.

^c*P* < 0.05 for Tukey-Kramer or Games-Howel; vs VFA only.

B. Insulin Sensitivity in Adipose Tissue, Muscle and Liver

Table 2 shows the data from the two-step hyperinsulinemic euglycemic clamp study. Hepatic insulin sensitivity, defined as percent reduction of endogenous glucose production/steady-state serum insulin at the first step [19], was relatively lower in the FL only and VFA plus FL groups than in the other two groups. On the other hand, muscle insulin sensitivity, defined as rate of disappearance/steady-state serum insulin at the second step [20], were lower in the FL only and VFA plus FL groups compared with the control group. Also, adipose tissue insulin sensitivity, defined as FFA suppression/insulin at the first step [19], was substantially lower in both the FL only and VFA plus FL groups compared with the control group. Consistently, our preliminary analysis showed that compared with one to one matched control subjects by age and BMI [21], the FL only group and VFA plus FL group had substantially or relatively impaired insulin sensitivity in muscle, liver, and adipose tissue, respectively; however, insulin sensitivity in those were comparable between age and BMI matched control and VFA only group (data not shown).

3. Discussion

In nonobese Asians, as in other ethnic groups, VFA and FL were reported to be associated with insulin resistance and metabolic abnormalities [3–7, 14], however, it is still unclear which parameter better predicts insulin resistance. In the current study, we found that subjects with FL but without VFA had insulin resistance in adipose tissue and muscle, as well as relatively lower hepatic insulin sensitivity. In contrast, subjects with VFA but without FL showed similar insulin sensitivity of muscle, liver, and adipose tissue as control subjects. Subjects with both VFA and FL had more body fat than other groups, but similar insulin resistance as subjects with FL alone. Although VFA has been recognized as a clinical marker to predict insulin resistance [3–7, 14], the current study showed that nonobese Japanese men with VFA alone (without FL) did not demonstrate insulin resistance and their insulin sensitivity was similar to men without VFA or FL. In contrast, the presence of FL alone was well associated with insulin resistance in adipose tissue and muscle.

The FL only group showed insulin resistance in adipose tissue as well as muscle, despite the low fat content in adipose tissue. Previously, it was demonstrated that compared with individuals of other ethnicities, including white, black, Hispanic, and Southeast Asian, East Asians have a lower fat-storage capacity in their subcutaneous adipose tissue and the highest accumulation of visceral fat with increasing adiposity [8]. However, in this study, subjects in the FL only group did not seem to store lipids sufficiently in either visceral or subcutaneous fat, which may have caused insulin resistance. Indeed, another indicator of adipose tissue dysfunction, namely decreased adiponectin levels, was observed in the FL only group. Adiponectin is one of the adipokines that promotes lipid oxidation in muscle and liver and maintains insulin sensitivity [22]. Indeed, decreased adiponectin levels were previously linked to FL and insulin resistance in humans [16, 23], and also associated with increased IMCL and impaired peripheral insulin sensitivity after a high-fat diet in nonobese men [24]. Although adiponectin levels were also decreased in the VFA only and VFA plus FL groups, they were lowest in the FL only group despite this group's lower adiposity. Thus, despite lower adiposity, the FL only group demonstrated obvious adipose tissue dysfunction.

Although VFA has been established as a marker of insulin resistance in nonobese East Asians [3–7], subjects with VFA but without FL did not have insulin resistance in the present study. In contrast, as discussed above, subjects with FL but without VFA had insulin resistance in adipose tissue and muscle. However, this is not surprising if adipose tissue insulin resistance is considered as upstream of insulin resistance in muscle and liver as well as IHL accumulation. For example, circulating FFAs are a major source of liver fat in NAFLD patients [25]. Adipose tissue insulin resistance is associated with insulin resistance in muscle and liver in obese subject [26]. The FFA elevation by lipid infusion induces insulin resistance in muscle and liver in healthy individuals [27, 28]. Our recent study also revealed that in nonobese apparently

healthy Japanese men, reduced adipose tissue insulin sensitivity was associated with moderate IHL accumulation and muscle insulin resistance [19]. Taken together, it is speculated that FL but not VFA alone is a good marker to predict adipose tissue insulin resistance, thus FL is a better predictor for insulin resistance in peripheral tissues than VFA.

Unlike our results, Ding *et al.* [29] demonstrated that VFA is a better marker for insulin resistance than IHL accumulation in nonobese Asian. We do not know exact reason why this discrepancy occurred, however, we suppose that the differences in study subjects and protocol may contribute to the opposite results. For example, one large difference was observed in the degree of IHL accumulation. In the study, the IHL level in the high IHL group was 4.4% (median), whereas it was 12.5% (mean) in the current study (Table 1). In addition, ~40% of study subjects were female and BMI and VO₂max were matched among the groups [29].

The current study has several limitations. First, we included only men. Fat distribution differs between women and men and therefore it is unclear if our data can be applied to women. Second, both the FL and VFA plus FL groups contained a relatively small number of subjects. However, because two-step hyperinsulinemic euglycemic clamp is a complicated technique, the number of subjects is considered reasonable. Third, this study showed clinical relevance of FL and VFA as a marker of insulin resistance, thus the causal relations involved have not been identified. There may be several potential confounders of the association between FL or VFA and insulin resistance [29–31].

In conclusion, in nonobese Japanese men without diabetes, subjects with FL alone or VFA plus FL demonstrated insulin resistance in adipose tissue and muscle, whereas subjects with VFA alone did not. These data suggest that FL (IHL \geq 5%) may be a useful marker than VFA (visceral fat area \geq 100 cm²) for predicting insulin resistance in nonobese Japanese men without diabetes.

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