

# Insulin, C-Peptide, and Leptin Concentrations Predict Increased Visceral Adiposity at 5- and 10-Year Follow-Ups in Nondiabetic Japanese Americans

Jenny Tong,<sup>1</sup> Wilfred Y. Fujimoto,<sup>1</sup> Steven E. Kahn,<sup>1</sup> David S. Weigle,<sup>1</sup> Marguerite J. McNeely,<sup>2</sup> Donna L. Leonetti,<sup>3</sup> Jane B. Shofer,<sup>4</sup> and Edward J. Boyko<sup>5</sup>

We prospectively examined the relationship between leptin and markers of insulin resistance and secretion and future visceral adipose tissue accumulation. In this study, 518 nondiabetic Japanese-American men and women underwent the following measurements at baseline and at 5- and 10-year follow-ups: plasma glucose and insulin measured after an overnight fast and during a 75-g oral glucose tolerance test, insulin secretion ratio (ISR) [(30-min insulin – fasting insulin)/30-min glucose], fasting C-peptide levels, plasma leptin (baseline only), and fat areas (intra-abdominal and subcutaneous) measured by computed tomography. Predictors of future intra-abdominal fat (IAF) were determined using multiple linear regression. Fasting insulin and C-peptide levels at baseline were significantly associated with IAF area at 5 years (coefficient = 0.041,  $P = 0.001$  and coefficient = 1.283,  $P < 0.001$ , respectively) and 10 years (coefficient = 0.031,  $P = 0.020$  and coefficient = 0.221,  $P = 0.035$ , respectively). ISR was not significantly associated with IAF at 5 or 10 years. Leptin level at baseline was positively associated with IAF at 5 years (coefficient = 0.055,  $P = 0.002$ ) and 10 years (coefficient = 0.059,  $P = 0.003$ ). In conclusion, higher levels of fasting insulin, C-peptide, and circulating leptin level predicted visceral fat accumulation independent from subcutaneous fat accumulation in nondiabetic Japanese-American men and women in both short-term (5 years) and long-term (10 years) follow-up. *Diabetes* 54:985–990, 2005

From the <sup>1</sup>Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, VA Puget Sound Health Care System and Harborview Medical Center, University of Washington, Seattle, Washington; the <sup>2</sup>Department of Medicine, University of Washington, Seattle, Washington; the <sup>3</sup>Department of Anthropology, University of Washington, Seattle, Washington; the <sup>4</sup>Research and Development Service, VA Puget Sound Health Care System, Seattle, Washington; and the <sup>5</sup>Epidemiologic Research and Information Center, VA Puget Sound Health Care System, Seattle, Washington.

Address correspondence and reprint requests to Jenny Tong, MD, VA Puget Sound (S-151), 1660 S. Columbian Way, Seattle, WA 98108. E-mail: tongj@u.washington.edu.

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CT, computed tomography; IAF, intra-abdominal fat; IGT, impaired glucose tolerance; ISR, insulin secretion ratio; NGT, normal glucose tolerance; SCF, subcutaneous fat.

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Visceral adiposity plays an important role in the development of type 2 diabetes and metabolic syndrome (1,2). Its association with insulin resistance and hyperinsulinemia is particularly strong as demonstrated by several studies (3–5). Imaging techniques such as computed tomography (CT) and magnetic resonance imaging allow the differentiation between accumulation of intra-abdominal (visceral) and subcutaneous abdominal fat, which may have distinct impacts on glucose and lipoprotein metabolism. Several analyses have shown that the effect of visceral fat on glucose tolerance is independent from total adiposity and subcutaneous fat (SCF) depots (6,7). Yet little is known about the cause and effect association between the two.

Obesity, genetic susceptibility, aging, and male sex were found to be associated with increased visceral fat accumulation (8–10). Despite having lower average BMI than whites, Asian women have a higher degree of central adiposity for a given BMI (11), which confers an increased risk for metabolic syndrome, type 2 diabetes, and cardiovascular disease (1,12,13).

Leptin, a product of the obesity (*ob*) gene, is an adipocyte-derived peptide hormone (14). It regulates body weight through binding to receptors in the hypothalamus and regulates caloric intake and energy expenditure (15,16). In general, plasma leptin levels correlate positively with fat mass with most obese subjects having elevated leptin levels. Chessler et al. (17) have demonstrated in Japanese Americans that relatively increased plasma leptin levels are associated with greater subsequent gains in weight and total adiposity. However, regional fat area change over time was not examined independently from that of total fat areas in this study.

We previously demonstrated in a prospective study of 137 Japanese-American men that greater insulin resistance, represented by higher fasting insulin and C-peptide levels, and reduced insulin secretion, measured by stimulated incremental insulin response, predicted a positive change in intra-abdominal fat (IAF) accumulation over 5 years (18). We have since completed a 10-year follow-up of these 137 subjects and have 5- and 10-year follow-up data on additional male and female subjects, including plasma leptin levels at baseline. This report describes the association between IAF accumulation at 5- and 10-year follow-

TABLE 1

Baseline characteristics of all study participants, those who completed the 5-year follow-up, those who completed the 10-year follow-up, and those who were lost to follow-up at 5 or 10 years

Characteristic	All subjects	Follow-up 5 years	Follow-up 10 years	Lost to follow-up at 5 years	Lost to follow-up at 10 years
<i>n</i>	518	465	415	53	62
Age (years)	52.2 ± 0.5	52.1 ± 0.6	51.7 ± 0.6	52.2 ± 1.8	55.2 ± 1.6
Male (%)	51.0	52.3	52.1	39.6	53.2
Weight (kg)	62.9 ± 0.5	63.2 ± 0.6	63.2 ± 0.6	60.3 ± 1.3	63.3 ± 1.3
BMI (kg/m <sup>2</sup> )	24.1 ± 0.1	24.1 ± 0.2	24.1 ± 0.2	23.6 ± 0.4	24.3 ± 0.3
IAF area (cm <sup>2</sup> )	81.2 ± 2.2	82.0 ± 2.3	80.3 ± 2.4	74.3 ± 7.3	94.3 ± 6.5
SCF area (cm <sup>2</sup> )	387.2 ± 7.8	385.0 ± 8.0	385.6 ± 8.6	407.7 ± 28.0	388.4 ± 22.7
Fasting insulin (pmol/l)	13.3 ± 0.3	13.4 ± 0.3	13.3 ± 0.3	12.5 ± 0.8	13.7 ± 0.8
ISR	0.5 ± 0.01	0.5 ± 0.02	0.5 ± 0.02	0.4 ± 0.04	0.5 ± 0.04
C-peptide (nmol/l)	2.6 ± 0.03	2.6 ± 0.04	2.6 ± 0.04	2.3 ± 0.1	2.6 ± 0.1
Leptin (pmol/l)*	7.3 ± 0.3	7.3 ± 0.3	7.2 ± 0.3	—	7.6 ± 0.9

Data are means ± SE unless otherwise indicated. \*Leptin measurements were obtained only for subjects who returned for the 5-year follow-up.

ups in relation to earlier measurements of fasting and stimulated insulin, C-peptide, and plasma leptin levels.

## RESEARCH DESIGN AND METHODS

The study population consisted of 288 second-generation (Nisei; age 61.8 ± 5.9 [mean ± SD] years) and 230 third-generation (Sansei; age 40.1 ± 4.2 years) nondiabetic Japanese-American men and women enrolled in the Japanese-American Community Diabetes Study between 1983 and 1988. Details about selection and recruitment of the sample population have been described previously (19). In brief, subjects were chosen from volunteers and were representative of Japanese Americans in King County, Washington, with regard to age, residence, and parental immigration pattern. All subjects were of 100% Japanese ancestry. Nisei men returned for follow-up examinations 5 and 10–11 years after a baseline evaluation. Nisei women and Sansei men and women returned at 6 and 10–11 years after a baseline evaluation. Of the original 518 subjects, 465 (90%) completed the 5-year and 415 (80%) completed the 10-year follow-up evaluation.

All evaluations were performed at the General Clinical Research Center, University of Washington, using a protocol approved by the University of Washington Human Subjects Review Committee. Signed informed consent was obtained from all participants. A 75-g oral glucose tolerance test performed in the morning after a 10-h overnight fast was used to classify all subjects as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or type 2 diabetes on the basis of the American Diabetes Association 1997 criteria (20). Diabetes was diagnosed if subjects reported a history of this condition and were taking oral hypoglycemic medication or insulin, if the fasting plasma glucose level was ≥126 mg/dl (7.0 mmol/l), or if the 2-h value was ≥200 mg/dl (11.1 mmol/l). Subjects with a fasting plasma glucose level <126 mg/dl (7.0 mmol/l) but a 2-h value from 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.1 mmol/l) were defined as having IGT.

Serum glucose was assayed by an automated glucose oxidase method at 0, 30, and 120 min during the oral glucose tolerance test. Plasma insulin and C-peptide levels were measured by radioimmunoassay as previously described (18). The insulin secretion ratio (ISR) was calculated as (30- to 0-min plasma insulin)/30-min glucose, which correlates well with direct measures of stimulated insulin secretion (21,22). Leptin measurements were obtained from frozen plasma of the 409 subjects who completed the 5-year follow-up. Plasma leptin levels were determined in duplicate using a radioimmunoassay kit (Linco Research, St. Charles, MO) (23). Plasma from fasting morning blood samples (7:30–8:30 A.M.) was stored at –80°C and thawed just before use for leptin measurement.

Body regional fat distribution was quantified by CT (24,25). Single 10-mm slices of the thorax on inspiration at the level of the nipples, the abdomen at the level of the umbilicus, and the mid-thigh at a level halfway between the greater trochanter and the superior margin of the patella were analyzed for cross-sectional area of adipose tissue (centimeters squared). IAF was measured using the transversalis fascia as the outer boundary at the umbilicus level. Total SCF area equaled the sum of the SCF areas from the thorax, abdomen, and midthigh. BMI was computed as weight (kilograms) divided by height (meters) squared.

Subjects were questioned regarding cigarette smoking status, amount

smoked per day, duration of smoking in years, average daily consumption of alcoholic beverages, and weekly work and recreational activity levels.

**Statistical analysis.** Paired and unpaired *t* tests were used to compare mean values. A univariate linear regression model was used to estimate the association between baseline and future IAF. Multiple linear regression analyses was used to model IAF measured at follow-up as a function of other variables of interest, such as fasting insulin, C-peptide, ISR, or leptin. Because insulin and C-peptide are highly correlated, they were entered into separate regression models. Leptin models included SCF changes and fasting insulin level in addition to baseline IAF, age, and sex due to the reported associations between this adipose depot and plasma leptin (4,26,27) and plasma insulin levels (27–29). Because SCF would be expected to increase in absolute terms by a greater degree than IAF, we performed adjustments for changes in SCF in determining predictors of IAF accumulation in Tables 3 and 4. This adjustment also permits assessment of changes in IAF in relation to predictors independent of changes in SCF. Models that included ISR were adjusted for fasting insulin level, because a significant association exists between fasting insulin level and insulin secretion (30).

When used as the dependent variable, IAF underwent square root transformation to satisfy the normality assumptions. To examine whether observed associations varied by IGT status, first-degree interactions between IGT status and main effects of interest were tested. Analysis of residuals was performed to test for model fit and regression assumptions. All *P* values reported are two-sided. Mean values were reported as means ± SE unless specified. All statistics were calculated using Stata version 8.0 (College Station, TX).

## RESULTS

A total of 518 subjects (age 52.2 ± 12.0 years [mean ± SE]; 51.0% males) were eligible for this study. Fifty-three subjects were lost to follow-up after 5 years and another 50 subjects were lost to follow-up after 10 years due to death or illness, inability to locate the subjects, or withdrawal from the study.

Baseline characteristics of the study subjects grouped by follow-up status are shown in Table 1. Subjects who completed 5- or 10-year follow-up had similar body composition and metabolic parameters compared with all subjects eligible for the study, as well as subjects who were lost to follow-up at either 5 or 10 years (*P* > 0.05). Serum leptin concentrations exhibited the expected sexual dimorphism in our study population: mean baseline plasma leptin level for men was 4.0 ± 2.7 (mean ± SD) pmol/l and 11.6 ± 7.3 pmol/l for women. A total of 211 (40.7%) subjects had IGT at baseline. Subjects with IGT were older; had higher BMI, IAF, and SCF areas; had higher fasting insulin, C-peptide, and leptin levels (*P* < 0.05); and had lower ISR (*P* < 0.05) compared with those

TABLE 2

Changes in body weight, BMI, IAF, and SCF areas over the study period among nondiabetic Japanese Americans who completed both the 5- and 10-year follow-ups

Characteristics	Baseline	Change 0–5 years*	Change 5–10 years*	Change 0–10 years*
Weight (kg)	62.9 ± 0.5	1.27 (3)	0.97 (2)	2.26 (4)
BMI (kg/m <sup>2</sup> )	24.1 ± 0.1	0.62 (3)	0.47 (2)	1.09 (5)
IAF area (cm <sup>2</sup> )	81.2 ± 2.2	10.9 (13)	4.99 (5)	17.3 (20)
SCF area (cm <sup>2</sup> )	387.2 ± 7.8	37.6 (9)	16.8 (4)	53.3 (14)

Data are means ± SE or mean (%). \*All comparisons were  $P < 0.01$  except for weight change between periods of 0–5 and 5–10 years.

who had NGT. Sixty-two subjects met the 1997 American Diabetes Association diagnostic criteria for diabetes at the 5-year follow-up and an additional 64 subjects met the diagnostic criteria at the 10-year follow-up.

Table 2 lists the changes in weight and adipose tissue measurements over the 10-year study periods. Average baseline BMI for the study population was  $24.1 \pm 3.2$  (mean ± SD) kg/m<sup>2</sup>, with men having a higher mean BMI ( $25.2 \pm 3.0$  kg/m<sup>2</sup>) than women ( $22.9 \pm 3.1$  kg/m<sup>2</sup>). Of all of the CT fat areas, the largest increase occurred in IAF area from baseline to 5 years (13%). Despite the larger absolute increase in SCF area over time, the relative increase in fat area was larger for IAF compared with SCF. Most of the weight and adipose tissue area increase occurred during the first 5 years of follow-up. Only subjects who had both 5- and 10-year follow-up data were included in the analysis.

In separate analyses using baseline IAF to predict IAF at follow-up, IAF at baseline was a strong predictor of future IAF. The correlation coefficients between baseline IAF and IAF measured at 5 and 10 years were  $r = 0.82$  and  $r = 0.76$ , respectively (data not shown). Correlations between baseline IAF and fasting insulin and C-peptide are moderate (0.314–0.415), between IAF and leptin are weak (0.140), and between IAF and ISR are close to zero (0.049).

Multiple linear regression analysis was used to estimate the associations between fasting insulin, ISR, C-peptide, or leptin and later measurements of IAF while adjusting for baseline IAF, age, sex, and SCF area change. Significant positive associations were found between baseline fasting insulin, C-peptide, leptin, and IAF at 5 years (Table 3). No significant association was seen between ISR and IAF.

Similar analysis was performed to evaluate the association between insulin measures obtained at 5 years and IAF measured at 10 years (results not shown). We again observed positive and significant associations between C-peptide and IAF ( $\beta = 0.192$ ,  $P = 0.005$ ) after adjusting

for SCF change, sex, and age. The association between fasting insulin and IAF had a trend toward significance ( $P = 0.064$ ). The association between ISR and IAF was not statistically significant. Leptin analyses were not performed due to the unavailability of these measurements at 5-year follow-up.

Analyses of baseline measurements in relation to IAF measured at the 10- to 11-year follow-up are shown in Table 4. We observed positive significant associations between baseline fasting insulin, C-peptide, plasma leptin, and 10-year IAF ( $P = 0.020$ ,  $P = 0.035$ , and  $P = 0.003$ , respectively). ISR was not significantly associated with IAF at 10 years in these models.

First-order interactions between IGT status and main effects and sex and main effects were tested in all models but were not found to be statistically significant, indicating that the associations of interest did not differ significantly on the basis of IGT status or sex. BMI change was inserted in all leptin models instead of SCF change but did not have any significant impact on the association between baseline leptin level and future IAF (result not shown). Adjustment for BMI change in model 1 in Table 3 did, however, reveal significant associations between future IAF and both fasting insulin and ISR. The potential for effect modification by sex on the association between leptin and IAF was tested by inserting an interaction term (leptin\*sex) in the multivariate regression models. No significant interaction between leptin and sex was detected using this method ( $\beta = 0.027$ ,  $P = 0.52$  for the interaction variable in the 0- to 5-year model and  $\beta = -0.0002$ ,  $P = 0.996$  in the 0- to 10-year model). The results of the models shown in Tables 3 and 4 did not substantially change when smoking status, amount smoked per week, amount of alcohol consumed per week, and weekly energy expenditure were entered into these models as additional covariates (data not shown).

TABLE 3

Multiple regression analyses of IAF area at 5-year follow-up on baseline insulin levels, C-peptide levels, ISR, and leptin levels while adjusting for baseline IAF area, age, sex, SCF change, and other important covariates

Independent variables	Regression coefficient (95% CI)	<i>P</i>	Model <i>R</i> <sup>2</sup>
Model 1*			0.718
Fasting insulin	0.041 (0.018–0.065)	0.001	
Insulin secretion ratio	−0.365 (−0.788 to −0.057)	0.090	
Model 2*			0.392
Fasting C-peptide	1.283 (1.028–1.538)	<0.001	
Model 3†			0.726
Leptin	0.055 (0.021–0.088)	0.002	
SCF change	0.007 (0.006–0.009)	<0.001	

\*Models 1 and 2 adjusted for baseline IAF area, SCF change, sex, and age. †Model 3 adjusted for baseline IAF area, fasting insulin level, sex, and age.



TABLE 4

Multiple regression analyses of IAF area at 10-year follow-up on baseline insulin levels, C-peptide levels, ISR, and leptin levels while adjusting for baseline IAF area, age, sex, SCF change, and other important covariates

Independent variables	Regression coefficient (95% CI)	P	Model R <sup>2</sup>
Model 1*			0.690
Fasting insulin	0.031 (0.005–0.057)	0.020	
Insulin secretion ratio	0.090 (–0.391–0.572)	0.712	
Model 2*			0.687
Fasting C-peptide	0.221 (0.015–0.426)	0.035	
Model 3†			0.701
Leptin	0.059 (0.020–0.099)	0.003	
SCF change	0.008 (0.007–0.010)	<0.001	

\*Models 1 and 2 adjusted for baseline IAF area, SCF change, sex, and age. †Model 3 adjusted for baseline IAF area, fasting insulin level, sex, and age.

## DISCUSSION

These results confirm that associations exist between future IAF accumulation in relation to fasting insulin and C-peptide levels and insulin secretion. We found that fasting insulin and C-peptide levels were positively associated with IAF accumulation over 5 and 10 years. These associations appeared to be independent of SCF change. No significant association was found between ISR and later IAF accumulation in models adjusted for SCF change. These results extend our previous findings in elderly Japanese-American men of associations between IAF accumulation at 5-year follow-up in relation to fasting insulin and C-peptide levels and insulin secretion to younger men and both younger and older women. In addition, these results show an association between higher leptin level and future IAF accumulation independent of either change in BMI or SCF over the same time frame. Thus, leptin appears related to gain not only in adiposity but also site of adipose deposition.

The mechanism responsible for the increased IAF in Japanese Americans cannot be ascertained from the results of this study. Higher fasting insulin and C-peptide levels seemed to predict future IAF independent of SCF accumulation in this population. Baseline IAF area remains the best predictor for future IAF accumulation in all of our prediction models. Although the percentage change in IAF area over the study period was greater than that in SCF as shown in Table 2, the absolute change in SCF may still be much larger depending on the relative size of the adipose tissue depots. The correlation analysis suggests that the biochemical measures used as predictors for IAF accumulation did not have strong associations with IAF at baseline. Therefore, they are not likely to be surrogate markers for IAF in predicting future IAF gain. The distribution of IAF proportionate to the overall adiposity in this population is probably genetically determined (31). The reason for the stronger association between C-peptide with IAF accumulation compared with fasting insulin and ISR is unclear. Fasting C-peptide level may be more strongly associated with insulin sensitivity than fasting insulin levels in the Japanese-American population (32). C-peptide does not undergo hepatic extraction like insulin and therefore may be less likely to be affected by changes in hepatic insulin extraction related to adiposity and insulin sensitivity (33).

Another key finding of our study was that the plasma leptin level measured at baseline was positively associated

with IAF accumulation at 5- and 10-year follow-ups. This association remained significant after adjusting for total SCF or BMI change and was not modified by sex. Plasma leptin concentration has been found to be associated with female sex, total and SCF volume in population as well as cohort studies (34). It has been postulated as a sensor of fat mass and a hormonal signal that exerts its effect on energy regulation through its hypothalamic receptor (15,16). Because the adipocyte is the only source of *ob* gene products, serum leptin level has a strong positive association with the amount of body fat and adipocyte leptin mRNA in humans as in rodents (35–37). Several studies have examined serum leptin level and future weight gain, but few have examined change in regional adiposity (17,38,39). Our study demonstrated that higher leptin level predicts future IAF accumulation as measured by CT independent from total and subcutaneous adiposity or fasting insulin levels or from concurrent increase in BMI or SCF.

Despite the difference in mean leptin levels between males and females (11.6 vs. 4 pmol/l), we did not observe differences in the association between leptin levels and IAF accumulation by sex as seen in nonsignificant interaction terms in regression models between leptin and sex as predictors of future IAF accumulation. It is possible that such a difference, if it exists, may have been detectable with a larger sample size.

We observed stronger associations between fasting insulin or C-peptide and IAF at 5-year follow-up than 10-year follow-up, but similar magnitude associations were observed at both follow-up intervals for the associations between leptin (or SCF change) and IAF. The more consistent leptin effect may be due to smaller variation in leptin levels over time compared with fasting insulin and C-peptide levels, a phenomenon that has been referred to as “regression dilution” (40).

One plausible explanation of our findings may be that our baseline data may not represent the “real” baseline measurements of metabolic parameters for each individual at the onset of our study. It is possible that we obtained our measurements on study subjects when they already had some weight gain and had already begun the journey along the path to body fat accumulation, insulin resistance, and eventually, diabetes. Thus, the baseline hormone levels associated with later visceral fat accumulation may represent markers for an underlying unidentified cause or causes of this outcome. We also

propose the hypothesis that insulin and leptin resistance result in both increased baseline levels of insulin and leptin in the periphery and a diminished satiety signal in the central nervous system. The diminished satiety signal would cause a slight positive energy balance that, over time, would lead to obesity and increased IAF. In addition, other adipokines such as adiponectin may also play a role in the associations that we observed (41,42). Plasma adiponectin levels were not measured for these subjects. Future studies are needed to provide a better understanding of the causal relationships between metabolic parameters and adipose tissue accumulation.

Several prospective studies have examined the association between insulin resistance, insulin secretion, and weight gain and/or IAF accumulation. A study of middle-aged Caucasians with NGT found that reduced first-phase insulin secretion was associated with increased risk of future weight gain, whereas fasting hyperinsulinemia was associated with increased waist-to-hip ratio over time in women (43). Researchers reported that fasting hyperinsulinemia was a predictor of increased weight gain and overall future obesity among Pima Indian children (44). In a prospective study of nondiabetic Asian Indian, Creole, and Chinese Mauritians, insulin resistance predicted weight gain but not waist-to-hip ratio change in Chinese men independently of baseline age and BMI (45). None of the above-mentioned studies used CT scan as a measurement of IAF area. Other studies have shown no ability of hyperinsulinemia to predict weight gain in both children (46) and adults. Three longitudinal studies of Pima Indians and of Hispanic and non-Hispanic whites (47–49) found markers of insulin resistance (euglycemic clamp or fasting insulin) among nondiabetic subjects to be inversely associated with the rate of weight gain. Researchers have also shown that lower insulin secretion predicted future weight gain in a prospective study of young, obese Pima Indians (50). Very few studies have specifically examined the temporal relationship between insulin resistance, insulin secretion, and future IAF change using a measurement of regional adiposity obtained from imaging technology.

There are several limitations to our study. We used surrogate markers such as fasting insulin and C-peptide levels to measure insulin resistance instead of the more quantitative approaches such as the hyperinsulinemic-euglycemic clamp or minimal model (51). This can potentially introduce measurement error. If the error in these surrogate measures was random, we would be more likely to underestimate the true association between insulin resistance, insulin secretion, and future IAF accumulation. Visceral fat volume was measured using single-slice CT imaging at the umbilicus level, but this method has been shown to have a high correlation with directly ascertained total visceral fat volume by CT or magnetic resonance imaging (52,53). Our study subjects were middle-aged, nonobese, nondiabetic Japanese Americans at high risk for type 2 diabetes. The ability to generalize our study findings to other populations is unclear. We cannot exclude the possibility of residual confounding causing bias in our results due to unmeasured or inaccurately measured covariates.

In conclusion, the presence of greater insulin resistance as reflected by higher fasting insulin and C-peptide levels

was positively associated with future IAF accumulation in initially nondiabetic Japanese Americans. These associations were present in both shorter (5-year) and longer (10-year) follow-up. Higher plasma leptin level also predicted increased future visceral adiposity. The above associations were independent from intra-abdominal adiposity at baseline and change in subcutaneous adiposity over time. These results suggest that the metabolic changes associated with visceral fat may precede its accumulation or act in a positive feedback manner to perpetuate or exacerbate this condition. If these results represent causal associations, they would therefore suggest that interventions that target insulin resistance and leptin signaling and/or resistance might result in lower IAF accumulation. Further confirmation of these findings is needed from studies that measure the visceral adipose tissue depot using direct imaging technology over time.

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