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The Concurrent Accumulation of Intra-Abdominal and Subcutaneous Fat Explains the Association Between Insulin Resistance and Plasma Leptin Concentrations

Distinct Metabolic Effects of Two Fat Compartments

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Obesity is associated with insulin resistance, particularly when body fat has a central distribution. However, insulin resistance also frequently occurs in apparently lean individuals. It has been proposed that these lean insulin-resistant individuals have greater amounts of body fat than lean insulin-sensitive subjects. Alternatively, their body fat distribution may be different. Obesity is associated with elevated plasma leptin levels, but some studies have suggested that insulin sensitivity is an additional determinant of circulating leptin concentrations. To examine how body fat distribution contributes to insulin sensitivity and how these variables are related to leptin levels, we studied 174 individuals (73 men, 101 women), a priori classified as lean insulin-sensitive (LIS, $n = 56$), lean insulin-resistant (LIR, $n = 61$), and obese insulin-resistant (OIR, $n = 57$) based on their BMI and insulin sensitivity index (S_I). Whereas the BMI of the two lean groups did not differ, the S_I of the LIR subjects was less than half that of the LIS group. The subcutaneous and intra-abdominal fat areas, determined by computed tomography, were 45 and 70% greater in the LIR subjects ($P < 0.001$) and 2.5- and 3-fold greater in the OIR group, as compared with the LIS group. Fasting plasma leptin levels were moderately increased in LIR subjects (10.8 ± 7.1 vs. 8.1 ± 6.4 ng/ml in LIS subjects; $P < 0.001$) and doubled in OIR subjects (21.9 ± 15.5 ng/ml; $P < 0.001$). Because of the confounding effect of body fat, we examined the relationships between adiposity, insulin sensitivity, and leptin concentrations by multiple regression analysis. Intra-abdominal fat was the best variable predicting insulin sensitivity in both genders and explained 54% of the variance in S_I. This inverse relationship was nonlinear ($r = -0.688$). On the other hand, in both genders, fasting leptin levels were strongly associated with subcutaneous fat area ($r = 0.760$) but not with intra-abdominal fat. In line with these analyses, when LIS and LIR subjects were matched for subcutaneous fat area, age, and gender, they had similar leptin levels, whereas their intra-abdominal fat and insulin sensitivity remained different. Thus, accumulation of intra-abdominal fat correlates with insulin resistance, whereas subcutaneous fat deposition correlates with circulating leptin levels. We conclude that the concurrent increase in these two metabolically distinct fat compartments is a major explanation for the association between insulin resistance and elevated circulating leptin concentrations in lean and obese subjects. *Diabetes* 51: 1005–1015, 2002

The role of insulin resistance in metabolic diseases has received considerable attention in recent years (1). Insulin resistance has been suggested to be an important risk factor in the development of the metabolic syndrome, a cluster of disorders comprising glucose intolerance, dyslipidemia, hypertension, and dysfibrinolysis that is associated with type 2 diabetes and cardiovascular disease (2). It is evident that obesity is a risk factor for these same conditions and that this association is not only related to the degree of obesity, but also appears to be critically dependent on body fat distribution. Thus, individuals with greater degrees of central adiposity appear to develop this syndrome more frequently than those with a peripheral body fat distribution (3,4).

Although the metabolic impact of central versus peripheral body fat has been firmly established, the importance of the site of abdominal fat accumulation in relation to insulin sensitivity is still a matter of some debate. In recent years, imaging techniques have become available to quantify abdominal fat localized within or outside of the peritoneum. Although there now seems to be a consensus that these two fat compartments are metabolically quite different, studies have differed in the assessment of their relative importance. Some studies have suggested that the intra-abdominal fat (IAF) depot is the major determinant of insulin resistance (5–7) and of other features of the

metabolic syndrome (8,9), whereas others have suggested that the subcutaneous fat (SCF) compartment is the most critical determinant of insulin sensitivity (10,11). Most of these studies evaluated relatively small numbers of subjects and frequently compared subjects who were different, being either lean and insulin sensitive or (very) obese and insulin resistant.

Insulin resistance also frequently occurs in apparently lean individuals (12–14). Because these lean insulin-resistant subjects have had a slightly greater BMI (12) or calculated fat mass (14), it has been suggested that they may be more obese than lean insulin-sensitive individuals (15). However, detailed studies examining abdominal fat distribution and its relationship to insulin sensitivity in these subjects are lacking.

Body adiposity has been shown to be a major determinant of circulating leptin (16), an adipocyte-derived hormone involved in body weight regulation. Whereas women have higher leptin concentrations, even after correction for body fat mass (17), in both genders the SCF depot seems to be a stronger predictor of leptin levels than IAF (18). Insulin sensitivity has been suggested to be an additional determinant of leptin concentrations (19), possibly through stimulation of leptin secretion from adipose cells by insulin (19,20). However, the association between insulin and leptin is difficult to evaluate because of the ability of adiposity to influence both leptin levels and insulin sensitivity. The relative contribution of different fat compartments, insulin sensitivity, and insulin levels on fasting leptin concentrations has not yet been examined in a large number of male and female subjects with a broad spectrum of insulin sensitivity and body size.

To systematically examine the relationships between body fat distribution, insulin sensitivity, and leptin concentrations, we quantified these variables in apparently healthy individuals who were classified a priori into three groups: lean insulin-sensitive (LIS), lean insulin-resistant (LIR), and obese insulin-resistant (OIR). By design, the lean groups would differ in insulin sensitivity, while the LIR and OIR would have a different BMI. All subjects underwent computed tomography (CT) scanning of the abdomen to quantify intra-abdominal and SCF depots. Our aims were *1*) to examine the relative effects of insulin sensitivity and obesity on plasma leptin levels, *2*) to assess the role of IAF versus SCF distribution on insulin sensitivity and plasma leptin levels, and *3*) to examine the possible effect of gender on these relationships.

RESEARCH DESIGN AND METHODS

Subjects. A total of 234 (99 men, 135 women) individuals were recruited by advertisement to participate in a study of the effect of egg consumption on plasma lipids in people with varying degrees of insulin sensitivity. This study was reviewed and approved by the Human Subjects Review Committee at the University of Washington. The data presented in this article are baseline measurements in 174 subjects (73 men, 101 women), for whom data on insulin sensitivity, body fat distribution, and plasma leptin concentrations were available.

The subjects, aged 30–75 years, were apparently healthy, with no history of diabetes, dyslipidemia, or uncontrolled hypertension. Further exclusion criteria were fasting plasma glucose ≥6.4 mmol/l, biochemical evidence of liver or renal disease, uncontrolled thyroid disease, coronary or other vascular disease, and anemia. Premenopausal women ($n = 40$) were not studied in any particular phase of the menstrual cycle. Of the postmenopausal women, 34% were not using hormone replacement therapy, 28% were taking estrogen, and 38% were taking estrogen and progestin.

Eligible subjects had their height and weight measured and underwent a frequently sampled intravenous glucose tolerance test (FSIGT) to quantify insulin sensitivity. Based on their BMI and insulin sensitivity, subjects were a priori subdivided into three groups: LIS, LIR, and OIR, as discussed above. Ten obese insulin-sensitive subjects were excluded from the study because of their small number. The cutoff points used were 27.5 kg/m^2 for BMI, based on the criteria used before the redefinition of overweight and obesity (National Health and Nutrition Examination Survey [NHANES] II), and 7×10^{-5} min^{-1} · [pmol/l]$^{-1}$ for the insulin sensitivity index (S_I), which represented the highest value for this parameter among a group of apparently healthy obese subjects studied in Seattle (13). After their subdivision into LIS, LIR and OIR groups, subjects underwent additional measures of body anthropometry and body fat distribution. The ethnicity of subjects was Caucasian in 96% of the LIS and 89% of the LIR and OIR groups and Asian American in 3%. In addition, in the LIR and OIR groups, 6% were African-American and 2% were Hispanic or Native American.

Study procedures

Measures of anthropometry and body fat distribution. The average of two weight and height measurements were used to calculate BMI as weight (kg)/[height (m)]^2.

Waist and hip circumferences were calculated as the average of two measurements. Waist circumference was measured at the smallest circumference of the waist, and hip circumference was measured at the widest level of the buttocks, using a protocol described in the NHANES III Anthropometric Measurements Videotape (National Center for Health Statistics).

A CT scan of the abdomen was performed at the level of the umbilicus to quantify SCF area, IAF area, and total tissue area. Total tissue area was computed as the area with an attenuation range of -250 to 1,500 Hounsfield units, whereas for fat, an attenuation range of -250 to -50 Hounsfield units was used. IAF and SCF areas were quantified by delineating the border of the peritoneal cavity. These measurements were performed by a single observer using standard GE 8800 computer software. The variability of these measures made by a single observer was 1.5%, and day-to-day variability was <1% (21).

Insulin sensitivity. Subjects underwent a tolbutamide-modified FSIGT to quantify insulin sensitivity as the S_I using Bergman's minimal model of glucose kinetics (22). During this test, three basal blood samples were drawn at -15, -5, and -1 min before intravenous glucose administration at time 0. Glucose (11.4 g/m^2 body surface area) was infused over 1 min, and blood sampling was continued at 2, 3, 4, 5, 6, 8, 10, 14, 16, and 19 min after commencement of glucose administration. At time 20 min, tolbutamide (125 mg/m^2 body surface area) was administered over 30 s, and blood sampling was continued at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, and 240 min. Fasting glucose and immunoreactive insulin concentrations were calculated as the average of the three basal samples. In our laboratory, the day-to-day variability of S_I was 16.9% (23). The fasting plasma leptin concentration was determined on a basal sample obtained at the time of the FSIGT.

Assays. Glucose was measured in duplicate using the glucose oxidase method. Immunoreactive insulin was measured in duplicate by radioimmunoassay using a modification of the double antibody technique (24). Plasma leptin levels were measured by radioimmunoassay (25) (Linco Research, St. Charles, MO). Samples from subjects in each of the three different study groups were included in each assay to reduce the effect of interassay variability.

Calculations and statistics. Data are presented as means ± SD. Comparisons of LIS, LIR, and OIR groups and comparisons between genders were performed by ANOVA. There was no interaction between group and gender effect for any of the variables by two-way ANOVA. Neither was an interaction observed between group and menopausal status. This result suggests that the differences across LIS, LIR, and OIR groups were not affected by gender or menopausal status. Subsequently, we used one-way ANOVA followed by Bonferroni's post test to compare groups and to test for differences between genders within each group. S_I, fasting plasma insulin and leptin levels, and SCF and IAF areas were not normally distributed and were therefore log-transformed before ANOVA.

Scatterplots were made to visually evaluate the relationships between continuous variables in the group of 174 subjects. Correlations were performed by linear regression. These analyses were repeated after inclusion of the obese insulin-sensitive subjects ($n = 10$), who were previously excluded by study design. The results obtained when this group of subjects was included in the analyses were not different. For the assessment of the relationship between insulin sensitivity and measures of body fat, variables were log-transformed because the relationship between untransformed variables appeared nonlinear. To test whether the association between IAF and S_I is indeed best described by a nonlinear function, we inserted natural log (log$_e$) IAF in a multiple regression model that already contained IAF as a linear
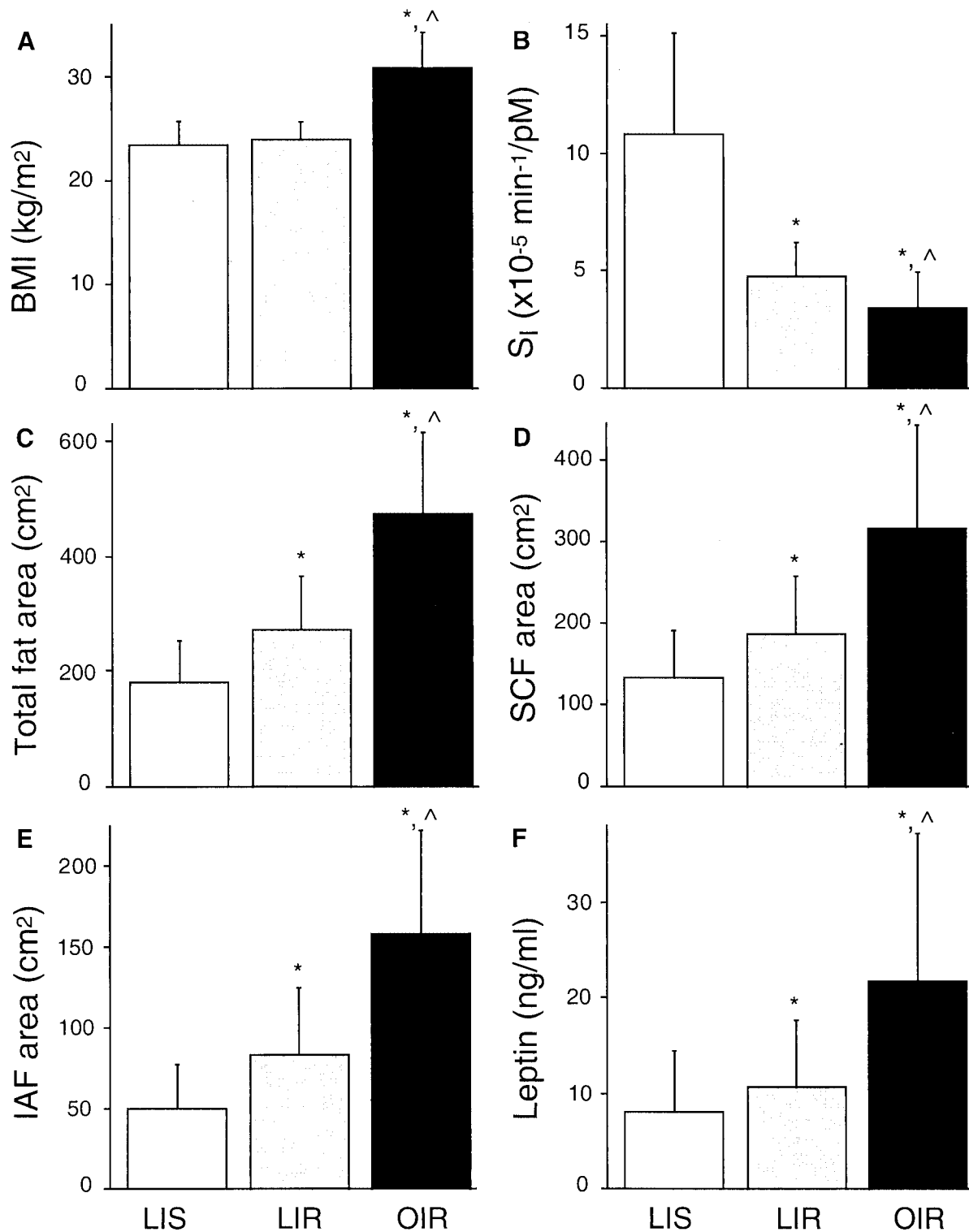
FIG. 1. BMI (A), S_I (B), total abdominal fat area (C), SCF area (D), IAF area (E), and fasting plasma leptin levels (F) in 56 LIS (□), 61 LIR (▨), and 57 OIR (■) subjects. *$P < 0.001$ vs. LIS; ^$P < 0.001$ vs. LIR. S_I, fat areas, and leptin levels were log-transformed before ANOVA because they were not normally distributed.

independent variable. This nonlinear regression model was compared with the linear model for prediction of $\log_e S_I$ using a partial F test.

The relative contribution of fat compartments on insulin sensitivity and leptin levels was examined by subanalysis of LIS and LIR subjects and of LIR and OIR subjects. The subjects were matched for gender and SCF or IAF area by pairwise selection of individuals with fat areas differing ≤10 cm². The subgroups were then compared by unpaired t test and by Mann-Whitney U test for non–normally distributed variables.

Multiple regression analysis was used to determine whether the association between the dependent and independent variables of interest remained significant after adjusting for other potentially confounding independent variables. In this analysis, the dependent variables were log-transformed to satisfy the necessary statistical assumptions of linear regression. Stepwise model building was used to estimate the relative contribution of the independent variables to the variability of the dependent variable. The multiple regression analyses were repeated after inclusion in the cohort of the obese

TABLE 1
Age, BMI, and metabolic characteristics of LIS, LIR, and OIR subjects subdivided by gender

	LIS	LIR	OIR
Men			
n	19	27	27
Age (years)	46.6 ± 7.3	55.7 ± 11.5†	53.9 ± 10.1
BMI (kg/m^2)	24.1 ± 2.3	24.8 ± 1.4	30.5 ± 2.7‡¶
S_I (× 10^{-5} min^{-1} · [pmol/l]$^{-1}$)	11.3 ± 5.2	4.3 ± 0.5‡	3.3 ± 1.3‖
Glucose (mmol/l)	5.36 ± 0.42	5.59 ± 0.39	5.77 ± 0.47†
Insulin (pmol/l)	45.0 ± 19.2	63.6 ± 25.2*	88.2 ± 69.6‡
Women			
n	37	34	30
Age (years)	51.0 ± 8.6	53.0 ± 11.5	53.3 ± 9.5
BMI (kg/m^2)	23.0 ± 2.2	23.8 ± 1.8#	31.5 ± 3.9‡¶
S_I (× 10^{-5} min^{-1} · [pmol/l]$^{-1}$)	10.7 ± 3.7	5.2 ± 1.2‡#	3.7 ± 1.7‡¶
Glucose (mmol/l)	5.14 ± 0.33#	5.28 ± 0.33**	5.52 ± 0.48‡§
Insulin (pmol/l)	39.6 ± 16.2	57.0 ± 29.4†	93.6 ± 48.6‡¶

Data are means ± SD. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ vs. LIS; §$P < 0.05$, ‖$P < 0.01$, ¶$P < 0.001$ vs. LIR; #$P < 0.05$, **$P < 0.01$ for women vs. men within the group. S_I and insulin were log-transformed to obtain normal distribution of the variable before ANOVA.

insulin-sensitive subjects ($n = 10$), who were previously excluded by study design. The results obtained when these subjects were included in the analyses were not different. A P value ≤0.05 was considered significant.

RESULTS

Demographic, adiposity, and metabolic characteristics. In this apparently healthy group of subjects, 68% were insulin resistant (defined as S_I ≤7 × 10^{-5} min^{-1} · [pmol/l]$^{-1}$) and 33% were obese (defined as BMI ≥27.5 kg/m^2). The mean age of the insulin-sensitive subjects was 49.5 ± 8.3 years, slightly less than that of the LIR and OIR subjects (54.2 ± 11.5 and 53.6 ± 9.7 years; $P < 0.05$, LIR vs. LIS). Conforming to the a priori classification of the subjects, the BMI of the obese group (31.0 ± 3.4) was higher than that of both lean groups (LIS 23.4 ± 2.3 and LIR 24.2 ± 1.7; Fig. 1*A*) as a result of a >20 kg higher mean weight in the obese subjects ($P < 0.001$) for a similar height. On the other hand, the BMI of the lean groups did not differ. By definition, the S_I of the insulin-sensitive subjects was more than two- and threefold greater than that of the LIR and OIR subjects, respectively (Fig. 1*B*). S_I was only 40% greater in the LIR group compared with the OIR group. The differences in age, BMI, and S_I between the LIS, LIR, and OIR groups were similar when men and women were analyzed separately (Table 1).

As expected, the insulin-resistant subjects were relatively hyperinsulinemic. Fasting insulin concentrations were twofold higher in the OIR subjects (91.2 ± 58.8 pmol/l) compared with the insulin-sensitive group (41.4 ± 17.4 pmol/l; $P < 0.001$), with an intermediate level in the LIR group (60.0 ± 27.6 pmol/l; $P < 0.001$ vs. OIR and LIS). Similarly, the mean fasting glucose concentration was higher in the LIR group than in the LIS group (5.42 ± 0.39 vs. 5.22 ± 0.37 mmol/l; $P < 0.01$) and was further increased in the OIR group (5.63 ± 0.49 mmol/l; $P < 0.01$ vs. LIR and $P < 0.001$ vs. LIS).

The waist-to-hip ratio was 0.78 ± 0.08 in the LIS subjects and increased to 0.83 ± 0.09 in the LIR group ($P < 0.001$ vs. LIS) and to 0.89 ± 0.08 in the OIR group ($P < 0.001$ vs.

TABLE 2
Body fat distribution and fasting plasma leptin concentrations in LIS, LIR, and OIR subjects subdivided by gender

	LIS	LIR	OIR
Men			
n	19	27	27
Waist-to-hip ratio	0.87 ± 0.06	0.90 ± 0.05	0.96 ± 0.05†‡
Total fat area (cm^2)	175 ± 64	264 ± 85†	431 ± 100†‡
SCF area (cm^2)	110 ± 51	166 ± 64†	248 ± 87†‡
IAF area (cm^2)	65 ± 28	98 ± 38*	183 ± 62†‡
Leptin (ng/ml)	3.1 ± 1.0	6.0 ± 3.9†	10.1 ± 6.2†‡
Women			
n	37	34	30
Waist-to-hip ratio	0.75 ± 0.06¶	0.77 ± 0.05¶	0.83 ± 0.05†‡¶
Total fat area (cm^2)	185 ± 75	283 ± 99†	517 ± 160†‡§
SCF area (cm^2)	143 ± 61	208 ± 72§	381 ± 124†‡¶
IAF area (cm^2)	42 ± 23‖	75 ± 42§	136 ± 59†‡¶
Leptin (ng/ml)	10.7 ± 6.6¶	14.6 ± 6.8*¶	32.5 ± 13.6†‡¶

Data are means ± SD. *$P < 0.01$, †$P < 0.001$ vs. LIS; ‡$P < 0.001$ vs. LIR; §$P < 0.05$, ‖$P < 0.01$, ¶$P < 0.001$ for women vs. men within the group. Fat areas and leptin levels were log-transformed to obtain normal distribution of the variable before ANOVA.

both), suggestive of a more central body fat distribution in the insulin-resistant groups. In the LIR subjects, the increased ratio resulted from a greater waist circumference (83.2 ± 9.1 vs. 77.7 ± 8.5 cm for LIS; $P < 0.01$) for an unchanged hip circumference (100.9 ± 4.9 vs. 99.3 ± 6.3 cm). As expected, women had markedly lower waist-to-hip ratios than men in all groups (Table 2).

In line with the waist circumference data, insulin-resistant subjects had greater amounts of abdominal fat measured by a CT scan (LIR 275 ± 93 cm^2 vs. LIS 181 ± 71 cm^2; $P < 0.001$; Fig. 1*C*). For similar BMI values, the LIR subjects had 45% more abdominal SCF than the LIS group (LIR 189 ± 71 cm^2 vs. LIS 132 ± 59 cm^2; $P < 0.001$; Fig. 1*D*). Their IAF area was also increased by 70% (LIR 85 ± 41 cm^2 vs. LIS 50 ± 27 cm^2; $P < 0.001$; Fig. 1*E*). This overall 50% increase in abdominal fat was present in both sexes, but women had less IAF and more SCF than men (Table 2). In the LIR group, postmenopausal women had more IAF than premenopausal women ($P < 0.05$), but, as mentioned in RESEARCH DESIGN AND METHODS, the differences between the LIS, LIR, and OIR groups were not affected by menopausal status. The increase in abdominal fat was even greater in the obese subjects. As compared with the LIS group, they had 2.5- and 3-fold greater SCF (318 ± 127 cm^2; $P < 0.001$ vs. LIS and LIR) and IAF areas (159 ± 64 cm^2; $P < 0.001$ vs. LIS and LIR), respectively (Fig. 1*D* and *E*). The total area of the abdomen, including all tissues, was also larger in the insulin-resistant groups and more so in the obese subjects. However, this was fully accounted for by the increase in abdominal fat because the nonadipose tissue areas did not differ between the LIS, LIR, and OIR groups (297 ± 56, 295 ± 52, and 315 ± 61 cm^2, respectively).

The increased fat area in the obese subjects was associated with a threefold elevation in their fasting leptin concentration (21.9 ± 15.5 vs. 8.1 ± 6.4 ng/ml for LIS and 10.8 ± 7.1 ng/ml for LIR; all comparisons $P < 0.001$; Fig. 1*F*). This
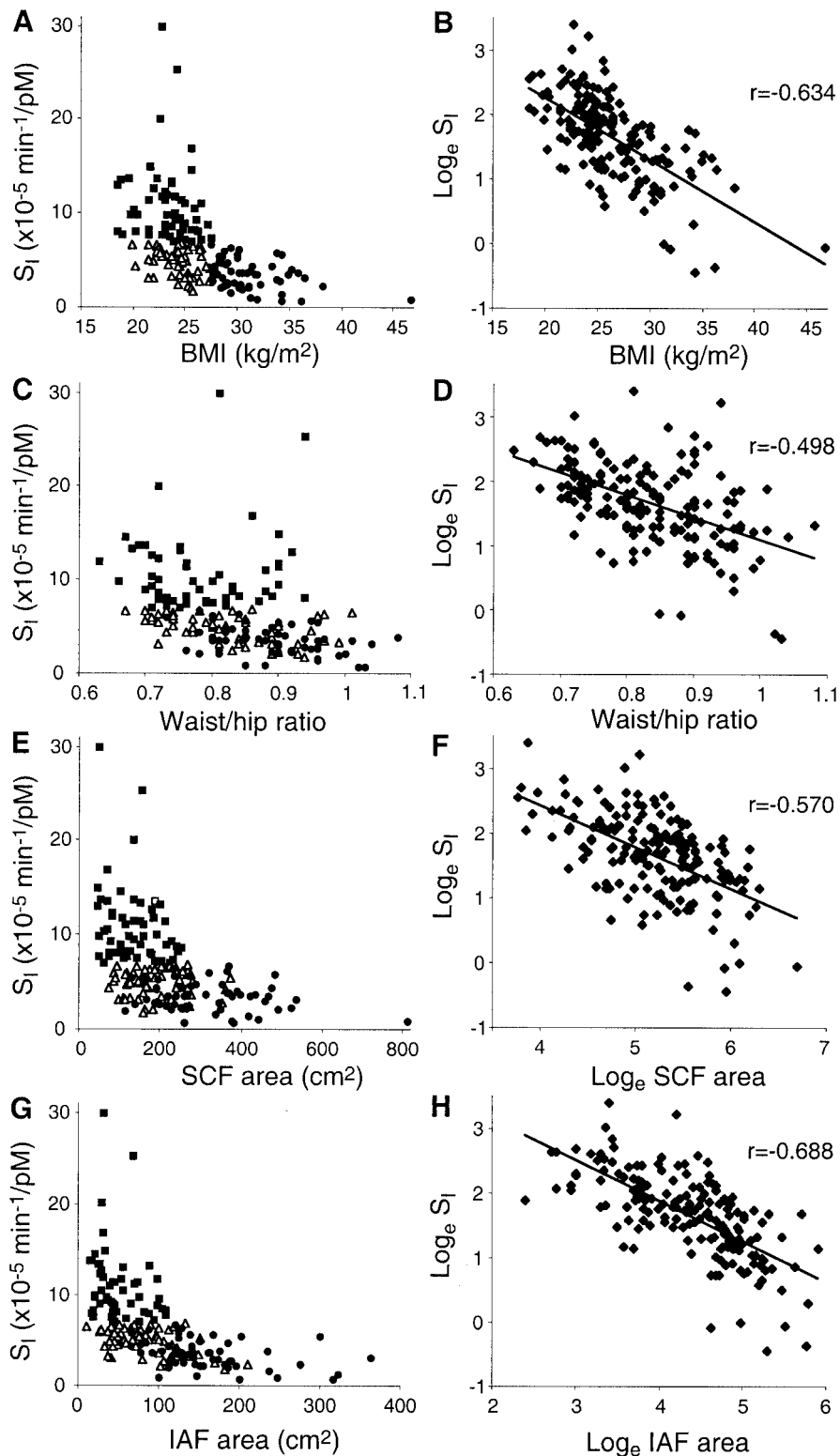
FIG. 2. Relationships between the S_I and BMI (A) and measures of body fat distribution:waist-to-hip ratio (C), SCF area (E), and IAF area (G) in 174 subjects classified as LIS (■), LIR (△), or OIR (●). B, D, F, and H display the correlations between S_I and adiposity measures after $\log_e$ transformation of non–normally distributed variables. $P < 0.001$ for all correlations.

increase in leptin levels occurred in both men and women (Table 2). Women had about threefold higher leptin levels than men, a gender difference that was present in all three groups (Table 2). Leptin levels were also elevated in LIR subjects compared with the LIS group, doubling in men, and increased by one-third in women (Table 2).

Relationship between body adiposity, fat distribution, and insulin sensitivity. In this cohort of 174 subjects, insulin sensitivity was inversely correlated with measures of body fat (Fig. 2). After $\log_e$ transformation of variables that were not normally distributed, the correlation coefficient for S_I and BMI was -0.634 ($P < 0.001$; Fig.

TABLE 3

Multiple linear regression analysis of the relationship between insulin sensitivity and age, gender, and measures of body fat distribution

Independent variables	Coefficient	SE	P
Age	−0.002	0.004	0.67
Gender	0.002	0.129	0.99
BMI	−0.021	0.016	0.19
Waist-to-hip ratio	−0.627	0.790	0.43
SCF area	−0.001	0.001	0.06
IAF area	−0.004	0.001	<0.001
Intercept	2.956	0.702	<0.001

The dependent variable is $\log_e S_I$. The model r^2 is 0.544. Sex was coded (0, 1) with the higher number indicating female sex. SE is the standard error for the regression coefficient.

TABLE 4

Body fat distribution and fasting plasma leptin concentrations in a subgroup of LIS and LIR subjects matched for SCF area and gender (14 men/24 women)

	LIS	LIR
n	38	38
Age (years)	49.6 ± 8.5	53.2 ± 12.0
BMI (kg/m^2)	24.1 ± 1.7	23.6 ± 1.7
S_I ($\times 10^{-5}$ min^{-1} · [pmol/l]$^{-1}$)	10.5 ± 3.8	5.2 ± 1.3*
SCF area (cm^2)	157 ± 52	160 ± 52
Waist-to-hip ratio	0.79 ± 0.08	0.80 ± 0.09
IAF area (cm^2)	56 ± 29	73 ± 39†
Insulin (pmol/l)	42.6 ± 19.2	53.4 ± 24.6†
Leptin (ng/ml)	8.8 ± 7.1	9.2 ± 5.9

Data are means ± SD. *$P < 0.001$; †$P < 0.05$.

2B), −0.498 for waist-to-hip ratio ($P < 0.001$; Fig. 2D), −0.570 for SCF ($P < 0.001$; Fig. 2F), and −0.688 for IAF ($P < 0.001$; Fig. 2H). These correlations were similar in both genders and in pre- and postmenopausal women. As shown in Fig. 2G, the relationship between the untransformed variables S_I and IAF area appeared to be nonlinear. Comparison of two regression models, containing either IAF alone (linear) or IAF and $\log_e$ IAF (nonlinear), indicated that the relationship was indeed better described by the nonlinear model ($P = 0.02$). Because of this nonlinearity, progressive but similar increments in the amount of IAF are associated with smaller decrements in insulin sensitivity. Despite a 6.8 kg/m^2 mean difference in BMI between the LIR and OIR subjects, there was a significant overlap in their IAF areas (Fig. 2G).

We further examined the contribution of IAF to S_I by individually matching two subgroups of LIR and OIR subjects ($n = 27$ per group) for gender (11 men and 16 women in each group) and IAF area (LIR 117 ± 35 cm^2 vs. OIR 120 ± 32 cm^2). Their S_I was not different (LIR 4.6 ± 1.5 × 10^{-5} min^{-1} · [pmol/l]$^{-1}$ vs. OIR 4.0 ± 1.4 × 10^{-5} min^{-1} · [pmol/l]$^{-1}$), although BMI (24.6 ± 1.4 vs. 30.0 ± 2.4 kg/m^2; $P < 0.001$), SCF area (213 ± 71 vs. 304 ± 94 cm^2; $P < 0.001$), and leptin levels (12.5 ± 7.5 vs. 20.4 ± 14.5 ng/ml; $P < 0.05$) were still higher in the obese individuals.

By multiple regression analysis, we analyzed the associations between measures of body fat distribution and insulin sensitivity simultaneously. In all subjects, S_I was highly inversely correlated with IAF ($P < 0.001$) but not with SCF, BMI, or waist-to-hip ratio (Table 3). In this model, 54% of the variance in S_I could be accounted for by IAF area. Using stepwise model building, an additional 5% of the variance in S_I could be attributed to SCF. Similarly, when the analysis was run in men and women separately, S_I was strongly predicted by IAF (regression coefficient −0.004 ± 0.001; $P \le 0.003$ in both genders) and not by any other variable, explaining 51 and 57% of its variability, respectively. In the regression model, IAF was the only variable that predicted S_I in subgroup analyses of pre- and postmenopausal women. When only lean subjects were included in the multiple regression analysis, S_I was predicted by IAF (regression coefficient −0.007 ± 0.001; $P < 0.001$) but not by age, sex, waist-to-hip ratio, or SCF.

Relationship between body adiposity, fat distribution, and leptin levels. First, we examined the relationships between leptin concentrations and measures of body fat distribution using simple linear regression in all subjects. A strong correlation was observed with BMI ($r = 0.555$; $P < 0.001$) and SCF ($r = 0.760$; $P < 0.001$) and IAF ($r = 0.301$; $P < 0.001$) areas. Thus, leptin levels were most strongly correlated with SCF area. Interestingly, the correlation coefficient for total abdominal fat area ($r = 0.679$) was not greater than that for SCF area alone. When genders were analyzed separately (Fig. 3), the correlation coefficients for leptin were greatest with SCF in both men and women ($r = 0.754$ for men, $r = 0.783$ for women; $P < 0.001$ for both; Fig. 3F). Similar correlation coefficients were observed for pre- and postmenopausal women.

Second, we examined the relationship between insulin sensitivity and leptin concentrations. Although their BMI was not different, the LIR group had leptin levels 33% higher than the LIS group (Fig. 1F). An increase in leptin levels was seen in both male and female LIR subjects, by 94 and 36%, respectively ($P < 0.01$, Table 2). However, because of the 50% greater abdominal fat area in insulin-resistant subjects, it was not possible to discern whether this hyperleptinemia was associated primarily with insulin resistance or with abdominal adiposity.

To examine whether the higher leptin levels in the LIR group were associated with increased adiposity or reduced insulin sensitivity, we examined whether leptin levels were different in two subgroups of 38 LIS and 38 LIR subjects with comparable SCF areas. As shown in Table 4, by definition, the LIR group still had a lower S_I. Age and BMI were not different, and the two groups were well matched for SCF area (Table 4). In contrast, the LIR subjects still had a 30% greater IAF area ($P < 0.05$). They were hyperinsulinemic, but their leptin levels were similar to those of LIS subjects matched for SCF area (Table 4). In contrast, in a subgroup of LIR subjects ($n = 37$; 15 men and 22 women) matched with LIS subjects for age, sex, BMI, and IAF area (LIR 62 ± 27 cm^2 vs. LIS 61 ± 26 cm^2), SCF area was significantly greater (174 ± 74 cm^2 vs. LIS 139 ± 57 cm^2; $P < 0.05$), as were leptin levels (11.1 ± 7.4 ng/ml vs. LIS 7.9 ± 6.0 ng/ml; $P < 0.05$).

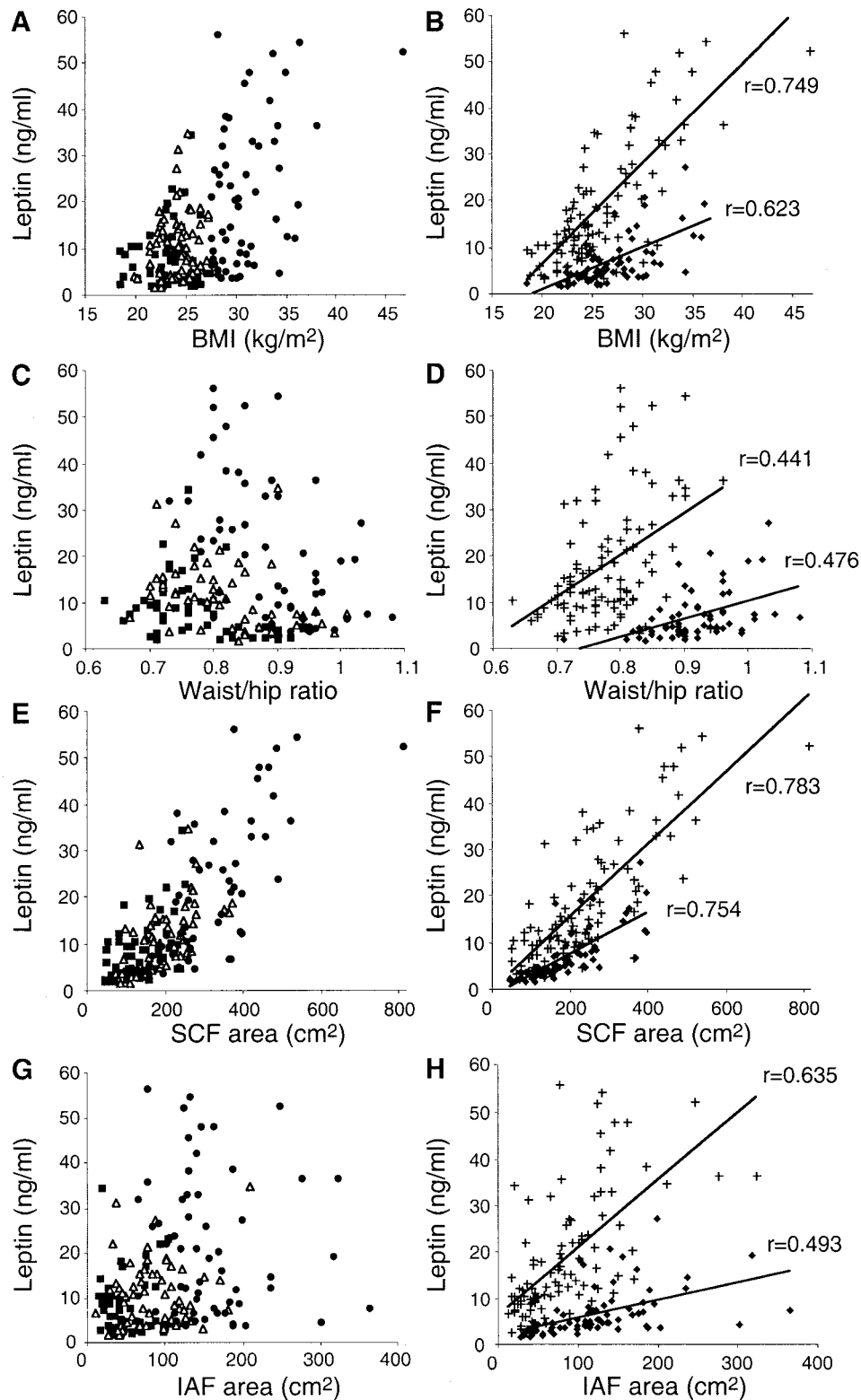We performed multiple regression analysis using step-

FIG. 3. Relationships between fasting plasma leptin levels and measures of body fat distribution in 174 subjects classified as LIS (■), LIR (△), or OIR (●). Correlation coefficients were determined for men (♦) and women (+). $P < 0.001$ for all correlations.

wise model building to analyze the association between leptin levels, body fat distribution, and insulin sensitivity simultaneously. As shown in Table 5, the strongest association was seen with SCF area in both sexes. By stepwise regression analysis of the same variables, SCF area accounted for 66% of the variance in leptin levels in men and

54% in women. The effect of other variables was minimal. In the stepwise regression model, 6% of the variance of leptin levels in men could be explained by IAF area and 2% by fasting insulin levels. In women, 4% was accounted for by S_I. In women, but not in men, age was negatively associated with leptin levels.

TABLE 5
Multiple regression analysis of the association between fasting plasma leptin levels and measures of body fat distribution and insulin sensitivity

Independent variables	Men			Women		
	Coefficient	SE	P	Coefficient	SE	P
Age	0.005	0.005	0.31	−0.012	0.005	0.03
BMI	−0.020	0.021	0.33	0.010	0.021	0.64
Waist-to-hip ratio	−0.788	1.033	0.45	−0.806	1.035	0.44
SCF area	0.005	0.001	<0.001	0.002	0.001	0.002
IAF area	0.002	0.001	0.046	0.003	0.002	0.07
S_I	−0.030	0.020	0.14	−0.062	0.025	0.02
Insulin	0.015	0.006	0.02	$−1 \times 10^{-5}$	0.011	1.0
Intercept	1.460	0.932	0.12	3.118	0.812	<0.001

The dependent variable is $\log_e$ leptin. The model r^2 is 0.764 for men and 0.620 for women. SE is the standard error for the regression coefficient.

DISCUSSION

We examined the relationship between body fat distribution, insulin sensitivity, and leptin concentrations in 174 apparently healthy individuals (73 men and 101 women). Based on their BMI and S_I, they were a priori classified as either LIS, LIR, or OIR. Although the BMI of the LIR subjects was similar to that of the LIS group, they had a more central body fat distribution. Waist circumference was greater, and this was attributable to a 50% increase in abdominal fat area, whereas nonfat tissue area was not different. However, the increase in waist-to-hip ratio between the LIS and LIR groups was small (0.02 for both genders). Among the measures of body fat distribution, IAF area was best correlated with S_I, and this was true for men and women and for pre- and postmenopausal women. This relatively small fat compartment [comprising 30% of abdominal and 10–20% of total body fat (10,26)] explained 54% of the variance of S_I.

Our finding that IAF is the major predictor of insulin sensitivity is in agreement with previous studies in 9 (6) and 16 men (26), in 25 black and white obese women (5), and in 55 postmenopausal women (7). It has been proposed that IAF is only associated with insulin resistance in the setting of obesity because indexes of glucose intolerance and dyslipidemia correlated with IAF in obese subjects but not in lean subjects (27). Bonora et al. (28) found that, in nonobese women, IAF is not correlated with glucose disposal after correction for total body fat, whereas in obese women, fat distribution rather than total fat mass assumes a critical role. Our data do not support this proposition and are in agreement with others (29,30) in that central abdominal fat distribution is a good predictor of insulin sensitivity in lean individuals. Although total body fat was not measured in our study, adjustment for BMI, as a measure of overall obesity, did not weaken the correlation between IAF and S_I in lean subjects. In addition, we have shown that this relationship is nonlinear. Thus, small increases in IAF area are associated with larger reductions in insulin sensitivity when small amounts of IAF are present, whereas the effect quantitatively diminishes as IAF accumulates.

Abate et al. (10) suggested that SCF is the major determinant of insulin sensitivity in obese men, whereas intra- and retroperitoneal fat have a lesser role (10). They also studied type 2 diabetic patients and found that they were more insulin resistant and had more SCF but

similar IAF than nondiabetic control subjects. Goodpaster et al. (11) found that total abdominal fat was strongly correlated with the insulin-stimulated glucose disposal rate but that IAF was not as good a predictor of insulin sensitivity as SCF in obese subjects, whereas in lean subjects, no correlations were detected. In contrast, an interventional study of diet-induced weight loss, by the same group, showed that the improvement in insulin sensitivity correlated with the decrease in IAF but not with other measures of body composition (31). The apparent contradiction between these reports may possibly be explained by the nonlinearity of the IAF/insulin sensitivity relationship [apparent in the study by Goodpaster et al. (11) and also in a rodent model of visceral fat accumulation and insulin resistance (32)]. Indeed, it is conceivable that a linear regression model using nontransformed variables, as in the study by Goodpaster et al., failed to detect the strength of this relationship. Our approach to examine the relationship between S_I and measures of body fat distribution using a nonlinear regression model clearly demonstrates that IAF is the critical variable predicting insulin sensitivity.

The mechanism(s) by which IAF causes insulin resistance is not clear. It has been suggested that elevated free fatty acid concentrations are involved in the association between IAF and insulin resistance. Intra-abdominal fat is relatively insensitive to insulin (26,33) and has a high lipolytic activity (26,34). In addition, subcutaneous adipocytes from women with visceral obesity exhibit higher lipolysis rates than those obtained from women with little visceral fat (35). The increased plasma free fatty acid concentrations associated with intra-abdominal adiposity may induce fat accumulation and insulin insensitivity in skeletal muscle (36,37) and liver (38,39). It remains to be determined whether enlargement of the IAF depot is causally related to the triglyceride accumulation in liver and muscle or whether it is a marker of the process of fat deposition in these and other nonadipose tissues. Other adipocyte-derived proteins may also play a role in the pathogenesis of insulin resistance. For instance, tumor necrosis factor-α impairs insulin receptor signaling in an autocrine or paracrine form (40); its expression in SCF has been shown to correlate with IAF mass in rodents (41), and some studies have suggested that circulating tumor necrosis factor-α levels correlate with

IAF in humans (42). Low plasma levels of adiponectin (43) have been associated with a state of insulin resistance in animal models (44) and in humans (43); in the animals, insulin resistance could be reversed by adiponectin infusion (44). Recently, another adipocyte-derived peptide, resistin, has been suggested to induce insulin resistance (45), but this was not confirmed in other rodent (46) and human (47) studies.

In addition to its role in glucose homeostasis, insulin acts as an adiposity signal in the brain, leading to a reduction in food intake (48). Another important signal of body adiposity and recent energy balance that is delivered to the central nervous system and limits food intake is circulating leptin (49). The relationship between body fat mass and leptin concentrations is well established (16,20). The larger SCF compartment has been implicated as the determinant of leptin levels (50), and this concept is supported by in vitro studies showing greater leptin secretion (51) and leptin gene expression (52) by subcutaneous adipocytes than by intra-abdominal adipocytes. However, others have found that leptin levels correlate better with the size of the IAF depot than with SCF in men (53). The latter finding is at variance with our finding that SCF is the major predictor of leptinemia, explaining 66% of its variance in men and 54% in women. In addition to the correlation with fat, leptin secretion can be regulated by insulin via insulin's effect to stimulate glucose metabolism in adipocytes (54), and, therefore, insulin sensitivity could be a determinant of leptinemia (19). Some studies have shown that this association disappeared after correction for body fat mass (20,55), but others suggested it was an independent determinant (56,57). The LIR subjects in our study were indeed hyperleptinemic compared with the LIS group, but their increased central adiposity made it difficult to simply attribute this to their insulin resistance. The fact that the difference in leptin levels disappeared when a subgroup of LIS and LIR subjects were matched for SCF illustrates the confounding effect of body fat on this association. However, whereas the effects of insulin sensitivity and fasting insulin concentrations on fasting leptin levels appear to be minimal, this study did not address the relationship between acute changes in insulin and leptin concentrations. Because circulating leptin levels are not constant throughout the day but exhibit a diurnal pattern that is dependent on the state of energy balance (58), it is possible that in the nonfasting state, leptin levels may be determined in part by insulin action.

Our observation that fasting insulin and leptin levels are elevated in obesity is in keeping with the findings of Schwartz et al. (55), who found that insulin sensitivity contributes to the association between body adiposity and plasma levels of insulin but not leptin. The fact that these signals of energy balance are elevated in obesity also supports the concept of insulin and leptin resistance in the central nervous system (59). Our finding that IAF determines insulin sensitivity while the SCF compartment correlates with plasma leptin levels suggests that these depots may result in a difference in the relative signaling to the central nervous system and suggests that, whereas insulin and leptin may both be important adiposity signals, their respective roles in body weight regulation may be different. Future research will help clarify whether a common mechanism leads to this impaired central leptin and insulin signaling and whether it shares similarities with the mechanisms resulting in peripheral insulin resistance.

In conclusion, we have shown that accumulation of IAF correlates best with insulin resistance and increased insulin levels and that SCF deposition is better associated with leptin levels in both genders. The concurrent increase in these metabolically distinct fat compartments appears to be the main explanation for the relationship between elevated leptin and insulin levels. Whereas increases in the two fat depots and in the respective adiposity signals are well recognized in obese individuals, the present study clearly demonstrates that the same phenomena can occur in lean subjects.

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