Levels of Free Fatty Acids (FFA) Are Associated with Insulin Resistance But Do Not Explain the Relationship between Adiposity and Insulin Resistance in Hispanic Americans: The IRAS Family Study

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Context and Objective: We investigated whether free fatty acids (FFA) mediate the association between adiposity and insulin resistance in the Hispanic-American families of the Insulin Resistance Atherosclerosis Family Study.

Design: In 815 Hispanic individuals in the Insulin Resistance Atherosclerosis Family Study, we tested for association between the following: 1) levels of adiposity [body mass index (BMI), visceral and sc adipose tissue area (VAT and SAT)] and circulating levels of FFA; 2) levels of circulating FFA and insulin sensitivity (S_I); and 3) levels of adiposity and S_I, additionally testing to see whether levels of FFA mediated or modified the relationship between adiposity and S_I.

Results: After adjusting for age, sex, clinic site, and admixture, increasing levels of BMI, VAT, and SAT were weakly associated with increasing levels of circulating FFA (BMI: $P = 0.024$; VAT: $P = 2.33 \times 10^{-3}$; SAT: $P = 0.018$; percent variation explained: ~1.00%). Increasing levels of circulating FFA were associated with decreasing S_I ($P = 8.10 \times 10^{-11}$). Increasing BMI, VAT, and SAT were also associated with decreasing S_I (BMI: $P = 4.98 \times 10^{-71}$; VAT: $P = 1.48 \times 10^{-64}$; SAT: $P = 4.21 \times 10^{-62}$), but this relationship was not significantly mediated by FFA. VAT, but not BMI or SAT, interacts with levels of FFA to influence S_I ($P = 0.021$).

Conclusions: Although levels of circulating FFA are associated both with increasing adiposity and decreasing S_I, they do not appear to mediate the association between levels of adiposity and S_I in this large cohort of Hispanic-Americans. These results may indicate that FFA contribute to insulin resistance independent of adiposity. **(*J Clin Endocrinol Metab* 97: 3285–3291, 2012)**

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Abbreviations: BMI, Body mass index; CI, confidence interval; CoA, acylation of coenzyme A; FFA, free fatty acid; FSIGT, frequently sampled iv glucose tolerance test; IRAS, Insulin Resistance Atherosclerosis Family Study; SAT, sc adipose tissue area; S_I, insulin sensitivity; VAT, visceral adipose tissue area; VSR, ratio of VAT to SAT.

The prevalence of type 2 diabetes mellitus has steadily risen in the United States and the world in the last decade and is associated with significant morbidity and mortality. Increased insulin resistance has been shown to precede the development of type 2 diabetes mellitus (1–4). Thus, understanding the biological mechanisms of insulin resistance may give great insight into the prevention of type 2 diabetes mellitus. Increasing obesity is associated with insulin resistance (reviewed in Ref. 5), and it has been hypothesized that plasma free fatty acids (FFA) may be an important link between the two (reviewed in Ref. 6).

FFA are used as the main source of fuel in the body during times of fasting or when glucose is not available. FFA are released from adipose tissue and subsequently they travel to target tissues such as skeletal muscle, liver, and others. Increased concentrations of FFA may induce insulin resistance through reduced glucose transport (7), through disruption of the insulin receptor substrate-1 signaling pathway (8), or through activation of the inhibitor of nuclear factor-κB kinase subunit-β/nuclear factor-κB inflammatory pathway (9, 10). Lowering levels of FFA has been shown to reduce insulin resistance; normalization of FFA levels improved insulin-stimulated glucose uptake in obese individuals without type 2 diabetes mellitus, individuals with type 2 diabetes mellitus, and lean individuals (11).

Increasing levels of obesity are often associated with increasing levels of circulating FFA (12, 13). This association has been attributed to increases in FFA release (5) and decreases in FFA clearance (14) resulting from increased adipocyte numbers in the enlarged adipose tissue mass. Rates of triglyceride uptake and lipolysis are higher in the visceral adipose tissue compared with the sc tissue depots (15–17). However, because the sc compartment is so much larger than the visceral compartment (reviewed in Ref. 18), the total amount of FFA turnover is greater in the sc compartment.

This work sought to carefully characterize the relationship between obesity, FFA, and insulin resistance in a large cohort of Hispanic-Americans in the Insulin Resistance Atherosclerosis (IRAS) Family Study and to investigate whether levels of FFA may mediate the relationship between obesity and insulin resistance or interact with adiposity to affect insulin resistance.

Procedures and Methods

Subject recruitment

Study design, recruitment, and phenotyping for the IRAS Family Study have been described in detail (19). Briefly, the IRAS Family Study is a multicenter study designed to investigate the genetic determinants of glucose homeostasis and adiposity. Probands who self-identified as Hispanic-American or African-American were first identified from the IRAS cohort study (20), and recruitment was supplemented with non-IRAS participants and their families. All families were recruited based on size and structure without regard to glucose tolerance or type 2 diabetes mellitus status. Hispanic participants were recruited from two clinic sites, one in San Antonio, TX, and one in San Luis Valley, CO. A total of 1081 individuals from San Antonio, TX (n = 560), or the San Luis Valley, CO (n = 521), making up 90 Hispanic families had a frequently sampled iv glucose tolerance test (FSIGT). All protocols were approved by the institutional review boards at each institution, and all participants provided written informed consent before participation.

Clinical protocols

Clinic examination of participants included a FSIGT using the reduced sampling protocol as previously described (21). In addition to anthropometric measures of adiposity, abdominal fat area was measured using a computed tomography scan for abdominal adiposity measurements at the L3/L4 and L4/L5 vertebral regions for sc adipose tissue area (SAT) and visceral adipose tissue area (VAT) as previously described (19). Plasma FFA levels were determined by using a colorimetric method (22) from Wako Diagnostics (Richmond, VA; catalog no. 991-34691) on the automated instrument, Elan ATAC8000 (Elan Diagnostics, Athlone, Ireland; interassay variation: 2.3–4.8%; intraassay variation: 5.1–8.6%). The method relies on the acylation of coenzyme A (CoA) by nonesterified fatty acids in the presence of added acyl-CoA synthatase. The resulting acyl-CoA is oxidized by added acyl-CoA oxidase with generation of hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide converts the substrate into a colored product, which can be read at 550 nm by the instrument. FFA data were available on 815 individuals. Characteristics of individuals missing FFA data are shown in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. After adjusting for demographic characteristics such as age, sex, and clinic site, the individuals with and without an FFA measure are not significantly different for characteristics such as body mass index (BMI) and insulin sensitivity (S_I).

The outcomes for this study were circulating levels of fasting FFA (described above) and S_I, a measure of insulin sensitivity derived using a minimal model (23) analysis of glucose and insulin data from the FSIGT (MINMOD Millennium, version 5.18).

Admixture adjustment

The IRAS Family Study has genotyped 80 unlinked single-nucleotide polymorphisms across the genome on a large fraction of IRAS Family Study participants. These markers were chosen as ancestry informative markers (Dr. Kent Taylor, personal communication) for the purposes of admixture adjustment. Based on a principal-components analysis of these 80 markers, the second principal component explained a significant proportion of the genetic variance in the Hispanic sample and represents the proportion of European admixture for each individual. We adjusted for admixture by including this principal component as a covariate in our association models (see below).

TABLE 1. Characteristics of 815 IRAS family study participants

Characteristic	Mean (SD)
Age (yr)	39.58 (13.00)
Male/female (n)	316/499
San Antonio/SanLuis Valley (n)	438/377
S_I ($\times 10^{-3}$ min^{-1}/pM)	3.69 (3.14)
FFA (μmol/liter)	738.50 (219.38)
BMI (kg/m^2) (n = 809)	28.47 (5.94)
VAT (cm^2) (n = 787)	104.02 (56.01)
SAT (cm^2) (n = 787)	334.21 (154.84)
VSR (n = 759)	0.35 (0.20)
Adiponectin (μg/ml)	13.44 (6.27)
Admixture principal component[a]	0.55 (0.13)

Values are mean (SD) unless indicated. All participants were nondiabetic, had a complete FSIGT, had a fasting FFA measure, and had been genotyped on 80 ancestry informative markers.

[a] Principal component is based on 80 ancestry informative markers.

Tests of association

A likelihood ratio test as implemented in the Simultaneous Oligogenic Linkage Analysis Routines software package (Texas Biomedical Research Institute, San Antonio, TX) was used to test for associations between adiposity and plasma levels of FFA, the associations between plasma levels of FFA and S_I, and the associations between adiposity and S_I. The Simultaneous Oligogenic Linkage Analysis Routines software package uses a variance components model, which directly models the covariance among related individuals. Continuous-outcome S_I was natural log transformed for analysis to best approximate the model assumptions (*i.e.* conditional multivariate normality and homogeneity of variance). Individuals with type 2 diabetes mellitus were excluded (61 from San Antonio and 39 from San Luis Valley). To directly compare the associations of each of the adiposity variables [BMI, VAT, SAT, and ratio of VAT to SAT (VSR)] with the outcomes of S_I or FFA, we calculated regression coefficients based on the SD difference in the adiposity characteristic.

Mediation analyses

A mediating variable (plasma levels of FFA) is an intermediate variable on the causal pathway between an independent variable (adiposity) and an outcome variable (S_I) (Supplemental Fig. 1) (24). To assess the potential for mediation of the relationship between adiposity and S_I by FFA, we first tested for association between each adiposity measure and plasma levels of FFA. We then tested for an association between levels of plasma FFA and S_I. For the adiposity measures that were associated with levels of plasma FFA ($P < 0.05$), we then tested for an association between that adiposity measure and S_I, recording the parameter estimate for the adiposity measure ($\beta_{adiposity}$). Finally, we tested for an association between each adiposity measure and S_I, adjusting for plasma levels of FFA, recording the FFA-adjusted parameter estimate for the adiposity measure ($\beta_{adiposity\_FFA\_adjusted}$). We used the change in estimate method [$(\beta_{adiposity} - \beta_{adiposity\_FFA\_adjusted})/\beta_{adiposity}$] to estimate the percentage of the association between adiposity and insulin sensitivity that is mediated through plasma levels of FFA (25). This method is identical with the method used to assess confounding; distinguishing between mediation and confounding is based on the biological pathway argument above. We considered a change in estimate of 20% or greater to be meaningful mediation. We also compared the mediation effects for plasma levels of FFA for each adiposity measure to assess whether plasma levels of FFA mediate the association between adiposity and S_I to a greater degree for certain measures. We use mediation in this context, recognizing that a mediation hypothesis inherently assumes a temporal relationship, with increases in adiposity occurring before increases in FFA and increases in FFA occurring before decreases in S_I. Because these data are cross-sectional, we tested the statistical associations while making the assumptions of temporality based on biological data (7, 8, 11–13).

Interaction analyses

We tested whether plasma levels of FFA modify the associations between adiposity measures and S_I by testing for interaction between the adiposity measure and FFA, using a likelihood ratio test comparing the models with and without the interaction term. The main effects for the FFA and the adiposity measure were also included in the model with the interaction.

Results

We report the results from 815 individuals of 1081 with a complete FSIGT and a fasting FFA measure who had been genotyped on the 80 ancestry informative markers and who did not have type 2 diabetes mellitus. The number of individuals per family ranged from three to 48 with a mean of 21. Descriptive characteristics for the sample are shown in Table 1.

TABLE 2. Associations of adiposity characteristics with FFA

Outcome	Adiposity measure	SD adiposity measure	$\beta_{adiposity}$[a]	*P* value	Percent variation explained: Full model (%)	Percent variation explained: Adiposity measure (%)[b]
FFA	BMI	6.18	17.73	0.024	9.89	0.68
FFA	VAT	61.42	28.34	2.33×10^{-3}	10.36	1.15
FFA	SAT	154.51	18.87	0.018	10.17	0.96
FFA	VSR	0.211	−3.50	0.760	9.65	0.44

[a] For a 1 SD change in adiposity measure, adjusted for age, sex, clinic, and admixture.

[b] Determined by subtracting the percent variation in FFA explained by the model without the adiposity measure (9.21%), which is adjusted for age, sex, clinic, and admixture, from the percent of variation in FFA explained by the full model including the adiposity measure.

TABLE 3. Association of FFA with S_I

Outcome	Predictor	SD FFA	β_{FFA}[a]	P value	Percent variation explained: Full model %	Percent variation explained: FFA %[b]
S_I	FFA	217	−0.140	8.10×10^{-11}	11.54	4.13

[a] For a 1 SD change in FFA, adjusted for age, sex, clinic, and admixture.

[b] Determined by subtracting the percent of variation in S_I explained by the model without FFA (7.40%), which is adjusted for age, sex, clinic, and admixture, from the percent variation in S_I explained by the FFA-adjusted full model.

In separate models, increasing levels of BMI, VAT, and SAT were each independently associated with increasing levels of FFA (Table 2), adjusting for age, sex, admixture, and clinic site. Levels of VSR were not associated with levels of FFA ($P = 0.760$). The proportion of the total variance in FFA that was explained by the adiposity measures was approximately 1% for BMI, VAT, and SAT. VAT had the strongest association with levels of FFA ($P = 2.33 \times 10^{-3}$). For a 1-SD increase in VAT, levels of FFA increased by 28 μmol/liter [95% confidence interval (CI) 10–47 μmol/liter], whereas for a 1-SD change in BMI or SAT, levels of FFA increased by 18 μmol/liter (95% CI 2–33 μmol/liter) and 19 μmol/liter (95% CI 3–34 μmol/liter), respectively.

Increasing levels of FFA were inversely associated with increasing levels of S_I (Table 3); FFA explained 4.13% of the variance in S_I.

Because levels of adiposity were associated with FFA and because levels of FFA were associated with S_I, we then assessed whether levels of FFA mediated the associations between levels of adiposity and S_I. Increasing levels of BMI, VAT, and SAT were each associated with decreasing levels of S_I (Table 4, adiposity alone; P values for adiposity: 4.98×10^{-71}, 1.48×10^{-64}, and 4.21×10^{-62}, respectively). VAT and SAT explain approximately 28% of the variation in S_I, and BMI explains approximately 30%. When we additionally adjust for FFA, all three adiposity measures remain significantly associated with S_I (P values: BMI: 1.97×10^{-70}; VAT: 1.58×10^{-70}; SAT: 4.77×10^{-61}) and explain between 26% (VAT, SAT) and 29% (BMI) of the variation in S_I (Table 4 adiposity adjusted for FFA). FFA are also still significantly associated with S_I in each of the models (P values: 6.22×10^{-8} to 6.20×10^{-9}; data not shown). However, the relationship between adiposity and S_I is not mediated by FFA to any significant degree (percent mediation: 2.46–3.20%).

We saw a weak statistical association between increasing levels of adiposity and increasing levels of FFA. Because levels of obesity and FFA are physiologically intertwined, we tested whether FFA interacted with measures of adiposity to influence levels of S_I. We found a significant interaction between VAT and FFA ($P = 0.021$) but not between BMI and FFA ($P = 0.065$) or between SAT and FFA ($P = 0.598$) (Table 5). To understand the effect of the interaction between VAT and FFA on levels of S_I, we determined the median level of FFA and VAT for the cohort (FFA = 742 μmol/liter; VAT = 94.08 cm^2). We then assigned individuals into four categories based on their FFA and VAT levels as follows: low FFA, low VAT (FFA ≤ 742 μmol/liter and VAT ≤ 94.08 cm^2); low FFA, high VAT (FFA ≤ 742 μmol/liter and VAT > 94.08 cm^2); high FFA, low VAT (FFA > 742 μmol/liter and VAT ≤ 94.08 cm^2); and high FFA, high VAT (FFA > 742 μmol/liter and VAT > 94.08 cm^2). For each of these four categories, we displayed the predicted S_I, adjusted for age, gender, ad-

TABLE 4. Association of adiposity measures with S_I (adiposity alone models) and associations of adiposity measures with S_I, accounting for the effects of FFA (adiposity adjusted for FFA)

Outcome	Adiposity measure	Adiposity alone[a]: $\beta_{adiposity}$[c]	Adiposity alone[a]: P value	Adiposity alone[a]: Variation full model (%)[d]	Adiposity adjusted for FFA[b]: $\beta_{adiposity\_FFA\_adjusted}$[e]	Adiposity adjusted for FFA[b]: P value	Adiposity adjusted for FFA[b]: Variation full model (%)[f]	Mediation by FFA (%)[g]
S_I	BMI	−0.367	4.98×10^{-71}	37.68	−0.358	1.97×10^{-70}	40.34	2.46
S_I	VAT	−0.413	1.48×10^{-64}	35.81	−0.399	1.58×10^{-62}	37.63	3.20
S_I	SAT	−0.349	4.21×10^{-62}	35.35	−0.340	4.77×10^{-61}	38.33	2.76

[a] Adjusted for age, sex, clinic, and admixture covariate.

[b] Adjusted for age, sex, admixture, and clinic.

[c] Represents a 1 SD change in adiposity measure in the adiposity-alone model.

[d] Percent of variation explained by age, sex, clinic, admixture covariate, and adiposity measure.

[e] Represents a 1 SD change in adiposity measure in the adiposity model adjusted for FFA.

[f] Percent variation explained by age, sex, clinic, admixture covariate, adiposity measure, and FFA.

[g] Determined by subtracting $\beta_{adiposity\_FFA\_adjusted}$ from $\beta_{adiposity}$ and dividing by $\beta_{adiposity}$.

TABLE 5. Levels of FFA interact with VAT but not BMI or SAT to predict S_I

Outcome	Interaction term[a]	Covariate	β-Coefficient[b]	P value[c]
S_I	BMI-FFA	BMI	−0.361	3.59×10^{-71}
		FFA	−0.105	5.02×10^{-9}
		BMI-FFA	0.033	0.065
S_I	VAT-FFA	VAT	−0.405	1.11×10^{-63}
		FFA	−0.101	3.40×10^{-8}
		VAT-FFA	0.042	**0.021**
S_I	SAT-FFA	SAT	−0.340	4.16×10^{-61}
		FFA	−0.106	9.86×10^{-9}
		SAT-FFA	0.009	0.598

Significant *P* values for interaction between adiposity measure and FFA are shown in bold.

[a] Each model includes age, sex, clinic, and admixture as covariates and is tested for the main effects of FFA, the adiposity measure, plus the interaction between adiposity and FFA.

[b] β-Coefficient is for a 1 SD change in the covariate.

[c] *P* values are for the fully adjusted models, adjusted for age, sex, clinic, admixture, adiposity, FFA, and the interaction of adiposity and FFA.

mixture, clinic site, VAT, FFA, and the interaction of VAT and FFA (Fig. 1). In conditions of high VAT, subjects with low FFA have similar S_I (mean S_I = 2.24; SD = 0.80) to individuals with high FFA (mean S_I = 1.79; SD = 0.75). However, in conditions of low VAT, individuals with low FFA have improved S_I (mean S_I: 4.63; SD = 1.02) relative to those with high FFA (mean S_I = 3.63; SD = 0.70).

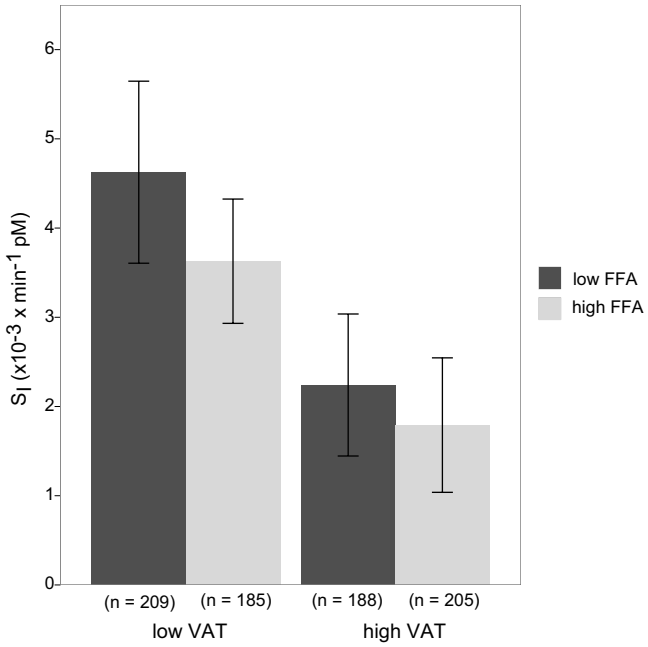

FIG. 1. Predicted S_I, accounting for the interaction between levels of FFA and VAT. The model is adjusted for age, gender, clinic, admixture, VAT, FFA, and the interaction between VAT and FFA. Individuals were assigned into one of four categories based on their FFA and VAT levels: low FFA, low VAT (FFA ≤ 742 μmol and VAT ≤ 94.08 cm^2); low FFA, high VAT (FFA ≤ 742 μmol and VAT > 94.08 cm^2); high FFA, low VAT (FFA > 742 μmol and VAT ≤ 94.08 cm^2); and high FFA, high VAT (FFA > 742 μmol and VAT > 94.08 cm^2). Values displayed are the mean predicted S_I for each group. *Error bars* represent the SD.

Discussion

We have demonstrated an association between increasing adiposity and increasing levels of circulating FFA, between increasing levels of FFA and decreasing levels of S_I, and between increasing levels of adiposity and decreasing levels of S_I. Although other studies have examined the relationships between obesity and FFA (12, 13) and between FFA and insulin resistance (7, 8, 26), very few large studies have examined how FFA may mediate or modify the relationship between obesity and insulin resistance. In addition, no studies have looked at these relationships in Hispanics, who show increased risk for obesity, insulin resistance and type 2 diabetes mellitus (27–29).

Contrary to our initial hypothesis, we found that levels of FFA do not mediate the association between adiposity and S_I. However, our findings suggest that FFA may interact with VAT to influence levels of S_I. Our study is one of the first to look at whether computer tomography-derived measures of adiposity or insulin sensitivity measures by an FSIGT are associated with fasting levels of FFA. It is also one of the first large studies to examine the relationships between adiposity, FFA, and S_I in Hispanics.

FFA have been shown to be associated both with measures of adiposity and with insulin sensitivity, and it has also been hypothesized that they are an important link between the two (6). Thus, we hypothesized that the association between adiposity and S_I might be mediated through FFA. There are several potential reasons that we have not demonstrated this in our present analysis.

First, although we demonstrated an association between increasing levels of adiposity and increased FFA, the relationship was not very strong. Each of the adiposity measures explained only approximately 1% of the variance in FFA. Based on the finding that adiposity is not a strong predictor of FFA in our population, we would not

expect circulating levels of FFA to strongly mediate the relationship between adiposity and S_I.

Likewise, there are several reasons for the relatively weak association between adiposity and FFA in our study population. Although obesity is thought to lead to an increase in circulating FFA due to increased number of adipocytes (5) and decreased clearance (14), levels of circulating FFA are likely influenced by several other factors, including overall physical activity (30), short- and long-term dietary intake (31, 32), and overall FFA metabolism. Thus, levels of obesity may not strongly correlate with levels of circulating FFA. Participants in this study were not asked to consume a nutrient-standardized meal previous to their fasting blood draw or FSIGT, and diet information was not collected at this clinic visit. Thus, we are not able to account for the effect of short- or long-term dietary intake, nor do we have any means to assess FFA and triglyceride metabolism. Fasting FFA represent only a short period of time during each day, and other time points, such as postprandial FFA, may be more relevant. Finally, our measure of fasting FFA does not take into account the complex nature between FFA, triglycerides, and adipocytes. After a meal, dietary FFA are esterified into triglycerides and then stored in adipocytes for later energy needs (reviewed in Ref. 33). Under fasting states, lipolysis breaks triglycerides into FFA, which then travel to metabolically active tissues such as skeletal muscle and liver. The level of plasma FFA depends on both rates of esterification and lipolysis. Other studies have used stable radiolabeled fatty acid isotopes to show that the rate of appearance of FFA into plasma is greater in obese individuals (34, 35). We cannot assess FFA trafficking in this study.

We did not find that increasing levels of FFA mediated the relationship between increased levels of adiposity and S_I, despite demonstrating a very strong association between adiposity and S_I. These results indicate that other factors likely contribute to the association between adiposity and S_I. Obesity is considered a state of chronic inflammation, whereby inflamed adipose tissue secretes FFA as well as proinflammatory cytokines such as TNF-α and IL-6 (reviewed in Refs. 36 and 37). FFA are known to induce oxidative stress and inflammation, and inflammatory signals disrupt the insulin signaling pathway (36–38). Thus, these proinflammatory cytokines likely also contribute to obesity-related insulin resistance. Unfortunately, this study did not collect inflammatory measures, so we were unable to test this hypothesis.

Given that both obesity and FFA are associated with insulin resistance, we also hypothesized that FFA might modify the association between obesity and S_I. We found that FFA interact with VAT to influence levels of S_I. Individuals with higher VAT (above the median) have similar levels of S_I, regardless of whether they have high or low levels of FFA. However, in individuals with lower VAT, individuals with low levels of FFA have improved levels of S_I relative to those with high levels of FFA. Because VAT is considered a relatively insulin-resistant tissue, individuals with high levels of VAT may be predisposed to insulin resistance, such that levels of FFA do not additionally impact insulin resistance. However, in individuals with low levels of VAT, increased levels of FFA may additionally contribute to insulin resistance through decreased glucose transport (7) or disruption of normal insulin signaling (8). The fact that adipocytes isolated from VAT are more insulin resistant than those isolated from SAT (39, 40) may explain why we see an interaction between FFA and VAT but no interaction between FFA and SAT.

Increased levels of obesity have long been associated with increased risk of insulin resistance. Our work demonstrates that circulating levels of FFA are also associated with insulin resistance in a large population of Hispanic-Americans. In addition to weight loss, lowering levels of FFA may represent a mechanism to reduce insulin resistance.

Although we did not find that FFA mediate the relationship between obesity and S_I, we did demonstrate a strong inverse association between levels of circulating FFA and S_I. Although the mechanisms by which increased levels of FFA disrupt normal insulin signaling are well established, very few population-based studies have examined the associations between circulating levels of FFA and S_I. Our work has demonstrated that circulating levels of FFA explain 4% of the variance in S_I. This association persists after adjustment for adiposity, suggesting that the association between FFA and S_I may be independent of adiposity.

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