Ethnic Differences in Pancreatic Fat Accumulation and Its Relationship With Other Fat Depots and Inflammatory Markers

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OBJECTIVE—Visceral adipose tissue (VAT) and hepatic fat are associated with insulin resistance and vary by sex and ethnicity. Recently, pancreatic fat fraction (PFF) has also been linked with increasing obesity. Our aim was to assess ethnic and sex differences in PFF and its relationship to other fat depots, circulating free fatty acids (FFA), insulin secretion and sensitivity, and inflammation in obese adolescents and young adults.

RESEARCH DESIGN AND METHODS—We examined 138 (40 males, 98 females) obese Hispanics and African Americans (13–25 years). Subcutaneous adipose tissue and VAT volumes, hepatic fat fraction (HFF), and PFF were determined by magnetic resonance imaging. Insulin sensitivity and β-cell function were assessed during an intravenous glucose tolerance test.

RESULTS—Hispanics had higher PFF than African Americans (7.3 ± 3.8 vs. 6.2 ± 2.6%, $P = 0.03$); this ethnic difference was higher in young adults compared with children and adolescents (ethnicity × age: $P = 0.01$). Males had higher PFF than females ($P < 0.0001$). PFF was positively correlated with VAT ($r = 0.45$, $P < 0.0001$), HFF ($r = 0.29$, $P < 0.0001$), and FFA ($r = 0.32$, $P = 0.001$). PFF positively correlated with inflammatory markers but lost significance when adjusted for VAT. In multiple stepwise regression analysis, VAT and FFA were the best predictors of PFF (adjusted $R^2 = 0.40$). There were no significant correlations between PFF and markers of insulin sensitivity or β-cell function.

CONCLUSIONS—PFF is higher in Hispanics than African Americans, and this difference increases with age. In young obese individuals, PFF is related to VAT, HFF, and circulating FFA, thus possibly contributing to their increased risk for type 2 diabetes and related metabolic disorders.

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Accumulation of lipids in tissues others than subcutaneous adipose tissue (SAT), such as in the visceral adipose tissue (VAT), skeletal muscle, or liver, is linked to insulin resistance, which is central to the pathophysiology of type 2 diabetes (1–3). The unifying mechanisms are that in obese individuals, alteration of adipose tissue lipolysis increases circulating free fatty acids (FFA), which may play a role in liver fat accumulation (4) and inflammatory processes (5).

Most prior work in the area of ectopic fat has focused on muscle and liver, but there is also evidence that pancreatic fat deposition is related to obesity (6). Early autopsy studies from the 1930s demonstrated an association between body weight and pancreas weight in humans and a higher fat content in the pancreas of obese versus lean cadavers. More recent studies using imaging techniques confirmed that the pancreatic fat content increased with BMI and age (6–9).

Hispanics and African Americans are two ethnic groups with high prevalence of obesity and type 2 diabetes (10). However, regional fat distribution significantly differs between these two ethnicities: Hispanics tend to accumulate more VAT and have higher hepatic fat fraction (HFF), whereas African Americans have lower levels of VAT and HFF but higher levels of SAT (11). Whether pancreatic fat differs between these two at-risk minorities remains unknown.

The primary focus of the current study was to determine ethnic differences in pancreatic fat fraction (PFF) in obese Hispanic and African American adolescents and young adults. The secondary aims were to assess the associations between pancreatic fat and other fat compartments including liver, VAT, and SAT, as well as circulating FFA and insulin sensitivity, β-cell function, and circulating markers of inflammation.

RESEARCH DESIGN AND METHODS

Study subjects

This cross-sectional analysis includes 138 overweight (age- and sex-specific BMI ≥85th percentile based on Centers for Disease Control and Prevention charts or BMI ≥30 kg/m^2 in adults), African American or Hispanic ethnicity (self-report and based on all four grandparents being of the same ethnic group), aged 13–25 years old. Participants were excluded if they had taken medications known to effect body composition, been diagnosed with any major illness since birth, or had any diagnostic criteria for diabetes. Written informed consent and assent was received from both parents and children. This study was approved by the Institutional Review Board.

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Anthropometry and fat quantification

Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a beam medical scale and wall-mounted stadiometer, and BMI was calculated (12). Whole body fat and soft lean tissue was measured by dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR 4500 W (Hologic, Bedford, MA).

Abdominal magnetic resonance imaging data were obtained by the Dixon method, with a sensitive three-point chemical-shift fat-water separation method using a 1.5 Tesla Siemens Symphony Maestro whole-body scanner (Siemens AG, Erlangen, Germany) with Numaris 4 software. A two-dimensional multislice breath-hold protocol previously reported by Hussain et al. (13) was adopted to obtain 19 axial images across the abdomen from the dome of the liver to the L2-L3 vertebrae. The standard body transmit and receive coil was used, along with a rectangular field-of-view of 420 mm (right/left) by 315 mm (anterior/posterior). The slice thickness was 10 mm with no interslice gaps. The fat-only dataset was used in the subsequent quantification of SAT and VAT volume, whereas the fat fraction dataset was used to assess percent hepatic and pancreatic fat content (HFF, PFF). The computations for SAT, VAT, HFF, and PFF were performed by a trained operator (M.P.) at the Image Analysis Laboratory of St. Lukes-Roosevelt Hospital Center in New York. A commercially available image segmentation and quantification software (SliceOmatic, Tomovision) was used. SAT and VAT volumes were computed across all 19 image slices in each subject. HFF and PFF were computed as the mean fat fraction of all imaging slices within which the liver and the pancreas was present, respectively.

Frequently sampled intravenous glucose tolerance test

An insulin-modified frequently sampled intravenous glucose tolerance test (IVGTT) (14,15) was performed after an overnight fast. Upon arrival, a topical anesthetic (EMLA cream; Aztrozeneca, Wilmington, DE) was applied to the antecubital area of both arms and an hour later a flexible intravenous catheter was inserted into both of the arms. At time 0 min, glucose (25% dextrose, 0.3 g/kg body wt) was administered intravenously. Insulin (0.02 units/kg body wt, Humulin R [regular insulin for human injection]; Eli Lilly, Indianapolis, IN) was injected intravenously at 20 min. Blood samples of glucose and insulin were collected at time points −15, −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min and of FFA at time −15.

Blood analysis

Blood samples from all time points taken during the IVGTT were centrifuged immediately for 10 min at 860 *g* and 8–10°C, and plasma was collected and frozen at −70°C until assayed. Glucose was assayed in duplicate on a Yellow Springs Instrument 2700 Analyzer (Yellow Springs Instrument, Yellow Springs, OH) using the glucose oxidase method. Insulin was assayed in duplicate using a specific human insulin ELISA kit from Linco (St. Charles, MO) and FFA using a colorimetric kit (NEFA-HR [2], Wako Diagnostics). Circulating inflammatory mediators including plasminogen activator inhibitor 1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and hepatocyte growth factor (HGF) were measured in batch using multiplex Luminex assays (Linco Research) (16). High-sensitivity C-reactive protein (hs-CRP) was measured chemically using ADVIA 1800 Chemistry System (Siemens Healthcare Diagnostics, Deerfield, IL). Because of missing samples, cytokines were obtained only in a subgroup of 108 participants.

Calculations

Plasma collected during the IVGTT was analyzed for glucose and insulin, and values were entered into the MINMOD Millenium 2003 computer program (version 5.16, Bergman) to determine insulin sensitivity index (ISI), glucose effectiveness (S_g), acute insulin response (AIR), and disposition index (DI) (17). ISI was defined as the net capacity for insulin to promote the disposal of glucose and to inhibit the endogenous production of glucose, whereas S_g indicated the capacity of glucose to mediate its own disposal. AIR was defined as the area under the plasma insulin curve between 0 and 10 min, and DI, an index of β-cell function, was calculated as the product of AIR and ISI.

Statistical methods

ANOVA was used to compare unadjusted insulin sensitivity, fat distribution, and body composition variables by sex and ethnicity. Post hoc comparisons were done using Tukey-Kramer test. Because HFF, PFF, PAI-1, and hs-CRP were not normally distributed, they were log-transformed before all statistical analyses. Adjustments of comparisons for potential confounders including age, sex, ethnicity, BMI, total fat, visceral fat, and lean body mass were performed using ANCOVA when appropriate. Pearson correlation tests were used to assess the relationships between PFF and the other parameters. After potential ethnic and sex interactions were tested, these calculations were performed in the entire sample when interactions were found not significant. To examine the main predictor of pancreatic fat, we performed a stepwise multiple regression analysis including ethnicity, sex, age, total fat, visceral fat, hepatic fat, FFA, and DI as independent variables. All data are means ± SD, and statistical analyses were performed using STATA version 11.0 (Stata Corp, College Station, TX), with a significance level of $P < 0.05$.

RESULTS

Ethnic, sex, and age differences in fat depots

Characteristics of subjects by ethnicity and sex subgroups are shown in Table 1. All subgroups were of similar age and BMI. Hispanics had higher levels of total body fat (40.2 ± 6.0 vs. 37.5 ± 6.5%, $P = 0.007$), VAT (2.6 ± 1.3 vs. 1.5 ± 0.9 L, $P < 0.0001$), and HFF (7.3 ± 5.3 vs. 4.5 ± 3.2%, $P < 0.0001$) than African Americans as well as a trend for lower subcutaneous fat (13.9 ± 5.1 vs. 15.4 ± 5.9 L, $P = 0.056$) (Fig. 1). PFF was higher in males compared with females (8.3 ± 3.8 vs. 6.4 ± 3.2%, $P = 0.006$). These results remained significant when adjusted for sex, BMI, and age. In both ethnicities, there was a significant ($P < 0.05$) effect of age on all fat compartments. Participants aged 18–25 years compared with the 13–17 years group had higher levels of SAT (16.1 ± 13.9 vs. 13.9 ± 5.8 L), VAT (2.9 ± 1.6 vs. 1.8 ± 1.1 L), HFF (7.5 ± 6.0 vs. 5.7 ± 4.3%), and PFF (8.7 ± 4.5 vs. 6.4 ± 2.9%). These differences remained significant after adjusting for ethnicity, sex, BMI, and lean body mass. Hispanics over age 18–25 years have higher PFF than younger Hispanics aged 13–17 years or African Americans of similar age: in ANCOVA analysis of PFF including ethnicity, age, and the ethnicity × age interaction, all factors were significant (ethnicity: $P = 0.02$; age: $P = 0.001$; ethnicity × age: $P = 0.01$) (Fig. 1).

Table 1—*Anthropometric and metabolic parameters of subjects*

	Hispanic		African American			
	Males (n = 20)	Females (n = 54)	Males (n = 20)	Females (n = 44)	P value ethnicity	P value sex
Anthropometric parameters						
Age (years)	17.1 ± 2.7	16.8 ± 3.2	17.7 ± 4.4	17.2 ± 2.9	NS	NS
BMI (kg/m^2)	34.2 ± 4.3	35.1 ± 5.5	36.0 ± 5.3	34.8 ± 6.7	NS	NS
Fat depots						
Total fat (%) (n = 99)	31.8 ± 3.6	42.5 ± 4.4	31.5 ± 7.3	39.0 ± 5.2	<0.0001	0.01
SAT (L)	13.7 ± 4.7	14.0 ± 5.3	15.2 ± 5.2	15.9 ± 6.2	NS	NS
VAT (L)	3.4 ± 1.5	2.2 ± 1.1	2.1 ± 1.2	1.2 ± 0.08	<0.0001	<0.0001
HFF (%)	8.9 ± 6.6	6.7 ± 4.8	6.0 ± 4.0	3.8 ± 1.8	<0.001	<0.0001
PFF (%)	8.0 ± 4.0	7.0 ± 3.8	7.9 ± 3.6	5.5 ± 1.6	<0.0001	<0.005
Plasma FFA (mmol/L)	0.80 ± 0.13	0.78 ± 0.18	0.76 ± 0.15	0.69 ± 0.18	0.04	NS
Glucose and insulin homeostasis						
Fasting glucose (mmol/L)	5.1 ± 0.3	5.1 ± 0.4	5.0 ± 0.5	5.4 ± 0.4	0.01	NS
Fasting insulin (pmol/L)	127 ± 69	152 ± 91	137 ± 118	127 ± 69	NS	NS
AIR (pmol/L × 10 min)	10,438 ± 5,535	9,208 ± 5,286	16,619 ± 10,872	13,486 ± 10,885	0.001	NS
S_g (% per min)	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	NS	NS
ISI ($\times 10^{-4}$min^{-1}/μU/mL)	1.6 ± 0.8	1.8 ± 1.5	1.5 ± 1.0	1.4 ± 0.9	NS	NS
DI ($\times 10^{-4}$min^{-1})	2,047 ± 1,018	1,708 ± 754	2,696 ± 1,295	2,009 ± 1,189	0.02	0.01

Data are means ± SD. Fasting glucose and fasting insulin values for both Hispanics and African Americans were tested using a two-way ANOVA, followed by Tukey-Kramer tests.

Relationships with other fat depots and indexes of obesity

In univariate analysis, there was a significant relationship between PFF and several body composition variables, including BMI, SAT, VAT, and HFF, as well as circulating FFA (Table 2). After adjustment for ethnicity, sex, age, BMI, and overall total fat, PFF remained correlated with BMI (r = 0.28, P = 0.01), SAT (r = 0.21, P = 0.06), and VAT (r = 0.35, P = 0.002). HFF and PFF were positively correlated with each other (r = 0.34, $P < 0.0001$), but this correlation was no longer significant after adjusting for VAT (Fig. 2).

The relationship between PFF and VAT was significantly influenced by ethnicity (P = 0.03 for interaction), with a stronger relationship between PFF and VAT in Hispanics than African Americans (Hispanics: r = 0.58, $P < 0.0001$; African Americans: r = 0.32, P = 0.01). Multiple stepwise regression analysis using PFF as the dependant variable and ethnicity, sex, age, total fat, SAT, VAT, HFF, FFA, and DI as independent variables showed that only VAT and FFA were significant predictors of PFF (VAT: $P < 0.001$; FFA: $P < 0.05$), together explaining 40% of the variance in PFF.

Relationships between PFF and insulin sensitivity, β-cell function, and markers of inflammation

PFF was not correlated with any outcome related to glucose or insulin, including ISI and DI ($P > 0.05$), even after adjusting for ethnicity, sex, BMI, and age. Relationships between PFF and AIR or DI were further adjusted for ISI, which did not modify the results. Among inflammatory markers, PFF was positively correlated with PAI-1 (r = 0.26, P = 0.009), MCP-1 (r = 0.23, P = 0.02), IL-8 (r = 0.28, P = 0.006), and HGF (r = 0.20, P = 0.04), and a trend was observed for TNF-α (r = 0.18, P = 0.07). These correlations, however, were no longer significant when adjusted for VAT. There was no significant relationship between PFF and hs-CRP ($P > 0.05$).

CONCLUSIONS—This study shows that obese Hispanic young adults have higher pancreatic fat accumulation than obese African Americans and that this ethnic difference becomes greater with increasing age. Pancreatic fat was positively associated with VAT volume and liver fat deposition, as well as increased concentrations of plasma FFA.

Previous studies have consistently reported that Hispanics accumulate more VAT and HFF than African Americans, whereas the latter have higher levels of SAT (11). VAT is known to be more deleterious than SAT, because of its high rate of lipolysis and delivery of portal inflammatory cytokines (18). This may contribute to higher liver fat deposition, a strong predictor of insulin resistance (18). Our results support these ethnic differences in fat partitioning and further underline the fact that they are detected early in life. Furthermore, we show that pancreatic fat deposition is higher in Hispanics than African Americans. Pancreatic fat has recently been identified as a novel obesity-related fat depot (6–9). It is higher in males than females and linearly increases with age and BMI (6,7), consistent with our results. In this study, we further show that ethnic differences in PFF are exacerbated with increased age even in young populations, with a twofold higher PFF in Hispanics versus African Americans from the 19–25 year group. This suggests that in Hispanic populations, PFF may cluster with high amounts of VAT and liver fat, thus possibly contributing to their increased risk for type 2 diabetes and related metabolic disorders as they grow up.

We therefore addressed the relationships between these various fat depots. HFF increases with obesity, and more specifically VAT (19–21). VAT has a high rate of lipolysis, which allows direct delivery of fatty acids into the portal vein, leading to hepatic lipid accumulation (18,22). Similar relationships exist in regard to pancreatic fat: recent imaging studies showed that both pancreatic volume and fat content increased with BMI (6,7), waist:hip ratio (6), and VAT deposition (9,23). We further extend this

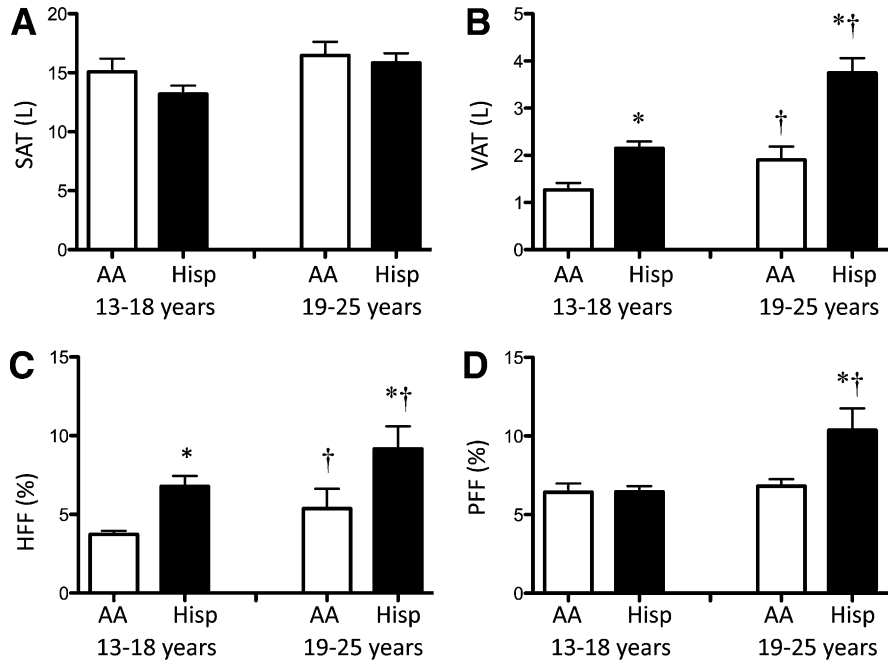

Figure 1—*SAT* (A)*, VAT* (B)*, HFF* (C)*, and PFF* (D) *by age and ethnic groups. All data show means ± SE and were analyzed by using 2-factor ANOVA; the Tukey-Kramer test was used during post hoc comparisons. AA, African American; Hisp, Hispanic. *Significant* ($P < 0.05$) *effect of ethnicity; †significant* ($P < 0.05$) *effect of age.*

finding and show that PFF positively correlates with both VAT and HFF, independently of BMI, total fat, and SAT content in an at-risk minority population. A previous study also reported simultaneous occurrence of fatty liver and fatty pancreas in overweight individuals (23), suggesting a common etiology to fat accumulation in these two organs. These results contrast with those from the Tushuizen study (8), who did not report any relationship between PFF and HFF. These contradictory results may possibly arise from methodology differences since Tushuizen et al. used magnetic resonance spectropscopy or from differences in population age and ethnicity. Because Hispanics are highly prone to visceral and liver fat accumulation, they may be genetically predisposed to altered fat partitioning in ectopic tissues as a whole.

We subsequently addressed whether PFF was related to abnormal endocrine function. PFF has been linked to low insulin sensitivity, measured by 2-h postchallenge glucose concentration (6,9) or homeostasis model assessment of insulin resistance (23), as well as insulin secretion (8). However, this last observation was found in healthy individuals but not in type 2 diabetic patients. In contrast, Heni et al. reported an inverse relationship between PFF and insulin secretion in individuals with impaired fasting glucose or impaired glucose tolerance but not normoglycemic participants (9). The authors postulated that PFF may have adverse metabolic effects when insulin resistance is present. Our results support this hypothesis: despite being overweight, all study participants were normoglycemic. Moreover, this study involved adolescents and young adults, who may be at more heterogeneous stages in the progression of β-cell failure, compared with older adults. Taken together, these results suggest that pancreatic fat is negatively correlated with insulin secretion in individuals with impaired glucose tolerance but not in normoglycemic individuals or type 2 diabetic patients. Thus pancreatic fat accumulation may play a pivotal role during the intermediary step of the disease and negatively affect insulin secretion only by persistent insulin resistance, when β-cells cannot anymore compensate for the increased insulin demand.

To further identify metabolic perturbations linked to PFF, we assessed the relationship between PFF and FFA. FFA are elevated in obese patients and are strong predictors of hepatic fat deposition (4). We show that in a multiple stepwise regression analysis, FFA and VAT were the strongest predictors of PFF. Several animal studies have assessed the effect of lipotoxicity on β-cell function. In Zucker diabetic fatty rats, plasma FFA were elevated 3 to 4 weeks before they became diabetic (22). The elevated FFA concentrations were followed within 2 weeks by islet triglyceride accumulation and impaired insulin secretion. Interestingly, these alterations were reversed by reducing plasma FFA, suggesting a causal role of high plasma FFA on altered β-cell function. The authors proposed that high circulating FFA may lead to β-cell lipid

Table 2—*Pearson correlation coefficients between PFF and other metabolic parameters*

	Pearson coefficient	*P* value
Body composition		
BMI (kg/m^2)	0.24	0.02
Total fat (%)	—	NS
SAT (L)	0.21	0.001
VAT (L)	0.45	<0.0001
HFF (%)	0.29	0.006
Glucose and insulin homeostasis		
AIR (μU/mL × 10 min)	—	NS
ISI ($\times 10^{-4}$min^{-1}/μU/mL)	—	NS
DI ($\times 10^{-4}$min^{-1})	—	NS
Lipid metabolism		
FFA (mmol/L)	0.32	0.0008
Inflammation		
MCP-1 (pg/mL)	0.23	0.003
IL-8 (pg/mL)	0.29	0.003
TNF-α (pg/mL)	0.18	0.06
HGF (pg/mL)	0.20	0.04
PAI-1 (pg/mL)	0.26	0.01
hs-CRP	—	NS

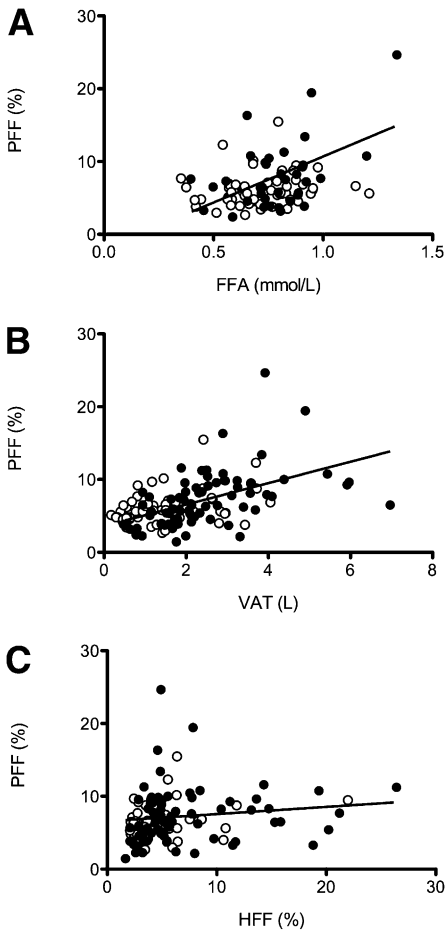

Figure 2—*Unadjusted relationships between PFF and FFA* (A)*, VAT* (B)*, and HFF* (C)*. In the overall group, PFF was positively correlated with FFA* (r = *0.32,* P = *0.001), VAT* (r = *0.45,* P < *0.0001), and HFF* (r = *0.29,* P < *0.0001).* ●*, Hispanics;* ○*, African Americans.*

accumulation, which may subsequently impair β-cell function, possibly by stimulation of ceramide production, similarly to what has been described in the skeletal muscle (2) and liver (3).

Finally, markers of chronic inflammation have been associated with type 2 diabetes, β-cell dysfunction (24), and hepatic fat accumulation. We found that PFF was associated with proinflammatory cytokines MCP-1, IL-8, TNF-α, HGF, and thrombogenic factor PAI-1. These markers are usually elevated in obese patients and are linked to insulin resistance and liver fat. However, when adjusted for VAT, these relationships were no longer significant. This suggests that in our population, inflammatory markers merely reflect VAT-related inflammation rather than cytokine production by the pancreas. We therefore cannot state that there is a direct relationship between PFF and inflammation either as a cause or consequence.

A limitation of our study includes the assessment of insulin secretion by IVGTT. Although IVGTT is the gold-standard method to evaluate glucose-mediated insulin secretion, it does not allow measurement of incretin-stimulated insulin secretion that physiologically occurs after meal consumption, which potentiates insulin secretion (25). It remains therefore possible that pancreatic fat may affect specifically meal-induced insulin secretion.

Collectively, these results raise concern about the effects of pancreatic fat accumulation in humans. Hispanics accumulate more pancreatic fat than African Americans. Pancreatic fat is closely related to other deleterious fat depots, such as VAT and liver fat, which are elevated in Hispanics, a population at high risk for metabolic disease. Pancreatic fat is also positively related to circulating FFA, which may trigger lipotoxicity and affect pancreatic function in the long run.

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K.-A.L. collected and analyzed data, contributed to discussion, wrote the article, and read and reviewed the article. E.E.V. collected data, contributed to discussion, and read and reviewed the article. J.Q.F., J.N.D., M.J.W., and M.P. read and reviewed the article. H.H.H., K.S.N., and M.I.G. contributed to discussion and read and reviewed the article.

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