Ectopic Fat Storage in the Pancreas, Liver, and Abdominal Fat Depots: Impact on β-Cell Function in Individuals with Impaired Glucose Metabolism

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Context: Pancreatic fat content (PFC) may have deleterious effects on β-cell function.

Objective: We hypothesized that ectopic fat deposition, in particular pancreatic fat accumulation, is related to β-cell dysfunction in individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT).

Design, Setting and Participants: This was a cross-sectional study in 64 age- and body mass index-matched individuals, with normal glucose tolerance (NGT; n = 16, 60% males), IFG (n = 29, 52% males), or IFG/IGT (n = 19, 63% males) was conducted.

Intervention and Main Outcome Measures: Participants underwent the following: 1) a combined hyperinsulinemic-euglycemic and hyperglycemic clamp, with subsequent arginine stimulation to quantify insulin sensitivity and β-cell function; 2) proton-magnetic resonance spectroscopy to assess PFC and liver fat content (LFC); and 3) magnetic resonance imaging to quantify visceral (VAT) and sc (SAT) adipose tissue. The disposition index (DI; insulin sensitivity adjusted β-cell function) was assessed.

Results: IFG and IFG/IGT were more insulin resistant ($P < 0.001$) compared with NGT. Individuals with IFG/IGT had the lowest values of glucose- and arginine-stimulated C-peptide secretion (both $P < 0.03$) and DI ($P < 0.001$), relative to IFG and NGT. PFC and LFC gradually increased between NGT, IFG, and IFG/IGT ($P = 0.02$ and $P = 0.01$, respectively), whereas VAT and SAT were similar between groups. No direct associations were found between PFC, LFC, VAT, and SAT and C-peptide secretion. The DI was inversely correlated with PFC, LFC, and VAT (all $P < 0.05$).

Conclusions: PFC was increased in individuals with IFG and/or IGT, without a direct relation with β-cell function. **(*J Clin Endocrinol Metab* 96: 459–467, 2011)**

Chronically elevated nonesterified fatty acids (NEFAs) have been implicated in the pathogenesis of β-cell dysfunction (1). *In vitro*, prolonged exposure to elevated levels of NEFAs result in decreased insulin gene expression, blunted glucose-stimulated insulin secretion (GSIS), and increased apoptosis (2, 3). These deleterious effects of NEFAs have been referred to as lipotoxicity (4, 5). *In vivo*, although acute infusion of intralipids was shown to enhance (6, 7), prolonged infusion of intralipids was shown to impair glucose-stimulated insulin secretion (7, 8). In

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Abbreviations: AIR_{arg}, C-peptide response to arginine; AUC, area under the curve; BMI, body mass index; DI, disposition index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; ^{1}H-MRS, magnetic resonance spectroscopy; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MRI, magnetic resonance imaging; M-value, glucose infusion rate; NEFA, nonesterified fatty acid; $NEFA_{fast}$, NEFAs in the fasting state; $NEFA_{ins}$, NEFAs during the hyperinsulinemic-euglycemic clamp; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SAT, sc adipose tissue; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue; VOI, volume of interest.

visceral obesity, excessive NEFA and lipid supply may exceed the oxidative capacity, which, in the presence of an impaired lipid storage capacity of adipose tissue, will lead to lipid accumulation in nonadipose tissues (9, 10). Furthermore, this ectopic lipid accumulation may result in functional impairments, including insulin resistance in skeletal muscle and liver, and decreased diastolic function of the heart (11–13). At present, it is unclear whether lipid accumulation occurs in pancreatic islets and whether this contributes to β-cell dysfunction. In leptin-deficient rodent models, lipid accumulation in pancreatic islets was associated with deterioration of β-cell mass and function (4, 14). However, data in humans are less conclusive.

First, it is under debate whether, in glucometabolic disorders, pancreatic lipid accumulation exists independent of factors such as aging and obesity. Previously, increased pancreatic lipid accumulation, quantified by proton-magnetic resonance spectroscopy (^{1}H-MRS), was reported in age- and body mass index (BMI)-matched individuals with impaired glucose metabolism (15) and type 2 diabetes mellitus (T2DM) (16) compared with healthy controls. In contrast, increased pancreatic lipid content has also been reported only to associate with aging and obesity, without a relation to glucose tolerance (17).

Second, it is unclear whether pancreatic lipid accumulation associates with β-cell dysfunction. It could be hypothesized that the accumulated pancreatic lipids may act as a source of NEFAs or other lipid-derived metabolites, interfering with β-cell function through lipotoxic pathways. In our previous study, pancreatic fat content was inversely associated with model-derived parameters of β-cell function, assessed during an oral glucose tolerance test (OGTT), in healthy but not T2DM men (16). Similarly, in subjects with impaired glucose metabolism, an inverse relationship of pancreatic fat content and insulin secretion during an OGTT was described (18). However, although OGTT-derived β-cell function provides valuable information on various aspects of β-cell dynamics, the gold standard measurement of β-cell function is the hyperglycemic clamp method (19), which was used in the present study. For measurements of pancreatic fat content, we used ^{1}H-MRS. Although this method cannot discriminate between intraislet lipid accumulation and lipid accumulation outside the islets, ^{1}H-MRS-measured pancreatic fat content was validated against the gold standard biochemical staining (15). Furthermore, ^{1}H-MRS-measured fat content closely paralleled that of intraislet fat content (20). Consequently, ^{1}H-MRS is considered to be the most reliable and validated method to assess pancreatic fat content *in vivo*.

In this study, we assessed pancreatic fat content and the association with clamp-measured β-cell function in individuals with impaired glucose metabolism, *i.e.* those with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and healthy controls. Additionally, insulin sensitivity, liver fat content, and visceral (VAT) and sc adipose tissue (SAT) were assessed.

Subjects and Methods

Study population

Overweight Caucasian individuals with a family history of T2DM were recruited by advertisements in local newspapers. After obtaining written informed consent, 149 individuals underwent a 2-h, 75-g OGTT during a screening visit. Individuals with IFG [fasting plasma glucose (FPG) ≥6.1 and <7.0 mmol/liter] and/or individuals with IFG (FPG ≥5.6 and <7.0 mmol/liter) and a family history of T2DM (*i.e.* first and second degree relatives) and/or IGT (2 h plasma glucose level ≥7.8–11.1 mmol/liter) were eligible. Individuals were allowed to use only blood pressure-lowering or lipid-lowering medication (statins). Twenty-nine individuals with IFG (52% males), three individuals with isolated IGT, and 16 individuals with combined IFG/IGT (63% males) were enrolled; 16 NGT individuals (60% males), matched for age, gender, and BMI served as controls. Exclusion criteria included claustrophobia; excess alcohol intake (>20 U/wk); history of diabetes, hepatitis, and/or pancreatitis; abnormal liver and renal function tests (>2 times upper limits of normal); and recent (<3 months) changes in weight (≥5%). The local ethics committee approved the study, and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study design

Within 1 month after inclusion, participants arrived at the research institute after an overnight fast (from 22 h the previous evening). All participants underwent a ^{1}H-MRS and a combined hyperinsulinemic-euglycemic and hyperglycemic clamp procedure, with additional arginine stimulation.

Pancreas and liver ^{1}H-MRS

Magnetic resonance imaging (MRI), for determination of abdominal fat compartments, and ^{1}H-MRS, to quantify fat content in the liver and the pancreas, were performed in the supine position using a 1.5-T whole-body system equipped with spine and body phased-array coils (Sonata; Siemens, Erlangen, Germany). All scans were performed by a single investigator (N.J.Z.). To diminish respiratory artifacts, spectroscopic volumes of interest (VOIs) were positioned on respiratory-triggered coronal and axial T2-weighted half-Fourier acquisition single-shot turbo spin-echo images. A respiratory-triggered point-resolved spectroscopy sequence was used for ^{1}H-MRS acquisition (repetition time ≥3 sec, determined by respiration interval, echo time 30 msec, eight measurements with one acquisition each were stored separately). Pancreatic fat content was measured in the distal part of the pancreas using an oblique 2.5-cm^3 VOI (2.5 × 1.0 × 1.0 cm), avoiding the spleen vessels and the lateral margin of the pancreas. Three 8-cm^3 VOIs (2.0 × 2.0 × 2.0 cm) were positioned in the liver (right anterior, right posterior, and medial or left anterior) avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. All eight separate spectra per VOI were quantified with LCModel (version 6.1) and subsequently

combined for the analyses of the mean pancreas and liver spectra (16, 21). Fat content was defined as the signal intensities at 0.9 and 1.3 ppm (methyl and saturated methylene protons of triglycerides) relative to the signal intensity of water at 4.65 ppm. Occasionally, one or two of eight measurements were discarded due to obvious visceral fat contamination. To determine reproducibility, ^{1}H-MRS measurements of pancreatic and liver fat content were performed twice in the same individuals (n = 10) during two independent sessions separated by more than 10 min. The mean intrasubject coefficient of variation between two repeated pancreatic fat measurements was 14% with a Pearson correlation coefficient (r) of 0.95. The coefficient of variation between two assessments of liver fat was 4%, with r = 0.99.

VAT and SAT tissue compartments

VAT and SAT were measured by MRI as described previously (22). Briefly, sagittal and coronal slices were used to localize anatomic sites for image acquisition (L4-L5). Three T1-weighted transverse spin-echo images (repetition time 40 msec, echo time 13 msec, 10 mm slice thickness, 20 mm spacing, one image per breath hold) were obtained. Quantification of VAT and SAT were performed using an image analysis program, running on a Sparc10 workstation (Sun Microsystems, Palo Alto, CA). A seed point was placed in a fat depot, and using a seed-growing procedure, this fat depot can be circumscribed by the selection of a pixel intensity range. The areas were expressed in square centimeters, and the average area of the three transverse images was used for statistical analyses. Processing of MRI data and calculation of SAT and VAT was performed by a single experienced investigator (M.H.A.M.).

Combined clamp procedures

A modified combined hyperinsulinemic-euglycemic and hyperglycemic clamp, with subsequent arginine stimulation, was performed as previously described (23) to assess insulin sensitivity and secretion. In brief, during the hyperinsulinemic-euglycemic clamp, the insulin infusion rate was maintained at 40 mU/min $\cdot$ m^2 body surface area for 120 min. Insulin sensitivity was defined as the glucose infusion rate (M-value; mg/kg^{-1} $\cdot$ min^{-1}) during the last 30 min of the hyperinsulinemic-euglycemic clamp, with blood glucose levels at a steady state of 5.0 mmol/liter. An hour after the hyperinsulinemic-euglycemic clamp, the hyperglycemic clamp was started with an iv glucose bolus, increasing the glucose levels to 15.0 mmol/liter. Blood glucose levels were sustained at a steady state of 15 mmol/liter, with variable 20% glucose infusion. During the hyperglycemic clamp, blood was collected to measure insulin and C-peptide levels, and first- and second-phase glucose-stimulated C-peptide secretion was assessed. First-phase C-peptide secretion was calculated as the C-peptide area under the curve (AUC; nanomoles per minute per liter) during the first 10 min after the iv glucose bolus. Second-phase C-peptide secretion was calculated as the C-peptide AUC during the 70 min after first-phase C-peptide secretion. Subsequently a 5-g arginine bolus was administered to measure maximum C-peptide secretory capacity at a steady-state blood glucose concentration of 15 mmol/liter (24). Combined hyperglycemia- and arginine-stimulated β-cell secretory capacity was calculated as the C-peptide AUC during the first 10 min after the arginine bolus (AIR_{arg}). Insulin sensitivity-adjusted β-cell function, the disposition index [DI; (picomoles per minute per liter)/(milligrams per kilogram per minute)], was calculated by multiplying first-phase insulin secretion and M-value (25).

Biochemical analyses

Fasting plasma glucose (FPG) concentrations were determined using a hexokinase method (Gluco-quant; Roche Diagnostics, Mannheim, Germany). Glycated hemoglobin (HbA_{1c}) was measured by cation exchange chromatography (Menarini Diagnostics, Florence, Italy; reference values: 4.3–6.1%). Total cholesterol, high-density lipoprotein (HDL)-cholesterol and NEFAs in the fasting state ($NEFA_{fast}$) and during the hyperinsulinemic-euglycemic clamp ($NEFA_{ins}$) were determined using an enzymatic colorimetric method.

Statistical analyses

Data are expressed as mean ± SD or, in case of skewed distribution, as median (interquartile range) for numerical variables and as proportions for categorical variables. Group differences were tested by ANOVAs with Bonferroni *post hoc* analyses. Differences in nonnormally distributed variables of subject characteristics were tested with the Kruskal-Wallis test. In the analyses isolated IGT and combined IFG/IGT were taken together, excluding the isolated IGT individuals from the analyses did not alter the results. Spearman's correlations were used to evaluate the relation between pancreatic fat content, liver fat content, VAT, SAT, and $NEFA_{ins}$ with β-cell function and relevant glucometabolic determinants (*i.e.* age, BMI, HDL-cholesterol, triglycerides, FPG, and insulin sensitivity). Multivariable regression analyses were used to assess independent determinants of pancreatic fat and to evaluate effect modification by group [*i.e.* normal glucose tolerance (NGT), IFG, and IFG/IGT, tested by adding the interaction term pancreatic fat content × group]. A $P < 0.1$ was considered to indicate effect modification. Statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Chicago, IL). A $P < 0.05$ was considered statistically significant.

Results

Subject characteristics

Baseline characteristics of the study population are summarized in Table 1. There were no differences between groups with respect to age, BMI, and waist and lipid profile. Individuals with impaired glucose metabolism had higher blood pressure, HbA_{1c}, FPG, and fasting plasma insulin compared with NGT. $NEFA_{fast}$ were increased in IFG; $NEFA_{ins}$ were highest in IFG/IGT compared with controls.

Pancreatic fat, liver fat, VAT, and SAT

Pancreatic fat content gradually increased with deterioration of glucose metabolism: NGT, 7.6% (2.9–13.4) *vs.* IFG, 12.1% (5.1–17.5) *vs.* IFG/IGT, 22.4% (7.3–36.2). Similarly, liver fat content increased between NGT, IFG, and IFG/IGT. However, VAT and SAT did not differ between the three groups (all Fig. 1).

TABLE 1. Clinical and biochemical characteristics

	NGT (n = 16)	IFG (n = 29)	IFG/IGT (n = 19)	P_{trend}
Sex (percent males)	60	52	63	0.668
Age (yr)	54.8 ± 7.3	57.0 ± 8.0	56.2 ± 6.4	0.655
BMI (kg/m^2)	27.5 ± 3.0	28.7 ± 3.7	28.2 ± 3.6	0.481
Waist circumference (cm)	98.3 ± 10.2	101.3 ± 10.3	100.6 ± 11.4	0.575
Systolic blood pressure (mm Hg)	123 ± 7	132 ± 13[a]	131 ± 9[a]	0.033
Diastolic blood pressure (mm Hg)	79 ± 6	83 ± 6	85 ± 7[a]	0.024
FPG (mmol/liter)	5.0 ± 0.3	6.3 ± 0.4[a]	6.2 ± 0.6[a]	<0.001
Fasting plasma insulin (pmol/liter)	44 (30–49)	64 (49–89)[a]	74 (48–97)[b]	0.003
Plasma glucose, 2 h (mmol/liter)	5.0 ± 1.5	6.3 ± 0.9[b]	9.5 ± 1.1[b,c]	<0.001
HbA_{1c} (%)	5.4 ± 0.3	5.8 ± 0.3[a]	5.8 ± 0.3[a]	<0.001
Alkaline phosphatase (U/liter)	64 (62–74)	78 (73–88)[a]	72 (54–82)	0.012
γ-GT (U/liter)	24 (20–30)	32 (22–44)	31 (20–42)	0.145
ALT (U/liter)	29 (17–42)	28 (22–37)	28 (20–43)	0.804
Total cholesterol (mmol/liter)	5.3 ± 0.8	5.3 ± 1.0	5.3 ± 1.0	0.985
HDL-cholesterol (mmol/liter)	1.6 ± 0.4	1.3 ± 0.4	1.4 ± 0.5	0.247
LDL-cholesterol (mmol/liter)	3.2 ± 0.9	3.3 ± 0.8	3.1 ± 0.8	0.820
Plasma triglycerides (mmol/liter)	1.2 ± 0.5	1.5 ± 0.7	1.7 ± 0.9	0.169
$NEFA_{fast}$ (mmol/liter)	0.45 (0.31–0.53)	0.52 (0.45–0.7)[a]	0.53 (0.35–0.65)	0.100
$NEFA_{ins}$ (mmol/liter)	0.03 (0.02–0.04)	0.05 (0.03–0.07)	0.06 (0.04–0.08)[a]	0.095
M-value (mg/kg^{-1} · min^{-1})	9.4 (6.9–11.3)	5.0 (4.0–5.6)[b]	5.3 (3.4–5.8)[s,b]	<0.001
Blood pressure-lowering therapy (%)	0	22	29	0.084
Lipid-lowering medication (%)	0	7	0	0.298

Values are mean ± SD or median (interquartile range). ALT, Alanine transaminase; GT, γ-glutamyl transferase; LDL, low-density lipoprotein.

[a] $P < 0.05$, compared with NGT.

[b] $P < 0.001$, compared with NGT.

[c] $P < 0.001$, IFG/IGT compared with IFG.

Pancreatic fat, liver fat, and VAT were positively associated with age, BMI, FPG, and triglycerides and negatively related to HDL-cholesterol (Table 2). In addition $NEFA_{ins}$ associated with increased liver fat content, triglycerides, VAT, and FPG and decreased HDL-cholesterol (Table 2).

Using multivariate analysis, independent determinants of pancreatic fat content were assessed. Because group (*i.e.* NGT, IFG, and/or IFG/IGT) was not an effect modifier ($P > 0.1$), the analysis was performed in the total study population. However, to correct for possible confounding, group was added to the model. Age, BMI, 2-h plasma glucose levels, and HDL-cholesterol were significant determinants of pancreatic fat content (adjusted R^2 of the model: 0.33, $P_{model} = 0.001$, unstandardized beta (b) group: b = 5.8, $P = 0.073$; age: b = 0.38, $P = 0.062$; BMI: b = 1.16, $P = 0.025$; 2-h plasma glucose level: b = 0.29, $P = 0.05$; HDL-cholesterol: b = −11.6, $P = 0.01$).

Clamp-measured aspects of β-cell function, insulin sensitivity, and DI

Differences in C-peptide secretion between the three groups are shown in Fig. 2; IFG individuals had decreased first-phase C-peptide secretion compared with NGT. In IFG/IGT, both glucose- and arginine-stimulated C-peptide secretion were diminished, compared with IFG and NGT. As expected, IFG and IFG/IGT were more insulin resistant compared with controls (M-value, IFG/IGT: 5.3 mg/kg^{-1} · min^{-1} (3.4–5.8) *vs.* IFG: 5.0 mg/kg^{-1} · min^{-1} (4.0–5.6) *vs.* NGT: 9.4 mg/kg^{-1} · min^{-1} (6.9–11.3), $P_{trend} < 0.001$). The DI decreased with deterioration of glucose metabolism: NGT, 1958 (picomoles per minute per liter)/(milligram per kilogram^{-1} per minute^{-1}) (1779–2887) *vs.* IFG, 773 (picomoles per minute per liter)/(milligrams per kilogram^{-1} per minute^{-1}) (404–1129) *vs.* IFG/IGT, 483 (picomoles per minute per liter)/(milligrams per kilogram^{-1} per minute^{-1}) (331–730) ($P < 0.001$).

Associations of ectopic fat with variables of β-cell function, insulin sensitivity, and DI

No significant correlations were found between pancreatic fat content and glucose- or arginine-stimulated C-peptide secretion (Table 2). Pancreatic fat content, liver fat content, VAT, SAT, and triglycerides were inversely associated with insulin sensitivity (Table 2). There was an inverse relationship between pancreatic fat content and DI. However, in multivariable regression analyses, this association did not sustain after adjustment for age and BMI (data not shown). Similarly, inverse associations were observed between liver fat content, VAT, $NEFA_{ins}$, and the DI.

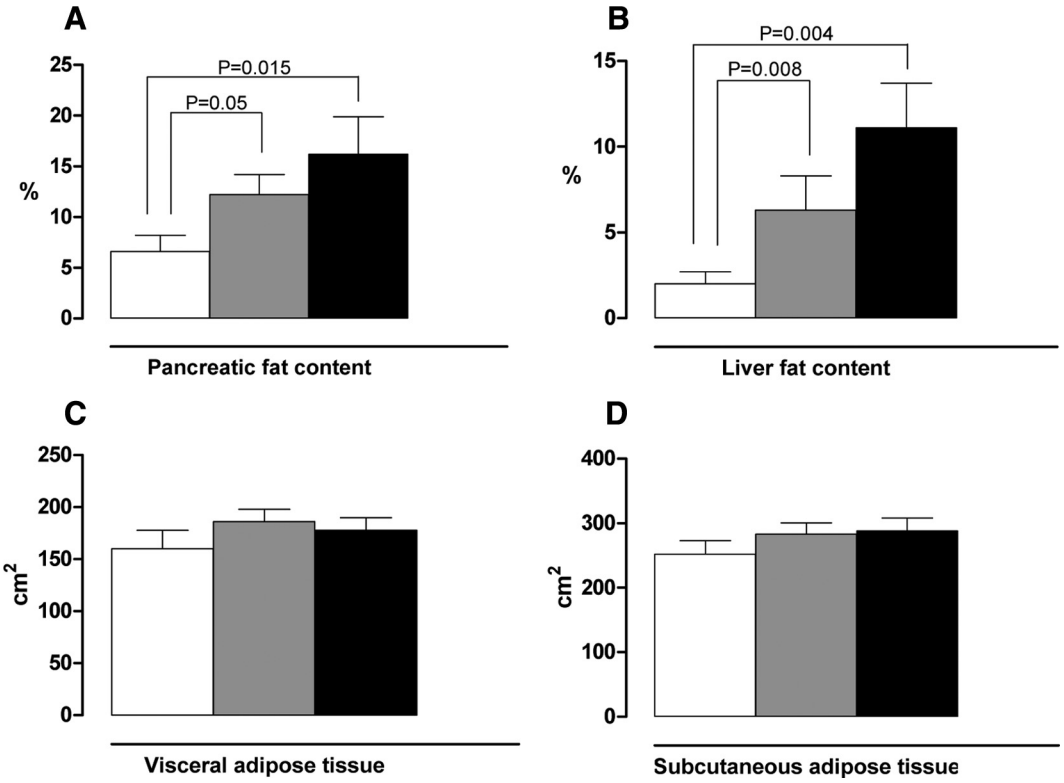

FIG. 1. Pancreatic fat content (A), liver fat content (B), VAT (C), and SAT (D) in normoglycemic healthy controls and IFG, and IFG/IGT subjects. Values are mean ± SD. NGT, *White bar*; IFG, *gray bar*; IFG/IGT, *black bar*.

Discussion

The present study demonstrates that the impairments in β-cell function in individuals with IFG and IFG/IGT are accompanied by fat accumulation in the pancreas. However, a relation between pancreatic fat accumulation and β-cell function could not be established. The ectopic fat depots showed strong inverse relations with insulin sensitivity.

Our participants were well matched for age and BMI and classified based on impairments in fasting and post-load glucose levels. VAT did not alter between the three groups, where there was an increase in liver fat content. The discrepancy between VAT and liver fat content has been described previously, outlining liver fat content as a more accurate marker in the detection of metabolic derangements than VAT in obese subjects (26, 27).

TABLE 2. Univariate correlations between abdominal fat measurements, β-cell function, and insulin sensitivity

	PFC (%)	LFC (%)	VAT (cm^2)	SAT (cm^2)	TG (mmol/liter)	$NEFA_{ins}$ (mmol/liter)
Age (yr)	0.413[a]	0.333[b]	0.175	0.124	−0.071	−0.194
BMI (kg/m^2)	0.471[a]	0.423[a]	0.468[a]	0.716[a]	0.300[b]	0.155
HDL-cholesterol (mmol/liter)	−0.356[b]	−0.494[a]	−0.473[a]	0.043	−0.423[a]	−0.426[a]
Triglycerides (mmol/liter)	0.231[b]	0.226[b]	0.271[b]	0.128		0.343[b]
$NEFA_{fast}$ (mmol/liter)	0.018	0.070	−0.086	0.074	−0.104	0.077
$NEFA_{ins}$ (mmol/liter)	0.084	0.335[b]	0.186	−0.029	0.343[a]	
FPG (mmol/liter)	0.264[b]	0.296[b]	0.116	0.035	0.229	0.329[b]
M-value ($mg/kg^{-1} \cdot min^{-1}$)	−0.301[b]	−0.562[a]	−0.291[b]	−0.266[b]	−0.368[a]	−0.577[a]
First-phase (nmol/min · liter)	0.075	0.022	0.012	0.087	0.137	0.101
Second-phase (nmol/min · liter)	0.197	0.268	0.095	0.068	0.232	0.257
AIR_{arg} (nmol/min · liter)	0.248	0.249	0.089	0.014	0.199	0.184
DI (pmol/min · liter)/($mg/kg^{-1} \cdot min^{-1}$)	−0.249[b]	−0.274[b]	−0.310[a]	−0.140	−0.097	−0.269[b]

r, Spearman's correlation coefficient; LFC, liver fat content; PFC, pancreatic fat content; first phase, AUC of glucose-stimulated first-phase C-peptide secretion; second phase, AUC of glucose-stimulated second-phase C-peptide secretion; TG, triglycerides; AIR_{arg}, AUC of C-peptide response to arginine at 15 mmol/liter glucose level.

[a] $P < 0.01$.

[b] $P < 0.05$.

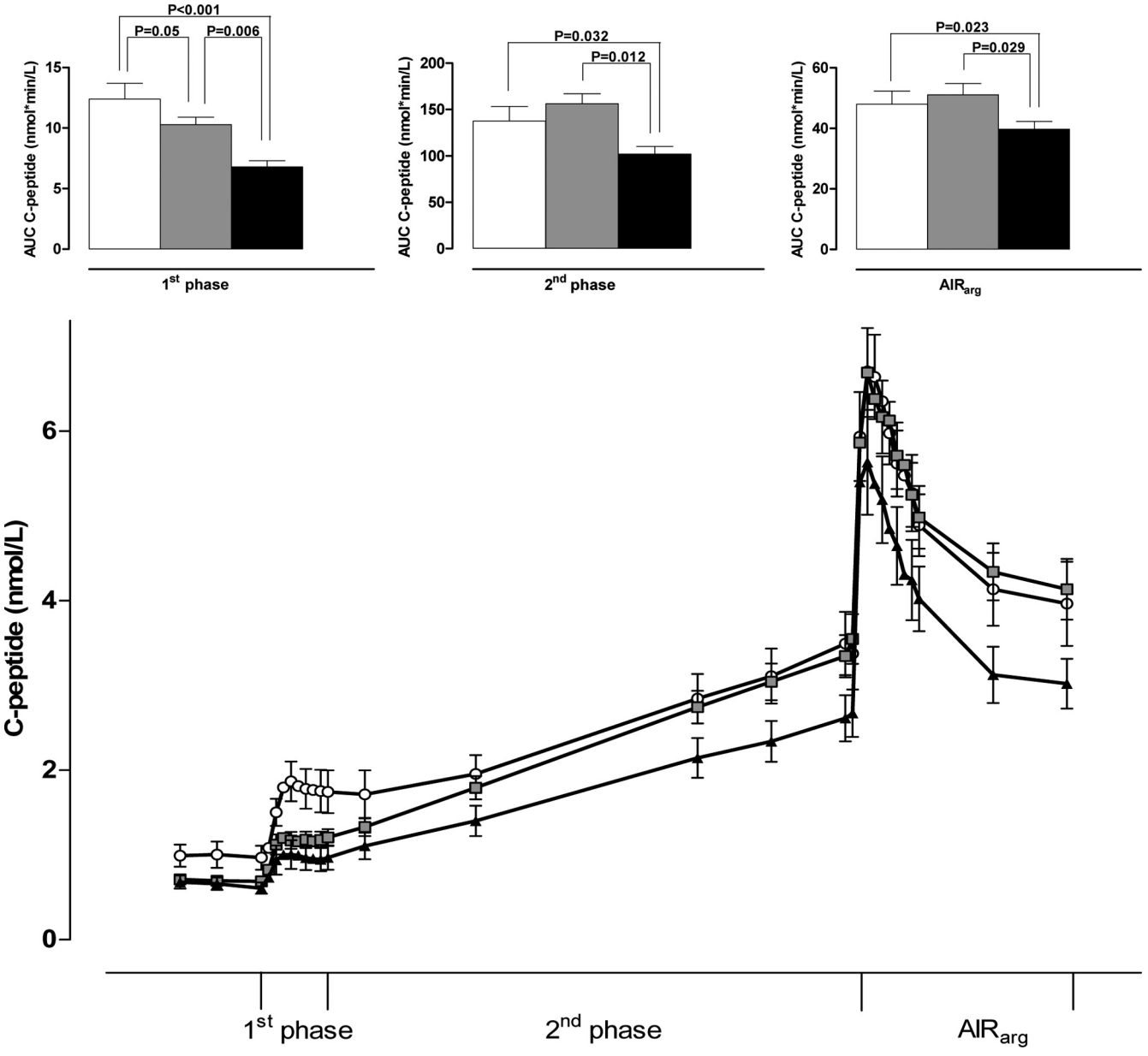

FIG. 2. C-peptide secretion during the hyperglycemic clamp in NGT, IFG, and IFG/IGT subjects. C-peptide concentrations during the hyperglycemic clamp with subsequent arginine stimulation in NGT (*white bar/dot*), IFG (*gray bar/squares*), and IFG/IGT (*black bar/triangles*). Data are mean ± SD. 1st phase, First-phase C-peptide response to glucose; 2nd phase, second-phase C-peptide response to glucose; AIR_{arg}, C-peptide response to arginine at 15 mmol/liter glucose concentration. See *Subjects and Methods* for calculations of β-cell function parameters.

In obesity and insulin-resistant states, in the additional presence of adipose tissue dysfunction with impaired lipid storage capacity, lipids will accumulate in nonadipose tissues (10). This ectopic lipid accumulation enhances with aging, obesity, and glucometabolic disorders (28, 29). However, whether the latter also applies to the pancreas is currently under debate. Previously we and others showed a gradual increase in ^{1}H-MRS-measured pancreatic fat content with deterioration of glucose metabolism (ranging from NGT, IFG, and/or IGT and T2DM (15, 16, 18). In contrast, Saisho *et al.* (17) found no relation between computed tomography measured pancreatic fat content and glucometabolic disorders. In the current study, we were able to confirm our previous findings, showing an increase in pancreatic fat content in individuals with IFG and/or IGT compared with age- and BMI-matched normoglycemic controls. Similar to Lingvay *et al.* (15), postload glucose levels were independently associated with pancreatic fat content, delineating an independent relation with glucometabolic changes. These data outline the reciprocal association of glucose and fatty acid metabolism, in which increasing glucose availability may promote the shuttling of fatty acids into esterification to triglycerides, rather than to oxidation, leading to intracellular triglyceride accumulation (30). Therefore, in

glucometabolic disorders both hyperglycemia and hyperlipidemia may contribute to ectopic lipid accumulation and organ dysfunction. Differences between our results and the findings of Saisho *et al.* (17) are possibly due to the use of different techniques to assess the pancreatic fat content. Saisho *et al.* (17) used computed tomography, measuring lipid content primarily outside the parenchyma, whereas we used ^{1}H-MRS, measuring lipid content within the parenchyma (31).

Excessive pancreatic lipid content may enter a continuous vicious cycle of hydrolysis and fatty acid reesterification, thereby generating toxic intermediates (*i.e.* ceramide, diacylglycerol), which may induce β-cell dysfunction and apoptosis (32, 33). These are among the lipotoxic mechanisms that have been shown to exist in pancreatic islets *in vitro* and in rodents *in vivo* (3–5, 33). Conclusive data in humans *in vivo*, regarding the occurrence of lipotoxicity as a causal factor of β-cell dysfunction are lacking. Studies with prolonged intralipid infusions show conflicting results because an increase (34), no effect (35), or a decrease (8) in glucose-stimulated insulin secretion are all described. However, these studies measured the effect of infused lipids rather than lipids stored in the pancreas. We and others (15, 16, 18) related pancreatic lipid accumulation to OGTT-measured β-cell function. Although inverse relations were seen with β-cell function, data were not conclusive. OGTT-derived β-cell function provides valuable information; however, the gold standard measurement for β-cell function is the hyperglycemic clamp method. Therefore, in this study we related clamp-measured β-cell function to pancreatic fat content.

Based on our previous study, in which we found an inverse relation between pancreatic fat content and OGTT-derived β-cell function in nondiabetic men (16), we expected to find an inverse relation with β-cell function in subjects with IFG and/or IGT. However, we could not establish a relation between pancreatic fat content and direct measurements of β-cell function. Several explanations for the lack of association with unadjusted C-peptide secretion variables may be postulated. Although ^{1}H-MRS measures parenchymal triglyceride accumulation, ^{1}H-MRS cannot discriminate between intra- and interislet fat accumulation (31). Because β-cells constitute only 2% of the total pancreas mass (36), ^{1}H-MRS measured lipid deposition in the pancreas is most likely located outside β-cells, *e.g.* in exocrine or stromal cells or in adipocytes infiltrating the pancreas. This infiltrating adipose tissue is presumably closely associated with visceral fat, which is known to be metabolically active and release NEFAs and adipocytokines (37), all of which may subsequently adversely affect islet structure, survival, and β-cell function. Unfortunately, in this study these contributing factors were not assessed. Nevertheless, determination of circulating factors will not allow to establish their origin and therefore will not enable us to discriminate between the contribution of the different fat compartments to β-cell dysfunction.

Finally, it has been suggested that triglyceride depositions reflect a protective buffer, diverting excess NEFAs from metabolic pathways leading to lipotoxicity affecting β-cell function and survival. Instead, NEFAs are channeled into lipid stores in nonadipose tissue, including the pancreas. Therefore, although pancreatic lipid content reflects derangements in lipid metabolism, it is unknown whether these depositions in fact are inert or harmful.

In the current study, we focused on individuals with impaired glucose metabolism, a population that is characterized by defects in both β-cell function and insulin sensitivity (38). Subjects with impaired glucose metabolism showed altered C-peptide secretion during the combined clamp procedure as well as a higher degree of insulin resistance, compared with normoglycemic controls. It is widely accepted that insulin resistant states, such as obesity, are accompanied by increased insulin secretion. Therefore, correcting C-peptide secretion for accompanying insulin resistance (DI), might give additional information (39).

In our study, subjects with IFG and IFG/IGT showed a gradual decrease in the DI, compared with NGT. The DI was inversely associated with pancreatic and liver fat content, VAT, and SAT. This suggests an inverse relation between derangements in glucose and lipid metabolism and β-cell function. However, the DI is based on both insulin secretion and insulin sensitivity. Because all fat compartments were inversely related to insulin sensitivity, the relation between DI and fat compartments might be driven by decreased insulin sensitivity.

Taken together, in individuals with impaired glucose metabolism, pancreatic fat content is increased compared with healthy controls, independent of aging and obesity. In this study, we could not establish a direct relation between pancreatic fat content and diminished β-cell function in subjects with impaired glucose metabolism. Longitudinal studies are needed to gain more insight in the sequence of events and the contributing role of ectopic fat that may occur during the progressive decline in β-cell function.

Acknowledgments

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