Pancreatic Fat Content and β-Cell Function in Men With and Without Type 2 Diabetes

MAARTEN E. TUSHUIZEN, MD[1]
MATHIJS C. BUNCK, MD[1]
PETRA J. POUWELS, PHD[2]
SASKIA BONTEMPS, MSC[1]
JAN HEIN T. VAN WAESBERGHE, MD, PHD[3]
ROGER K. SCHINDHELM, MD, PHD[1]
ANDREA MARI, PHD[4]
ROBERT J. HEINE, MD, PHD, FRCP[1]
MICHAELA DIAMANT, MD, PHD[1]

OBJECTIVE — Insulin resistance, associated with increased lipolysis, results in a high exposure of nonadipose tissue to lipids. Experimental data indicate that fatty infiltration of pancreatic islets may also contribute to β-cell dysfunction, but whether this occurs in humans in vivo is unknown.

RESEARCH DESIGN AND METHODS — Using proton magnetic resonance spectroscopy and oral glucose tolerance tests, we studied the association of pancreatic lipid accumulation in vivo and various aspects of β-cell function in 12 insulin-naive type 2 diabetic and 24 age- and BMI-matched nondiabetic men.

RESULTS — Patients versus control subjects had higher A1C, fasting plasma glucose, and insulin and triglyceride levels and lower HDL cholesterol, but similar waist circumference. Median (interquartile range) pancreatic fat content in patients and control subjects was 20.4% (13.4–43.6) and 9.7% (7.0–20.2), respectively ($P = 0.032$). Pancreatic fat correlated negatively with β-cell function parameters, including the insulinogenic index adjusted for insulin resistance, early glucose-stimulated insulin secretion, β-cell glucose sensitivity, and rate sensitivity (all $P < 0.05$), but not potentiation. However, these associations were significantly affected by the diabetic state, such that a significant association of pancreatic fat with β-cell dysfunction was only present in the nondiabetic group (all $P < 0.01$), suggesting that once diabetes occurs, factors additional to pancreatic fat account for further β-cell function decline. In control subjects, the association of pancreatic fat and β-cell function remained significant after correction for BMI, fasting plasma glucose, and triglycerides ($P = 0.006$).

CONCLUSIONS — These findings indicate that pancreatic lipid content may contribute to β-cell dysfunction and possibly to the subsequent development of type 2 diabetes in susceptible humans.

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Progressive β-cell dysfunction, in the context of insulin resistance, is a hallmark of type 2 diabetes (1). Glucose toxicity, ensuing from diabetes-related hyperglycemia, has been regarded as a contributor to β-cell damage (2). In contrast, chronic exposure of the pancreatic islets to nonesterified fatty acids (NEFAs) is considered as a potential primary cause of β-cell dysfunction (3). In obese individuals, increased lipolysis contributes to high levels of circulating NEFAs, whereas liver insulin resistance leads to elevated hepatic output of triglyceride-rich particles (4). When NEFA supply exceeds utilization, nonadipose tissues, including the pancreatic islets, start accumulating triglycerides (3), which is aggravated by the simultaneous presence of hyperglycemia (2,5,6). Subsequently, various mechanisms including the formation of reactive long-chain fatty acyl-CoAs and toxic metabolites, such as ceramide, the activation of protein kinase C-δ, and increased oxidative stress, may all contribute to apoptosis and the decline of β-cell mass (2,3,5–7). Finally, experimental and autopsy data indicate that fatty infiltration of the pancreas may contribute to a decrease in β-cell mass and function, possibly by local release of NEFAs and by adipocyte-derived proinflammatory and vasoactive factors (2,6–8). Collectively, these mechanisms may cause β-cell dysfunction in susceptible individuals.

To date, there are no in vivo data available confirming the proposed relationship of pancreatic lipid accumulation and β-cell dysfunction in humans, due to the inaccessibility of the pancreatic islets in vivo. Proton magnetic resonance spectroscopy (^{1}H-MRS) has been validated against direct determination of triglyceride content in human liver biopsies and has become the method of choice to determine hepatic fat content (9,10). Using ^{1}H-MRS and a modified oral glucose tolerance test (OGTT), we quantified pancreatic fat content and various aspects of β-cell function, respectively, and assessed their associations in men with and without (insulin-naive) type 2 diabetes.

RESEARCH DESIGN AND METHODS

— A total of 36 Caucasian men, aged 35–65 years, with ($n = 12$) and without ($n = 24$) type 2 diabetes, based on American Diabetes Association criteria (11), were recruited by advertisement and studied after obtaining written informed consent. Diet, sulfonylurea, and/or metformin were the only glucose-lowering treatments allowed. Exclusion criteria included claustrophobia, excess alcohol intake (>20 units/week), history of hepatitis and/or pancreatitis, abnormal

From the [1]Department of Endocrinology/Diabetes Center, VU University Medical Center, Amsterdam, the Netherlands; the [2]Department of Physics and Medical Technology, VU University Medical Center, Amsterdam, the Netherlands; the [3]Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands; and the [4]Institute of Biomedical Engineering, National Research Council, Padova, Italy.

Address correspondence and reprint requests to M. Diamant, MD, PhD, Endocrinology/Diabetes Center, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands. E-mail: m.diamant@vumc.nl.

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Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; HOMA-IR, homeostasis model assessment for insulin resistance; NEFA, nonesterified fatty acid; OGGT, oral glucose tolerance test; VOI, volume of interest.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

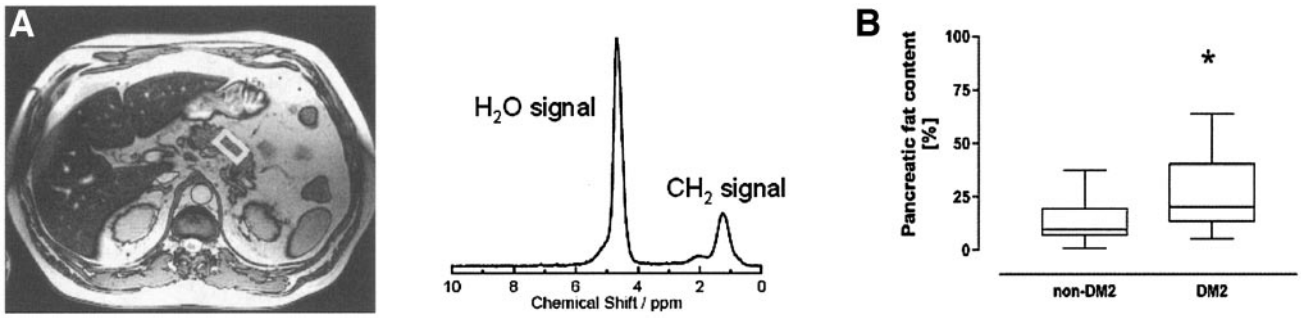

Figure 1—*^{1}H–magnetic resonance spectra (CH_2 peak at 1.3 ppm is main signal of lipids) were obtained from abdominal magnetic resonance imaging scans indicating VOI in the pancreas* (A)*. Median (interquartile range) lipid content of the pancreas* (B) *in type 2 diabetic versus nondiabetic men is shown (*P < 0.05). DM2, type 2 diabetes.*

liver and renal function tests (more than two times the upper limits of normal), recent (<3 months) changes in weight (≥5%) and/or medication, and history or current use of glucocorticosteroids, insulin, and/or thiazolidinediones. The local ethics committee approved the study, and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study design

After an overnight fast (from 8:00 P.M. the previous evening), all participants arrived at the research center at 7:30 A.M. and underwent ^{1}H-MRS and an OGTT during one single visit. They were instructed to omit their medication on the morning of the examination and to refrain from heavy physical activities during the previous 24 h (additional information on ^{1}H-MRS can be found in an online-only appendix at http://dx.doi.org/10.2337/dc07-0326).

Pancreas and liver ^{1}H-MRS

Pancreatic fat content was measured by a single investigator (M.E.T.) in the distal part of the pancreas using an oblique 2 cm^3 (1.0 × 1.0 × 2.0 cm^3) volume of interest (VOI), avoiding the spleen vessels and the lateral margin of the pancreas (Fig. 1*A*). Location of VOIs was determined in axial and coronal images. Occasionally, individual spectra showed a dramatic increase of lipid signals, probably because of the sudden deep breathing of the subject. These spectra were removed, and only reproducible spectra were subsequently quantified and used in the analyses of the (mean) pancreas spectrum (9,10,12,13). At three positions in the liver (right anterior, right posterior, and medial or left anterior), a 15-cm^3 VOI (2.5 × 2.5 × 2.5 cm^3) was positioned, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. ^{1}H-MRS measurements of pancreatic and liver fat content were performed twice in the same individuals (n = 8) on two independent occasions separated by >10 min (10). The coefficient of variation (CV) between two repeated pancreatic fat measurements was 15.2%. The CV between two assessments of liver fat was 4.7%.

Table 1—*Baseline characteristics of the study population*

	Nondiabetic	Type 2 diabetic	*P*
n	24	12	—
Age (years)	55.4 ± 2	54.6 ± 2	0.78
Diabetes duration (years)	—	5.6 ± 1	—
BMI (kg/m^2)	29.1 ± 1	31.3 ± 1	0.071
Waist (cm)	106.5 ± 2	111.8 ± 2	0.10
Systolic blood pressure (mmHg)	130 ± 3	136 ± 4	0.29
Diastolic blood pressure (mmHg)	79 ± 2	82 ± 2	0.38
A1C (%)	5.6 ± 0.1	7.2 ± 0.3	<0.001
Fasting plasma glucose (mmol/l)	5.4 ± 0.1	8.2 ± 0.5	<0.001
Fasting plasma insulin (pmol/l)	56 ± 5	100 ± 17	<0.01
HOMA-IR	1.0 ± 0.1	2.0 ± 0.3	<0.01
Oral glucose insulin sensitivity (ml/min per m^2)	427 ± 12	328 ± 11	<0.001
NEFAs (μmol/l)	609 ± 39	676 ± 79	0.40
Total cholesterol (mmol/l)	5.0 ± 0.2	4.7 ± 0.3	0.25
HDL cholesterol (mmol/l)	1.37 ± 0.1	1.05 ± 0.1	<0.01
LDL cholesterol (mmol/l)	3.1 ± 0.1	2.8 ± 0.3	0.29
Triglycerides (mmol/l)	1.2 ± 0.1	2.4 ± 0.5	0.001
Alanine aminotransferase (units/l)	27.6 ± 2	32.0 ± 3	0.24

Data are means ± SE.

OGTT

After the ^{1}H-MRS measurements, a 75-g OGTT was performed (14,15). Whole blood samples were drawn at 0, 10, 20, 30, 60, 90, and 120 min to measure glucose (YSI 2300 STAT Plus Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH), serum C-peptide, and insulin concentrations (both by immunoradiometric assay [Centaur; Bayer Diagnostics, Mijdrecht, the Netherlands]). Baseline plasma glucose concentrations were measured by the hexokinase-based technique (Roche Diagnostics, Mannheim, Germany). A1C was measured with cation exchange chromatography (Menarini Diagnostics, Florence, Italy; reference values: 4.3–6.1%). Plasma total cholesterol, HDL cholesterol, and triglycerides were determined by enzymatic methods (Modular storage; Hitachi). LDL cholesterol was calculated by the Friedewald formula. NEFAs were assessed by enzyme-linked immunosorbent assay (WAKO Chemicals, Neuss, Germany).

β-Cell function parameters

Various β-cell function parameters were calculated. Insulin secretion rates were determined from C-peptide deconvolution (16). The insulinogenic index was calculated as the insulin increment at 30

Table 2—*β-Cell function parameters and their univariate correlation with pancreatic fat*

	Nondiabetic subjects	Type 2 diabetic subjects	Pancreatic fat content (log %) All subjects *R*	All subjects *P*	Nondiabetic subjects *R*	Nondiabetic subjects *P*	Type 2 diabetic subjects *R*	Type 2 diabetic subjects *P*
Empirical parameters of β-cell function								
Insulinogenic index adjusted for insulin resistance ($\Delta I_{0-30}/\Delta G_{0-30}$/HOMA-IR)	98 ± 18	11 ± 3*	−0.606	<0.001	−0.644	0.001	0.201	NS
Early-phase insulin secretion (nmol/m^2)	8.9 ± 0.7	5.3 ± 0.4*	−0.348	0.038	−0.162	NS	−0.407	NS
Late-phase insulin secretion (nmol/m^2)	40.1 ± 3.5	28.1 ± 2.6†	−0.145	NS	0.084	NS	−0.366	NS
Total insulin secretion (nmol/m^2)	49.0 ± 3.7	33.4 ± 2.8‡	−0.186	NS	0.046	NS	−0.403	NS
Model parameters of β-cell function								
Insulin secretion at the reference glucose level (pmol/min per m^2)	161 ± 18	124 ± 13§‖	NA	NA	−0.619	0.001	−0.310	NS
β-Cell glucose sensitivity (pmol/min/m^2/mmol/l)	122 ± 15	30 ± 3*	−0.580	<0.001	−0.595	0.002	0.100	NS
Rate sensitivity (pmol/m^2/mmol/l)	1,107 ± 131	194 ± 42*	−0.411	0.014	−0.326	NS	−0.081	NS
Potentiation factor	1.5 ± 0.2	1.0 ± 0.1†	−0.147	NS	−0.071	NS	0.134	NS

Data are means ± SE unless otherwise indicated. For calculations of the various β-cell parameters, see RESEARCH DESIGN AND METHODS. *$P < 0.001$; †$P < 0.05$; ‡$P < 0.02$. §Parameter calculated at 5 and 7 mmol/l glucose for nondiabetic and type 2 diabetic subjects, respectively. ‖Not comparable with control subjects because of different reference glucose level. NA, not applicable because of different reference glucose level; NS, not significant; *R*, Pearson's correlation coefficient.

min above basal divided by the corresponding glucose increment and was adjusted for homeostasis model assessment (HOMA) (17) for insulin resistance ($\Delta I_{0-30}/\Delta G_{0-30}$/HOMA-IR) (15). Oral glucose insulin sensitivity index was derived from OGTT glucose and insulin values (15). Early- and late-phase insulin secretion rates were calculated as the insulin secretion rate integrals from 0 to 30 min (insulin secretion rate area under the curve $[AUC]_{0-30}$) and 30 to 120 min (insulin secretion rate AUC_{30-120}), respectively. β-Cell function parameters were also obtained by modeling as previously described (14). In particular, β-cell glucose sensitivity (i.e., the slope of the dose-response function relating insulin secretion rate to glucose concentration), insulin secretion rate at a reference (close to basal) glucose level (calculated from the β-cell dose-response), and parameters quantifying the ability of the β-cell to anticipate a phase of rising insulin secretion (or rate sensitivity) and to memorize the glucose stimulus as well as reading incretin signals (potentiation) were calculated (additional information on modeling of β-cell function parameters can be found in an online-only appendix at http://doi.org/10.2337/dc07-0326).

Statistical analysis

Results are presented as means ± SE or medians (interquartile range). Differences between groups were calculated by Student's *t* test for unpaired comparisons. Non-normally distributed data were log-transformed. The association of pancreatic fat content and β-cell function was assessed by univariate correlation and multivariate linear regression analyses. The possible effect modification by diabetic state was evaluated by adding product terms (pancreatic fat × diabetic state) to the linear regression models. $P < 0.1$ was considered to indicate effect modification. $P < 0.05$ was considered statistically significant.

RESULTS — The diabetic and healthy groups did not differ significantly with respect to age, BMI, waist, blood pressure, total cholesterol, and liver enzymes (Table 1). Diabetic men had higher A1C, fasting plasma glucose, insulin, and triglyceride concentrations and lower HDL cholesterol and were more insulin resistant than nondiabetic men. Both empirical and model-derived β-cell function parameters differed significantly between the groups (Table 2). In addition to the insulinogenic index adjusted for HOMA-IR, and early- and late-phase glucose-stimulated insulin secretion, all model β-cell parameters were significantly impaired in patients versus control subjects.

Median pancreatic fat content was significantly higher in diabetic compared with nondiabetic men: 20.4% (13.4–43.6 [interquartile range]) vs. 9.7% (7.0–20.2) ($P = 0.032$, Fig. 1*B*). No associations were found for pancreatic fat with BMI, waist, triglycerides, or NEFAs.

In the univariate analysis, initially performed in the total study population, pancreatic fat was inversely associated with the HOMA-IR–adjusted insulinogenic index, early-phase insulin secretion, and β-cell glucose sensitivity and rate sensitivity, but not with potentiation (Table 2, Fig. 2*A–C*). Multivariate analysis, with β-cell function as the dependent variable and pancreatic fat × diabetes as an interaction term, demonstrated a significant association of pancreatic fat and various β-cell function parameters in the nondiabetic individuals only (Table 2), suggesting that once diabetes occurs, β-cell dysfunction is determined by factors other than pancreatic fat. Subsequent separate multivariate analyses in the nondiabetic group, with β-cell function (β-cell glucose sensitivity) as the dependent variable and pancreatic fat as the independent variable, were performed. Additional adjustment for age, BMI, fasting plasma glucose, and triglycerides in a multivariate model did not change the association of pancreatic fat with β-cell function to a significant extent (data not shown).

Median liver fat content was elevated in diabetic compared with nondiabetic men: 16.6% (interquartile range 9.8–29.8) versus 7.7% (4.1–1.4) ($P = 0.012$). As expected, liver fat content was positively associated with fasting plasma glu-

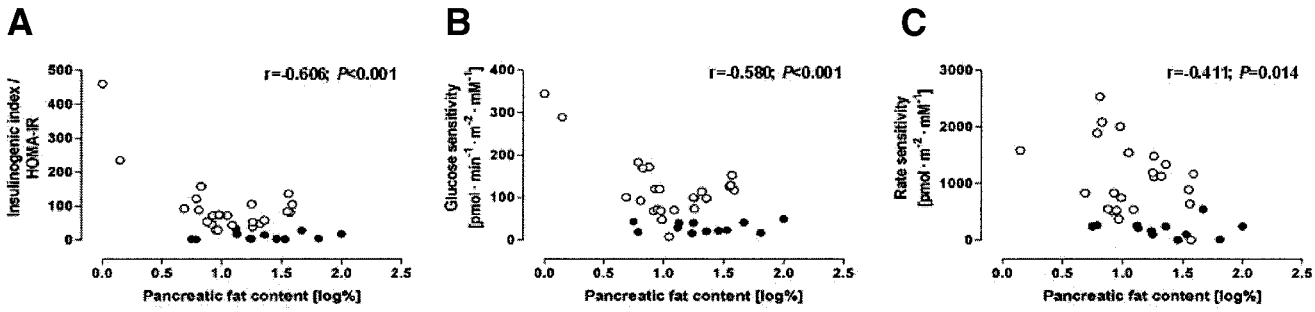

Figure 2—*Scatter plots representing the relationship between pancreatic fat content and β-cell function parameters, including insulin secretion rate corrected for HOMA-IR* (A)*, β-cell glucose sensitivity* (B)*, and rate sensitivity* (C) *in type 2 diabetic* (●) *versus nondiabetic* (○) *men. For statistical analysis of univariate associations, see Table 2 and text.*

cose, triglycerides, NEFAs, alanine transaminase concentrations, HOMA-IR, and oral glucose insulin sensitivity ($r = 0.39$, $P = 0.03$; $r = 0.47$, $P < 0.01$; $r = 0.41$, $P = 0.013$; $r = 0.58$, $P < 0.001$; $r = 0.43$, $P < 0.02$; $r = -0.67$, $P < 0.001$, respectively). Interestingly, no correlation was found between hepatic and pancreatic fat content ($r = -0.01$, $P = 0.99$).

CONCLUSIONS — This is the first report to show that, in addition to liver fat, pancreatic fat content is increased in men with type 2 diabetes, relative to nondiabetic men. In nondiabetic men, the pancreatic fat content was inversely associated with various features of β-cell function.

Although pancreatic fat was associated with all but one model/parameter of β-cell function, β-cell glucose sensitivity correlated most strongly with pancreatic fat. This parameter of β-cell function has been demonstrated to be most reproducible (14) and a good predictor of progression to type 2 diabetes in nondiabetic subjects (18).

The ^{1}H-MRS method does not allow discrimination between steatosis-like intracellular fat accumulation in β-cells and adipose tissue infiltration. Previous reports describe fatty replacement of the exocrine pancreas (19–21), which seems to occur primarily in the anterior part of the head of the pancreas, i.e., where acinar tissue is most abundant (21). We performed our measurements in the caudal part of the pancreas because of its relative abundance of islets. However, the islet volume comprises only ~2% of the total pancreas mass, suggesting that if ^{1}H-MRS is able to quantify fat in the pancreas, this fat should be largely located outside the islets. Theoretically, this extra-islet pancreatic fat could consist of infiltrating adipose tissue, presumably in relation with visceral fat, and/or fatty replacement of damaged tissue. In our study, we found no association between pancreatic and visceral fat (see below). Because intrapancreatic insulin signaling may affect viability and growth of cells present in the vicinity of the islets, including acinar cells, when insulin secretion is decreased, as occurs in diabetes, apoptosis and subsequent fatty replacement may occur. The latter mechanism may, in addition to glycotoxic cell injury, be one of the explanations why the diabetic subjects had more pancreatic fat than those without diabetes (19,20). Recently, evidence for a possible association between pancreatic lipomatosis and β-cell function was demonstrated (22). Using magnetic resonance imaging in 11 nondiabetic nonobese children with heterozygous carboxyl-ester lipase mutation, i.e., a rare mutation that causes diabetes and exocrine pancreatic dysfunction in association with pancreatic lipomatosis in adults, the authors found increased pancreatic fat content and a significant reduction of the first-phase insulin response to intravenous glucose in the mutation carriers. Importantly, these children had exocrine pancreatic dysfunction, in contrast to our subjects, suggesting that if the pancreatic fat measured indeed represents fatty replacement of injured tissue, cells other than acinar cells may be involved.

Although β-cell lipotoxicity has been convincingly demonstrated in animal models lacking the anti-steatotic hormone leptin (3,6), its contribution to β-cell dysfunction in human diabetes in vivo has been debated (5,6). At present, the full scope of actions of NEFAs on β-cells still remains to be unveiled. NEFAs, whether released from adipocytes lying in the close vicinity to islets or derived from the circulation, may cause intracellular accumulation of triglycerides and toxic intermediates, thus contributing to apoptosis and β-cell dysfunction (3). Also, chronic NEFA oversupply inhibits insulin synthesis and secretion, among others, by increasing uncoupling protein-2 levels, leading to inhibition of ATP synthesis and β-cell apoptosis. Finally, NEFAs may impair microvascular function and as such indirectly promote β-cell dysfunction (23,24). In contrast, short-term exposure of β-cells to NEFAs actually promotes glucose-stimulated insulin release (6). Also, NEFAs are essential for β-cell metabolism, and acute lowering of plasma NEFAs results in a decreased insulin response to glucose (25). In our study, we found a positive association of circulating NEFAs with total insulin secretion (and early- and late-phase insulin secretion) in diabetic men only ($r = 0.87$, $P < 0.001$; $r = 0.70$, $P = 0.012$; $r = 0.87$, $P < 0.001$, respectively), confirming that high NEFA levels reflect insulin resistance. However, we did not observe an association of circulating NEFAs with various β-cell function parameters in nondiabetic individuals. This lack of association may be explained by the fact that plasma NEFAs may not represent the actual NEFA levels to which islets are exposed, i.e., those resulting from release by adipocytes infiltrating the pancreas.

Beside NEFAs, adipocytes, whether localized in pancreatic or abdominal fat, secrete adipocytokines, thereby inducing proinflammatory responses that adversely affect islet structure and β-cell function (8).

In the present study, we found no relationship between magnetic resonance imaging–quantified visceral fat and β-cell function parameters (data not shown). Although the presence of an association of intra-abdominal fat with β-cell function has been reported by some (26,27), it was not observed by others (28–30). This seeming discrepancy may be due to the

differences in populations studied and the methods by which β-cell function parameters were estimated. In particular, if (uncorrected) acute insulin response is used to estimate β-cell function, a positive association with visceral adipose tissue is found, reflecting insulin sensitivity (26). Interestingly, Carr et al. (31), who had previously described a positive association of visceral fat and β-cell function (27), in a later report found that a decrease in visceral fat in glucose-intolerant subjects, following a lifestyle intervention, did not result in improvement of β-cell function. Thus, the association of abdominal adipose tissue and β-cell function may not be unequivocal. When diabetes develops, hyperglycemia may further deteriorate β-cell function, independently of the presence of (central) obesity.

No association was observed between pancreatic and liver fat, nor between pancreatic and visceral fat. This finding is in line with previous observations showing that local fat accumulation, e.g., in the liver, may be different in people with similar BMI and waist circumference (32). Furthermore, whereas pancreatic fat may largely consist of fatty infiltration, liver fat or steatosis may rather be the consequence of portal NEFA oversupply, which may be exaggerated and prolonged in the postprandial state in insulin-resistant individuals. Of note, NEFA supply to the pancreas rather depends on the system circulation (33). Genetic and environmental aspects, and factors determining the rate of fat accumulation and local fatty replacement, may contribute to individual differences in local fat deposition.

The association of pancreatic fat and β-cell function was found in nondiabetic but not in diabetic men. This may be explained by both methodological (relatively small number of diabetic men and low numerical values for the β-cell parameters assessed with too little variation to allow detection of any association) and pathophysiological factors, which are more likely to account for the findings. In diabetes, the presence of pancreatic fat may be permissive to the deleterious action of hyperglycemia on the β-cell (glucolipotoxicity) (2). Thus, because of the simultaneous activation of many deleterious cascades, including oxidative stress, inflammation, and apoptosis but also hypoperfusion of the islets, β-cell function deterioration may develop at a rate disproportional to that of pancreatic fat accumulation. Conversely, hyperglycemia via malonyl-CoA inhibits carnitine palmitoyltransferase-1, leading to a decrease in mitochondrial β-oxidation and further stimulation of intracellular triglyceride accumulation. As stated above, this mechanism may, among others, contribute to the higher pancreatic fat content observed in diabetic relative to nondiabetic men.

Limitations of our study include the relative small number of subjects, which may not allow generalization of the data. We also mentioned the limitations of the ^{1}H-MRS method, which does not allow discrimination between steatosis-like intracellular fat accumulation in β-cells and adipose tissue infiltration.

In conclusion, we found that in type 2 diabetic men, ^{1}H-MRS–measured pancreatic lipid content is higher than in nondiabetic age- and BMI-matched control subjects. Pancreatic fat content was independently associated with various aspects of β-cell function in nondiabetic men only. Whether the presence of pancreatic fat is relevant for subsequent development of type 2 diabetes in susceptible humans still needs to be determined.

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