Pancreatic Metabolism, Blood Flow and β-Cell Function in Obese Humans

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Clinical trial registration numbers ClinicalTrials.gov: NCT00793143, NCT01373892

Context **Glucolipotoxicity is believed to induce pancreatic β-cell dysfunction in obesity. Previously, it has not been possible to study pancreatic metabolism and blood flow in humans.**

Objective **The objective of the study was to investigate whether pancreatic metabolism and blood flow are altered in obesity using positron emission tomography (PET). In the preclinical part, the method was validated in animals.**

Design **This was a cross-sectional study.** ***Setting*** **The study was conducted in clinical research center.**

Participants **Human studies consisted of 52 morbidly obese and 25 healthy age-matched controls. Validation experiment was done with rodents and pigs.** ***Interventions*** **PET and MRI studies using a glucose analogue [^{18}F]FDG, a palmitate analogue [^{18}F]FTHA and radiowater [^{15}O]H_2O were performed. In animals, comparison between *ex vivo* and *in vivo* data was performed.**

Main Outcome Measures **Pancreatic glucose/fatty acid uptake, fat accumulation and blood flow. Parameters of β-cell function.** ***Results*** **PET proved to be a feasible method to measure pancreatic metabolism. Compared to healthy, obese participants had elevated pancreatic fatty acid uptake ($P < 0.0001$), more fat accumulation ($P = 0.0001$), lowered glucose uptake both during fasting and euglycemic hyperinsulinemia, and blunted blood flow ($P < 0.01$) in the pancreas. Blood flow, fatty acid uptake and fat accumulation were negatively associated with multiple markers of β-cell function.**

Conclusions **Obesity leads to change in pancreatic energy metabolism with a substrate shift from glucose to fatty acids. In morbidly obese humans, impaired pancreatic blood flow may contribute to β-cell dysfunction and in the pathogenesis of type 2 diabetes.**

Overt type 2 diabetes is characterized by hyperglycemia resulting from pancreatic β-cell failure. Despite multiple theories behind the loss of the functional β-cell mass compromising the capacity to compensate for insulin resistance, only few of the proposed mechanisms have been proven to be significant in vivo (1).

As adipose tissue insulin sensitivity decreases, a signif-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received December 10, 2013. Accepted January 31, 2014.

Abbreviations: *Nonstandard abbreviations* ARG; autoradiography; CT, computed tomography; [^{18}F]FDG, [^{18}F]fluoro-2-deoxy-*D*-glucose; FA, fatty acid; FFA, free fatty acid; FI_P, pancreatic fat index; [^{18}F]FTHA, 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid; FUR, fractional uptake rate; [^{15}O]H_2O, radiowater; HDL, high-density lipoprotein; HEC, hyperinsulinemic euglycemic clamp; K_i, influx rate constant; LC, lumped constant; LDL, low-density lipoprotein; *M* value, whole-body glucose disposal; MRI, magnetic resonance imaging;

icant rise in plasma free fatty acid (FFA) levels ensues. Numerous studies have addressed the role of circulating FFAs and triglycerides and the resulting pancreatic steatosis on β-cell function in animal models and in obese humans, but the association between pancreatic lipid accumulation and islet cell function is currently unsettled (2–4). In cell cultures, short- and long-term exposure of β-cells to fatty acids (FA) leads to increased and decreased glucose-stimulated insulin secretion (GSIS), respectively (5). However, whether β-cells, during the in vivo situation, can tolerate the oversupply of FFA, which occurs during obesity and insulin resistance is unclear.

β-cell function is defined by their insulin secretion based on plasma glucose levels (6). While being the most potent stimulator of insulin secretion and a significant contributor to β-cell proliferation, excessive plasma glucose amounts leads to an acute impairment in β-cell function in healthy participants (7, 8).

The pancreas is perfused by multiple arteries derived from the abdominal aorta, and has auto- and paracrine regulation (9). Since the pancreatic islets comprise 2% of the pancreatic volume but consume nearly 20% of its arterial supply, even a slight impairment in parenchymal blood flow can affect markedly pancreatic endocrine function (10). The pancreas is a difficult organ to study because it is small and hidden in the retroperitoneal space, previous approaches to quantify pancreatic blood flow, noninvasively, have yielded conflicting results and a firm consensus on the relation between blood flow and β-cell function is lacking (11).

We hypothesized that morbid obesity leads to changes in pancreatic metabolism and blood flow rate, all of which may affect pancreatic endocrine function. To address these questions, we used positron emission tomography (PET) with multiple radiotracers to quantify pancreatic glucose/fatty acid metabolism and blood flow, respectively. First, validation of fluorine-18 –labeled radiotracers to measure pancreatic metabolism was conducted in pig (protocol previously reported (12)) and mouse (protocol reported here) models. Then, this methodology was used in the clinical protocol, including a total of 77 participants. This study introduces novel mechanisms behind the deterioration of β-cell function which are involved in the type 2 diabetes pathogenesis.

Materials and Methods

Human participants and their metabolic and anthropometric characteristics. 52 morbidly obese participants who were scheduled for bariatric surgery and 25 healthy age-matched participants were recruited from larger data collections named SLEEVEPASS (NCT00793143) and SleevePET2 (NCT01373892), as previously described (13). Of the obese participants, 20 had type 2 diabetes (ADA criteria), and 18 with normal and 14 with impaired glucose tolerance constituted the nondiabetic group. Compared to healthy participants, obese participants were significantly insulin resistant, hyperglycemic and hyperinsulinemic, and their FFA levels were higher (Table 1). Most obese participants met the IDF criteria for metabolic syndrome. Compared to healthy and nondiabetic participants, parameters of β-cell function were markedly decreased in the diabetic study group. The Ethics Committee of the Hospital District of Southwestern Finland approved the studies and all gave their written informed consent before participation.

Clinical study design. Human participants underwent abdominal PET imaging with either [^{18}F]fluoro-2-deoxy-*D*-glucose ([^{18}F]FDG) (fasting state and hyperinsulinemic euglycemic clamp [HEC] on two separate days) or 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) and radiowater ([^{15}O]H_2O) (fasting state). For the patients undergoing bariatric procedure, the imaging studies were performed before the onset of preoperative very low-calorie diet (VLCD).

Twenty-five obese and 10 healthy participants were studied twice, once during fasting state and once during hyperinsulinemic euglycemic clamp with [^{18}F]FDG using GE Advance PET scanner. After 90 minutes of hyperinsulinemia, an [^{18}F]FDG-bolus (187 ± 8 MBq) was injected. Seventy minutes after the injection, the upper abdomen and pancreas were imaged for 15 minutes (frames 5 × 180 seconds). Euglycemic hyperinsulinemic clamp (1 mU/kg*min, Actrapid®, Novo Nordisk, Bagsværd, Denmark) was done as previously described (14) and whole body glucose uptake (*M* value) representing systemic insulin sensitivity was calculated from glucose infusion rates during the last 120 minutes of the clamp (15).

Twenty-seven obese and 15 healthy participants received [^{15}O]H_2O and [^{18}F]FTHA during the fasting state. PET and computed tomography (CT) images were obtained using the combined PET/CT-scanner GE Discovery VCT. At baseline, a bolus injection of [^{15}O]H_2O (579 ± 133MBq) was followed by a dynamic image acquisition of 6 minutes (26 frames, 16). Approximately ten minutes after radiowater injection, an [^{18}F]FTHA-bolus (190 ± 59MBq) was injected and dynamic imaging lasting 110 minutes was acquired. At 68 ± 3 minutes after the injection, the upper abdomen and pancreas were imaged for 15 minutes (frames 5 × 180 seconds).

Whole body magnetic resonance imaging (MRI) scan (Gyroscan Intera CV Nova Dual, Philips, the Netherlands or Magnetom Verio, Siemens, Germany) was performed to obtain the pancreatic adiposity index and visceral adipose tissue volume. PET and MRI studies were performed using standard operating procedures (17, 18), and biochemical analyses were performed as previously described (12). More details are shown in Supplemental Figure 1A-C.

Image processing, and PET and MRI analysis. All data were corrected for dead time, decay, and measured photon attenuation and reconstructed in a 256 × 256 matrix. ROIs were defined on

OGTT; oral glucose tolerance test; PET, positron emission tomography; ROI, region-of-interest; SI, signal intensity; TAC, time-activity curve; VLCD, very low-calorie diet.

Table 1. Participant characteristics.

		Obese		
	Controls ($n = 25$)	All ($n = 52$)	Non-diabetic ($n = 32$)	Diabetic ($n = 20$)
Anthropometic characteristics				
Age (yr)	45.8 ± 10.2	44.2 ± 9.2	41.4 ± 9.7	48.7 ± 8.1¶
Weight (kg)	64.8 ± 7.7	118 ± 13.8§	120 ± 14.4§	114 ± 12.3§
BMI (kg/m2)	23.0 ± 2.5	42.3 ± 3.9§	42.9 ± 3.6§	41.2 ± 4.2§
Body fat (%)	31.1 ± 6.5	49.4 ± 5.6§	50.0 ± 4.6§	48.3 ± 6.9§
Waist circumference (cm)	77.4 ± 9.4	120 ± 10.1§	120 ± 10.9§	120 ± 9.0§
Metabolic syndrome	0 (0.0%)	30 (58%)	12 (38%)	18 (90%)
Biochemical characteristics				
Fasting glucose (mmol/liter)	5.2 ± 0.4	5.8 ± 1.3*	5.3 ± 0.7	6.5 ± 1.6‡#
HbA1c (mmol/mol)	38.2 ± 3.0	41.2 ± 7.0	37.7 ± 3.7	46.7 ± 7.5§**
Fasting insulin (IU/liter)	5.8 ± 3.6	16.2 ± 11.4§	14.0 ± 9.2§	19.8 ± 13.9§
Cholesterol (mmol/liter)	4.7 ± 0.8	4.3 ± 0.8*	4.3 ± 0.7*	4.3 ± 1.0
Triglycerides (mmol/liter)	0.7 ± 0.3	1.3 ± 0.5§	1.2 ± 0.5‡	1.5 ± 0.4§
HDL:cholesterol -ratio (%)	40.5 ± 9.1	30.0 ± 7.3§	30.8 ± 7.4§	28.6 ± 7.1§
hsCRP (mg/liter)	0.9 ± 0.9	4.6 ± 4.0§	4.9 ± 4.0§	4.2 ± 4.0‡
Leptin (ng/ml)	12.0 ± 7.9	54.4 ± 19.2§	55.2 ± 19.3§	53.1 ± 20.2§
FFA (mmol/liter)	0.5 ± 0.2	0.7 ± 0.2§	0.7 ± 0.2§	0.7 ± 0.2‡
Insulin resistance indices				
2 h OGIS (ml/min*m 2)	427 ± 58.0	327 ± 46.0§	341 ± 44.0§	305 ± 41.1§¶
M value (μmol/kg*min)	40 ± 9.5	13 ± 7.3§	15 ± 8.3§	10 ± 3.6§
β-cell function parameters				
Insulinogenic index (mmol/IU)	16 ± 13	23 ± 25	31 ± 28*	9.5 ± 5.1*¶
Glucose sensitivity (pmol/min*m 2*mmol/liter)	123 ± 43.8	101 ± 85.1	132 ± 93.3	52.2 ± 34.4§#
Rate sensitivity (pmol/m 2*mmol/liter)	880 ± 832	883 ± 1169	1251 ± 1369	314 ± 277†#
Potentiation factor ratio (arbitrary units)	2.6 ± 1.8	1.4 ± 0.6§	1.5 ± 0.8†	1.3 ± 0.4†

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$, §$P < 0.0001$ for all obese, non-diabetic or diabetic *vs.* controls; $P < 0.05$, ¶$P < 0.01$, #$P < 0.001$, **$P <$ 0.0001 for diabetic *vs.* non-diabetic. *M* value calculated from subjects in the [^{18}F]FDG protocol. Data are presented as mean ± SD with significance tested using Student's *t* test.

the pancreas (Figure 1A-B) using Carimas 2 software (Turku PET Centre, freely downloadable at http://www.turkupetcenter.fi). MR or CT images were used as an anatomical reference.

For glucose and fatty acid uptake analysis, the plasma input function was obtained from blood radioactivity measurements (metabolite-corrected for [^{18}F]FTHA, 17). To determine the input function for [^{15}O]H_2O analysis, small ROIs with a diameter of 2–3 mm including pixels of maximal radioactivity were drawn on multiple transaxial slices inside the abdominal aorta (19) (Figure 1C) and the delay between pancreatic and aortic time-activity curves (TACs) was corrected. Due to the large size of the aorta and fine resolution of the PET scanner, a recovery correction was not considered necessary. The pancreatic [^{18}F]FDG and [^{18}F]FTHA influx rate constants were calculated using fractional uptake rate (FUR) (20–23), and glucose and FA uptake rates were calculated by multiplying corresponding influx rate constant values by the mean plasma glucose or FFA level during the imaging period, respectively. The pancreatic lumped constant (24) was set to 1.0.

For human data, one-tissue compartmental analysis derived from simple Kety flow model (25) was used to measure pancreatic blood flow. Hepatic parenchymal blood flow is dependent on a dual arterial/portal input function, and was calculated with a validated gut compartment model (26).

Pancreatic fat content from humans was estimated from MR images (27), based on the different chemical shifts of water and fat leading to typical signal intensities (SI) in In-Phase and Out-of-Phase MR tomograms. Here, the pancreatic fat index was calculated as $FI_P = (SI_{in} - SI_{out}) / SI_{in} \times 100$, where SI_{in} and SI_{out} are signal intensities during In- and Out-of-Phase MR imaging, respectively. Log10 transformation was performed for the fat index. Visceral adipose tissue volumes were estimated manually using Slice-O-Matic 4.3 software (Tomovision, Montreal, Canada).

Oral glucose tolerance test (OGTT), insulin sensitivity and β-cell function parameters. A routine 75g OGTT lasting 2 hours

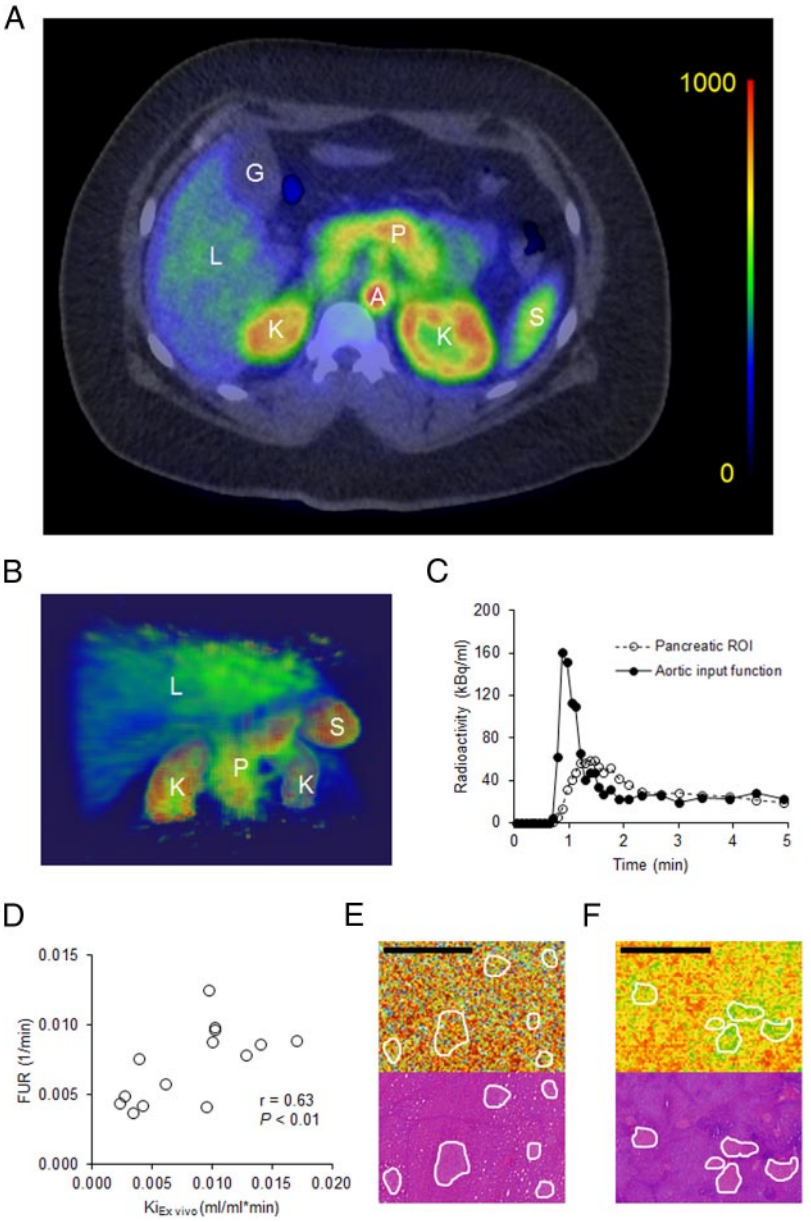

Figure 1. In humans, combined PET/CT and MRI techniques were used to measure pancreatic glucose/fatty acid metabolic and blood flow rates. An example of fusion [^{15}O]H_2O-PET/CT image shows that blood flow is higher in the pancreas compared to other GI organs (A-B) (A, abdominal aorta; G, gallbladder; K, kidney; L, liver; P, pancreas; S, spleen.). 3D-dimensional regions-of-interest (ROIs) were drawn on multiple planes of pancreatic images and the blood input function was obtained by blood sampling ([^{18}F]FDG and [^{18}F]FTHA) or PET images of the aorta (for [^{15}O]H_2O, C). In animal data, PET- (FUR) and ex vivo –derived influx rate constants were intercorrelated, supporting the use of PET in the characterization of pancreatic metabolism in vivo (D). Pancreatic [^{18}F]FDG-autoradiography analysis in both control and obese/diabetic pigs revealed that the endocrine and exocrine accumulation of the radiotracer was homogeneous (E). [^{18}F]FTHA was distributed homogenously in the pancreas of control mice. In contrast, in obese hyperlipidemic mice, [^{18}F]FTHA showed preferable accumulation in the exocrine pancreas, with lower uptake in pancreatic islets (F for [^{18}F]FTHA-ARG and corresponding hematoxylin-eosin stained slice of pancreatic parenchyma). Islets are encircled in white. Scalebar, 1000 μm.

was applied to human participants. The insulin sensitivity index (2h OGIS) and model parameters of β-cell function (*glucose sensitivity*, *rate sensitivity*, *and potentiation factor ratio;* Supplemental Data) were calculated as previously described (6), and the insulinogenic index was calculated as $\Delta Ins_{0-30}/\Delta Gluc_{0-30}$.

Animal studies. Validation of [^{18}F]FDG and [^{18}F]FTHA PET for the measurement of pancreatic glucose and fatty acid uptake was performed in 18 pigs, of which 10 obese and steptozotocin –treated, as previously described (12). Principles of laboratory animal care were followed and permission for the study was obtained from the State Provincial Office of Southern Finland (ESAVI-2010–03970/Ym-23).

Animals received a bolus of [^{18}F]FDG (890 ± 180MBq) and dynamic study using PET/CT scanner GE Discovery VCT (General Electric Medical Systems, Milwaukee, WI, USA) was contacted with arterial blood sampling (Supplemental Figure 2A) (12). After 40 minutes from the injection, a 20 minutes scanning of upper abdomen (4 × 300 seconds) was conducted.

At 240 minutes after the [^{18}F]FDG bolus, pigs were sacrificed the pancreas was excised. Radioactivity of tissue samples was measured as previously described (12), and ex vivo influx rate constants (denoted as K_i) and in vivo derived FUR (20–23) were calculated. To study the distribution of tracers in exocrine and endocrine pancreas, autoradiography analysis (28) was performed. For [^{18}F]FDG, pancreatic cross-sections of pigs were obtained. For [^{18}F]FTHA, 15 MBq of the tracer was injected in 12 adult male mice (3 healthy C57BL/6N and 9 atherosclerotic and diabetic IGF-II/LDLR$^{-/-}$ApoB$^{100/100}$ mice) (Supplemental Figure 2B). At 30 minutes after injection, mice were sacrificed and pancreatic cross-sections were obtained (Supplemental Figure 2B).

Autoradiographs were analyzed using the AIDA 4 software (Raywest, Straubenhardt, Germany) and the endocrine-to-exocrine radioactivity ratio was determined.

Statistical analysis. Statistical analyses were performed using SAS software for Windows (SAS Institute, Cary, NC, USA). Data are presented as mean±SD. Tests for equal variances and normality were performed. A paired *t* test was used to address the difference between *ex* and in vivo modalities in ^{18}F-PET validation. Student's *t* test was used for comparisons between healthy and obese groups. For correlation analysis, Pearson and Spearman correlation coefficients were calculated, as appropriate. Furthermore, multivariable linear regression analysis was conducted to determine the effects of independent factors on main measured outcomes. $P <$ .05 was considered statistically significant.

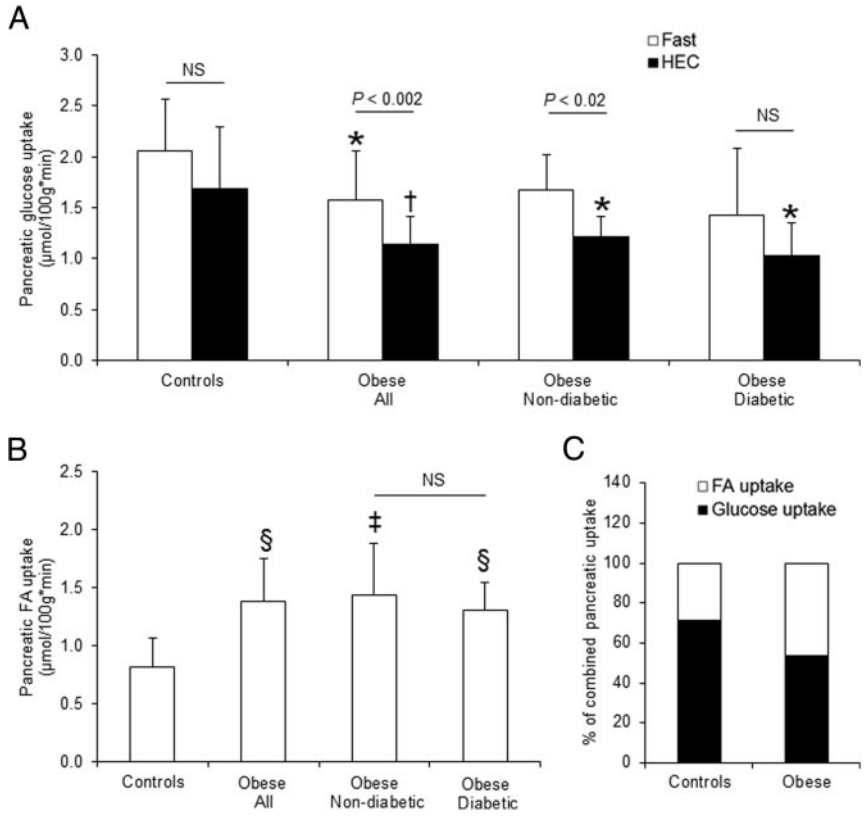

Figure 2. Compared to controls, obese human participants had lower pancreatic glucose uptake during the fasting state and hyperinsulinemic euglycemic clamp (HEC) (A). Insulin administration did not change pancreatic glucose uptake in healthy individuals. In contrast, FA uptake was 68% greater in obese participants than in controls with no difference between obese participants with and without type 2 diabetes (B). During obesity, the preferred pancreatic energy substrate was shifted from glucose to FAs (C). *$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$ for all obese, nondiabetic or diabetic vs controls. NS, not statistically significant.

Results

Based on animal data, PET is a valid method to study pancreatic metabolism. In the pig study, fractional glucose uptake derived from PET images was similar with those measured thereafter in tissue samples with tight correlation (Figure 1D, Supplemental Table 1). No hot spots were seen, and [^{18}F]FDG accumulated diffusely within the pancreatic parenchyma - both in control and obese diabetic pigs (Figure 1E).

In the mice study, obese mice were hyperlipidemic (plasma FFA 1.47 ± 0.50 vs 0.62 ± 0.07 mmol/l, $P < .02$). [^{18}F]FTHA accumulation was diffuse in the lean control mice, whereas in the diabetic mice, it was higher in the exocrine than endocrine parts of the pancreas (Figure 1F). Here, pancreatic islets contained only 70% of ^{18}F-derived radioactivity compared to the exocrine parts.

During obesity, pancreatic energy metabolism is shifted from glucose to fatty acids. In healthy participants, the hyperinsulinemic euglycemic clamp did not augment pancreatic glucose uptake from fasting values (Figure 2A). Compared to nonobese, obese participants had lower pancreatic glucose uptake rates both in the fasting state ($P < .03$) and during hyperinsulinemia ($P < .01$), but no difference was found between participants with and without diabetes. Obese participants had higher pancreatic glucose uptake during fasting than during hyperinsulinemia ($P < .01$), likely due to the mass action effect of glucose, as fractional uptake of [^{18}F]FDG was similar in fasting and during hyperinsulinemia (0.003 ± 0.001 vs 0.003 ± 0.001 1/min, NS). During hyperinsulinemia, pancreatic glucose uptake correlated inversely with fasting plasma glucose ($r = -0.55$, $P < .01$) and plasma triglycerides ($r = -0.44$, $P = .03$), but not with simultaneous plasma FFA.

In contrast to glucose uptake, pancreatic FA uptake was on average 68% greater in obese than in healthy participants with no difference between obese subgroups (Figure 2B). Since the fractional uptake of [^{18}F]FTHA was similar in both control and obese groups (0.016 ± 0.004 vs 0.018 ± 0.005 1/min, NS), the difference in FA uptake values was derived from the larger intravascular pool of circulating fatty acids in obese participants (Table 1). FA uptake correlated with fasting insulin levels ($r = 0.45$, $P < .004$), but not with fasting plasma glucose levels ($r = -0.04$, NS). Systemic insulin sensitivity (OGIS index) was inversely associated with pancreatic FA uptake ($r = 0.49$, $P < .003$).

From these data sets, the relative uptake of the two energy substrates (glucose and FA) was different between groups with glucose constituting 75 and 59% of whole pancreatic metabolism in healthy and obese participants, respectively (Figure 2C). Combined molar influx of the two substrates did not differ between healthy and obese participants (2.9 ± 0.7 vs 3.0 ± 0.4 µmol/100g*min, NS).

Obesity impairs pancreatic blood flow. Blood flow in the pancreatic parenchyma was lower in obese than in healthy participants (Figure 3A) having a mean coefficient of variation of 13% within the pancreatic parenchyma. Pancreatic flow correlated with splanchnic blood flow measured as portal vein blood flow (Figure 3B) and with whole hepatic blood flow ($r = 0.38$, $P < .02$) but not with hepatic arterial blood flow ($r = 0.22$, NS).

In pooled data, age was found to be the strongest predictive variable of pancreatic blood flow, with adjusted $R^2 = 0.36$ ($P = .0001$). Moreover, pancreatic blood flow showed a relation with HDL:cholesterol –ratio (r = 0.40, $P = .02$) and fat percentage (r = −0.39, $P < .02$), but not with fasting plasma glucose ($r = -0.12$, NS), triglycerides (r = −0.25, NS), free fatty acids (r = −0.24, NS), blood pressure (BP) (r = −0.04 and r = −0.12 for systolic and diastolic BP, respectively, both NS), or OGIS index (r = 0.21, NS).

In healthy but not in obese participants, fasting plasma glucose and insulin showed strong correlations with blood flow (r = 0.60, $P = .02$ and r = 0.80, $P = .002$), whereas in all obese participants, pancreatic blood flow correlated inversely with the intra-abdominal adipose tissue volume (Figure 3C) and with HbA1c (r = −0.48, $P = .01$).

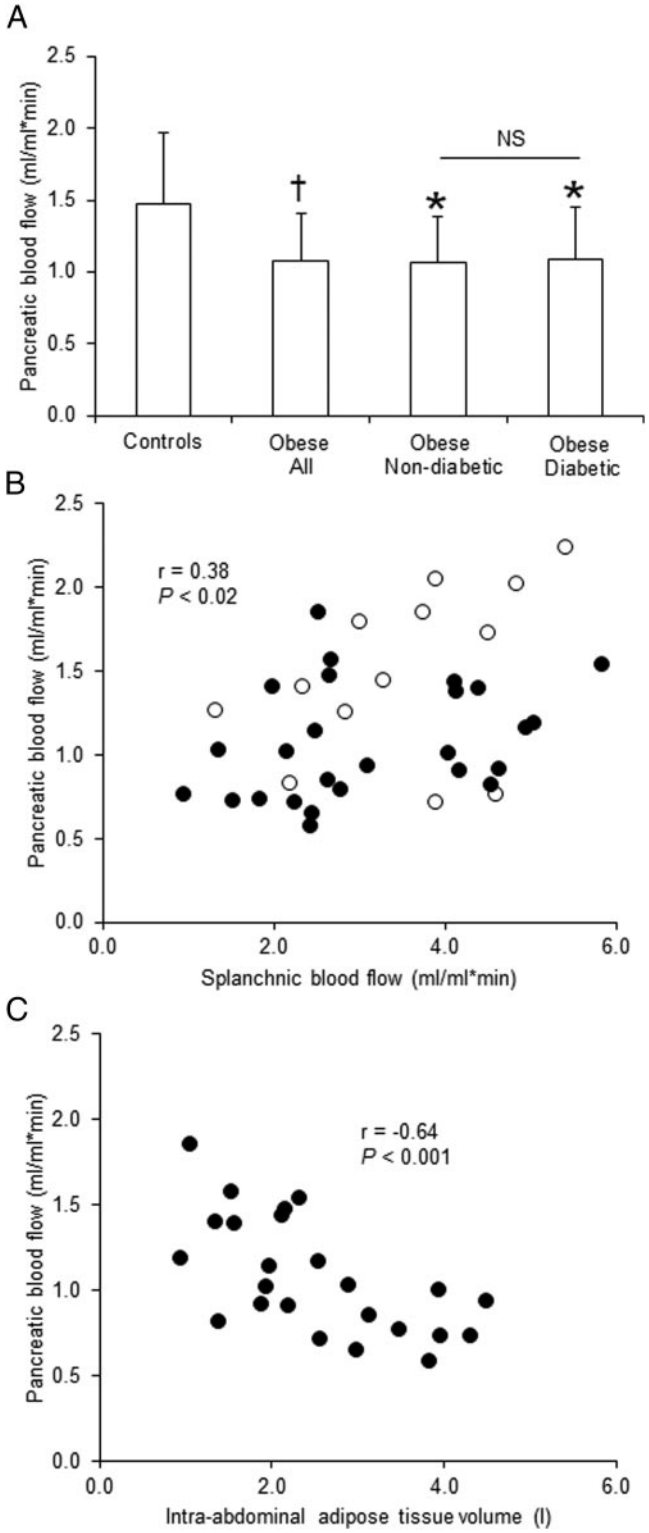

Figure 3. Pancreatic blood flow was significantly lower in obese participants than in controls with no difference between obese participants with and without type 2 diabetes (A). In pooled data, pancreatic blood flow was associated with portal vein blood flow (B). The intra-abdominal adipose tissue volume was negatively associated with pancreatic blood flow (C). White circles, controls; Black circles, obese. *$P < .05$, †$P < .01$ for all obese, nondiabetic or diabetic vs controls. NS, not statistically significant.

Pancreatic fat content is increased in obesity, in association with lower glucose uptake and blood flow but not with FA uptake. The pancreatic fat index, FI_P, was 5-times higher in obese as compared to nonobese participants (Figure 4A) but similar between obese with and without type 2 diabetes (NS). FI_P was inversely related with systemic insulin sensitivity (r = −0.48, $P = .007$) and HDL:cholesterol –ratio (r = −0.33, $P = .006$), and was associated positively with HbA1c (r = 0.28, $P = .02$). No relationship was found between FI_P and fasting plasma glucose or FFA levels (r = 0.18 and r = 0.04, respectively; both NS).

Pancreatic fat was a significant predictor of pancreatic blood flow (Figure 4B) and pancreatic glucose uptake during both fasting (r = −0.46, $P = .02$) and hyperinsulinemic states (Figure 4C). It was not associated with pancreatic FA uptake (Figure 4D).

High pancreatic FA uptake, fat accumulation (FI_P) and impaired blood flow are associated with β-cell dysfunction. Compared to healthy participants, insulin secretory capacity and model-derived β-cell function parameters (ie, *insulin secretion at reference glucose level, glucose sensitivity, rate sensitivity and potentiation factor ratio*) were considerably impaired in the obese group (Table 1). In pooled data, pancreatic FA uptake and FI_P correlated inversely with the potentiation factor ratio (Figure 5A) and associated positively with the mean incremental glucose during OGTT (Figure 5B), whereas glucose uptake during fasting and HEC was not associated with any of these β-cell function parameters (Supplemental Tables 2–5). Pancreatic blood flow was associated with β-cell glucose sensitivity (Figure 5C), insulinogenic index (Figure 5D) and mean incremental glucose during OGTT (r = −0.45, $P < .004$) (Supplemental Table 6).

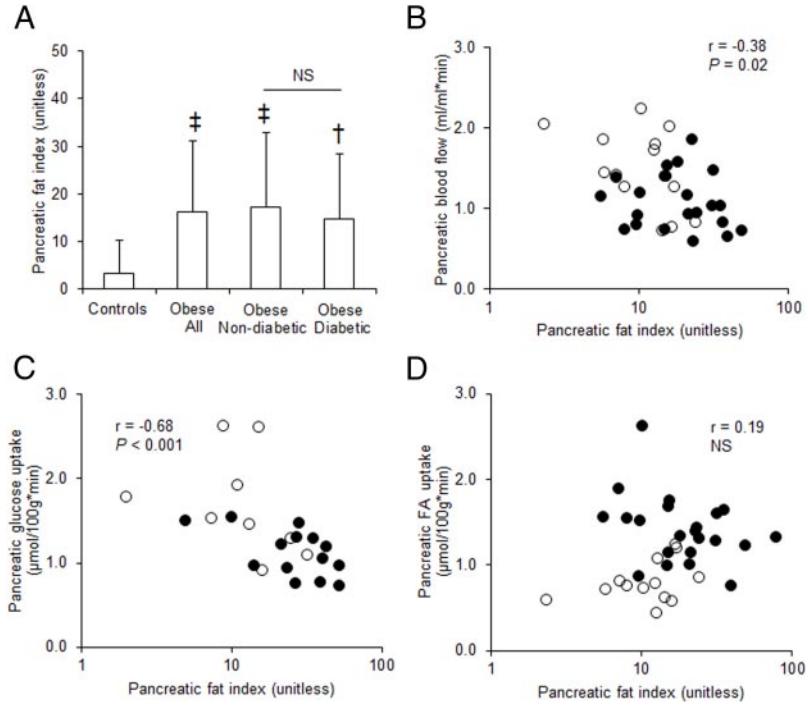

Figure 4. From human data, pancreatic fat content was estimated from In- and Out-of-Phase MR images showing great interindividual variability. It was 5-times higher in obese participants than in controls (A). No difference was found in fat content between participants with and without type 2 diabetes. Pancreatic fat content was associated inversely with both pancreatic glucose uptake (B, during HEC) and blood flow (C). Pancreatic fat content and FA uptake were not associated directly (D). †$P < .01$, ‡$P < .001$ for all obese, nondiabetic or diabetic vs controls. NS, not statistically significant.

Discussion

In this study, we showed for the first time in humans, that obesity and peripheral insulin resistance are associated with marked changes in pancreatic metabolism and impairment in pancreatic blood flow, with parallel defect in β-cell function. We did not find evidence for glucose-mediated toxicity.

The most salient finding of the study was that pancreatic blood flow was impaired in obese individuals, and this reduction in blood flow was associated with lower β-cell glucose sensitivity, insulinogenic index and mean incremental glucose during an OGTT. Previous mice studies show that islets with a greater perfusion have higher functional activity and that vascularization is necessary for the adequate function of pancreatic islets (9, 29). Pancreatic islets receive nearly 20% of whole pancreatic blood flow (10), and an adequate blood flow permits the entry of insulin secretagogues (glucose, incretins), oxygen and nutrients into the islets, affecting β-cell proliferation (9). The current PET technology – with a resolution 25 times larger than the mean size of an islet – cannot differentiate islets blood flow from whole pancreatic flow. However, the pancreas is characterized by very high blood flow compared to other GI organs (Figure 1A-B, 11), and even minor deteriorations in whole pancreatic blood flow will affect considerably islet blood flow.

In addition, the relationship between β-cell function parameters and pancreatic blood flow suggests strongly the involvement of islet hypoperfusion in the dysregulation of the endocrine pancreas. Experimental intermittent hypoxia leads to attenuated GSIS by down-regulating CD38 transcription (30). Recently, Dai et al showed that in response to insulin resistance, the pancreatic islet vasculature undergoes dilation (31). If this counter-regulatory mechanism occurred in our study, it was not sufficient to normalize pancreatic blood flow and β-cell function in our obese participants.

Together with obesity, age was a strong negative modulator of pancreatic blood flow. Other negative determinants included a large intra-abdominal adipose tissue volume, high pancreatic fat content and a poor lipid profile. In healthy participants, plasma glucose and insulin levels were associated positively with blood flow, confirming previous observations in animal studies (32). The imbalance between these factors in diabetic and nondiabetic obese groups may explain the lack of difference in blood flow in these obese subgroups.

Previous studies investigating the effects of fat on pancreatic endocrine function have been performed by measuring pancreatic fat content or percentage. Because pancreatic lipid accumulation occurs mostly in the exocrine (either extralobular or intracellular) parts of the organ, it is difficult to determine the direct consequences of pancreatic fat on islets function (33, 34). In addition to measuring pancreatic fat content using In-Phase and Out-of-Phase MRI, we utilized [^{18}F]FTHA-PET. This provided dynamic information, and showed that both pancreatic fat content and fatty acid uptake are greater in obese individuals. Our data indicate that the increase in pancreatic FA uptake observed in obese participants was due to the excess of FFA delivered through the bloodstream. Both plasma fatty acid levels and pancreatic FA uptake values correlated strongly with the OGIS index implying that the excessive pancreatic FA uptake occurs as consequence of insulin resistance in adipose tissue. In skeletal muscle, liver, myocardium, and brain, adipose tissue fatty acid

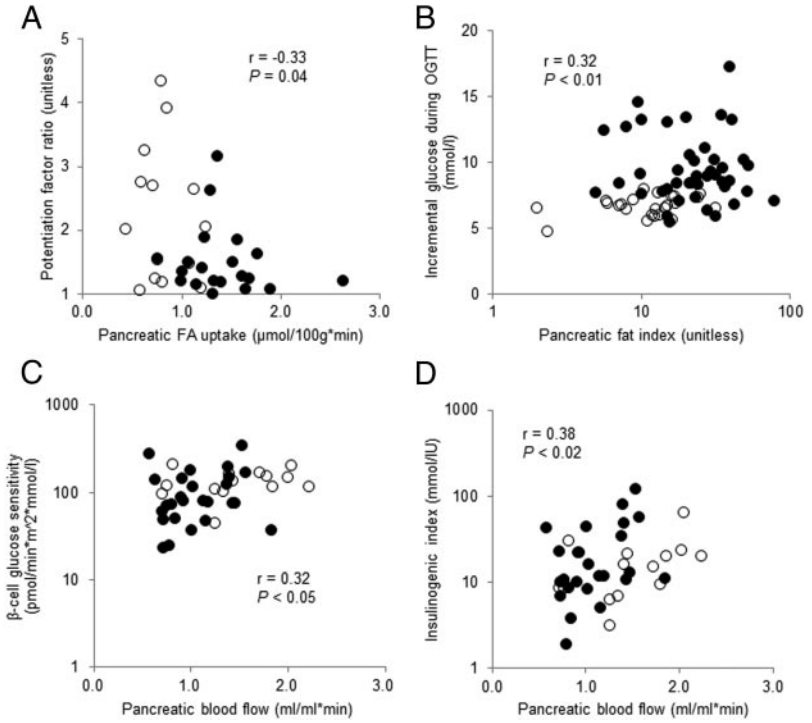

Figure 5. Pancreatic FA uptake, lipid accumulation and impaired blood flow were associated with a lower pancreatic endocrine function, as represented by several β-cell function parameters (more details are given in Supplemental Tables 2–7). In pooled data, FA uptake (A) and lipid accumulation (B) were correlated inversely with the potentiation factor ratio and mean incremental glucose during the OGTT. Pancreatic blood flow correlated with multiple parameters of β-cell function, including glucose sensitivity (C) and insulinogenic index (D).

release or spillover affects their function (17, 18, 35). Although in vitro islet exposure to fatty acids inhibits GSIS, consensus on the in vivo effects of fat on the endocrine functions of the pancreas is lacking (36). Tushuizen et al (4) showed that the pancreatic fat percentage was higher in diabetic than nondiabetic men, and that lipid accumulation correlated negatively with multiple β-cell function parameters. This was confirmed in our data, in which the pancreatic fat content was 5-times higher in obese participants together with higher FA uptake rates, and was associated with parameters of β-cell function (Supplemental Tables 2, 3), regardless of the presence of diabetes. These results imply that lipids per se produce toxicity to the pancreatic endocrine function, in line with animal studies showing that the overexpression of facilitated FA transport protein CD36 blunted insulin secretion (37). Autoradiography studies in mice showed that obesity was associated with a redistribution in FA uptake within the organ in favor of the exocrine pancreas, as a 30% greater gradient in [^{18}F]FTHA accumulation between the exocrine and the endocrine pancreas was found in obese vs lean mice. This finding suggests that islets respond to an FA overload by a partial down-regulation of FA uptake.

A previous study (38) has shown that an acute FA load reduces splanchnic glucose uptake and fractional extraction by 17% during oral glucose intake and hyperinsulinemic euglycemia. In line with this, we showed that pancreatic glucose uptake was 23 and 32% lower in obese participants than in lean controls during the fasting state and hyperinsulinemic euglycemia, respectively. The defect in glucose uptake likely involve pancreatic islets, since [^{18}F]FDG was evenly distributed between the exocrine and endocrine pancreas in our animal autoradiography experiments. No evidence was found to support glucotoxicity (Supplemental Tables 4, 5). Glucose is a significant stimulator of the fatty acid transport system (pm-FATP, TABP family, CD36; 39, 40) and the impairment in glucose uptake in our results suggest a mechanism that down-regulates FA uptake in islets and circumscribe the FA-dependent deterioration in β-cell function.

Due to the radiation dosage, [^{18}F]FDG and [^{18}F]FTHA studies were conducted in different carefully matched groups. Most participants were females, and this skewed sex distribution may have a modest effect on the interpretation of results. Notably intra-individual variation of pancreatic metabolic rate and blood flow was observed. This, in addition to minor error (5%–10%) associated with the reproducibility of PET data, may have weakened the strength of associations.

In conclusion, this is the first study to assess pancreatic metabolism and β-cell function, in humans. Morbid obesity shifts the preferred pancreatic energy substrate, leading to a predominant usage and accumulation of lipids over glucose. Impairment in pancreatic blood flow may affect β-cell dysfunction in long-standing obesity and insulin resistance. Further studies are warranted to investigate the reversibility of the pancreatic metabolic shift and of the defect in blood flow after weight-loss and the role of splanchnic blood flow as a regulator of intermediary metabolism.

Acknowledgments

The authors thank the staff of the Turku PET Centre and Turku University Central Hospital for performing PET, MRI and CT imaging and laboratory analyses, PhD Andrea Mari for his superb expertise in the area of β-cell modeling and assistance in the interpretation of results, MSc Ville Aalto for his statistical ex-

pertise, MSc Tam Pham for the performance of fat mass analyses, and study nurse Mia Koutu for her assistance. PhD Robert M. Badeau provided the scientific proofreading and language editing of this manuscript.

The results of this study were presented in an oral session (clinical findings) at the 49th European Association for the Study of Diabetes (EASD, Düsseldorf, Germany) annual meeting in Barcelona, 23–27 September 2013, and in a poster session (PET validation) at the 48th EASD annual meeting in Berlin, 1–5 October 2012.

AUTHOR CONTRIBUTIONS: H.H. acquired and researched data, wrote the manuscript, and contributed to the discussion. J.C.H. acquired and researched data, contributed to discussion, and edited the manuscript. M.T., H.K., N.K. and A.R. acquired data, contributed to the discussion, and edited the manuscript. P.S., M.S., and contributed to the discussion, and edited the manuscript. K.M., M.H.-S., and R.P. acquired and researched data, and edited the manuscript. V.S. and V.O. offered technical support, researched data, and contributed to the discussion. P.I. researched data, wrote the manuscript, and contributed to the discussion. P.N. was the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of manuscript.

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Disclosure statement Authors declare that there is no conflict of interest associated with this manuscript.

This work was supported by *Supporting grants* The study was conducted within the Finnish Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic Research and was supported by the Academy of Finland, Turku University Foundation, South-Western Finland Cultural Foundation, Jalmari and Rauha Ahokas Foundation, The Diabetes Research Foundation, Maud Kuistila Memorial Foundation and Turku University Hospital Foundation.

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