Pancreatic fat and β-cell function in overweight/obese children with nonalcoholic fatty liver disease

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Abstract

AIM: To analyze the associations of pancreatic fat with other fat depots and β-cell function in pediatric nonalcoholic fatty liver disease (NAFLD).

METHODS: We examined 158 overweight/obese children and adolescents, 80 with NAFLD [hepatic fat fraction (HFF) ≥5%] and 78 without fatty liver. Visceral adipose tissue (VAT), pancreatic fat fraction (PFF) and HFF were determined by magnetic resonance imaging. Estimates of insulin sensitivity were calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), defined by fasting insulin and fasting glucose and whole-body insulin sensitivity index (WBISI), based on mean values of insulin and glucose obtained from oral glucose tolerance test and the corresponding fasting values. Patients were considered to have prediabetes if they had either: (1) impaired fasting glucose, defined as a fasting glucose level ≥100 mg/dL to < 126 mg/dL; (2) impaired glucose tolerance, defined as a 2 h glucose concentration between ≥140 mg/dL and < 200 mg/dL; or (3) hemoglobin A1c value of ≥5.7% to < 6.5%.

RESULTS: PFF was significantly higher in NAFLD patients compared with subjects without liver involvement. PFF was significantly associated with HFF and VAT, as well as fasting insulin, C peptide, HOMA-IR, and WBISI. The association between PFF and HFF was no longer significant after adjusting for age, gender, Tanner stage, body mass index (BMI)-SD score, and VAT. In multiple regression analysis with...
WBISI or HOMA-IR as the dependent variables, against the covariates age, gender, Tanner stage, BMI-SD score, VAT, PFF, and HFF, the only variable significantly associated with WBISI (standardized coefficient B, -0.398; P = 0.001) as well as HOMA-IR (0.353; P = 0.003) was HFF. Children with prediabetes had higher PFF and HFF than those without. PFF and HFF were significantly associated with prediabetes after adjustment for clinical variables. When all fat depots where included in the same model, only HFF remained significantly associated with prediabetes (OR = 3.38; 95%CI: 1.10-10.4; P = 0.034).

CONCLUSION: In overweight/obese children with NAFLD, pancreatic fat is increased compared with those without liver involvement. However, only liver fat is independently related to prediabetes.

Key words: Nonalcoholic fatty liver disease; Pancreatic fat; Visceral fat; Beta-cell function; Children

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Core tip: Recent studies in children demonstrated that the prevalence of prediabetes increases with increasing hepatic fat content, and that fatty liver plays a central role in the impairment of liver, muscle and adipose insulin sensitivity. Pancreatic fat was identified as a novel obesity-related fat depot, which might contribute to the development of β-cell dysfunction. This study demonstrated that in overweight/obese children with nonalcoholic fatty liver disease (NAFLD), pancreatic fat is increased compared with those without NAFLD. However, only liver fat is independently related to prediabetes. Early intervention during childhood to recognize NAFLD might be critical in averting an unfavorable metabolic phenotype.

INTRODUCTION
Paralleling the worldwide epidemic of childhood obesity, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in children and adolescents. It is now clear that it is not only a risk factor for liver failure and liver carcinoma, but also a strong cardiovascular risk factor closely related to insulin resistance. In particular, recent studies in obese children and adolescents demonstrated that the prevalence of metabolic syndrome (MetS) increases with increasing hepatic fat content, and that fatty liver, independent of visceral and intramyocellular lipid content, plays a central role in the impairment of liver, muscle and adipose insulin sensitivity. Pancreatic fat has been identified as a novel obesity-related fat depot, which might contribute to the development of β-cell dysfunction. In humans, pancreatic fat content is closely associated with increasing body mass index (BMI), visceral fat, insulin resistance, MetS, and hepatic fat content. To date, however, there have been very few studies, especially in children, which addressed the association of pancreatic fat accumulation, as detected by magnetic resonance imaging (MRI), with either obesity or glucose intolerance. Moreover, there is currently a paucity of imaging data on the relationships of pancreatic fat accumulation with liver fat and other fat depots. Recognition of these relationships may be clinically important, because pancreatic fat accumulation might contribute to the development of metabolic disorders typically observed in patients with NAFLD.

In this study we sought to: (1) determine whether MRI-calculated pancreatic fat fraction (PFF) is greater in overweight/obese Caucasian children and adolescents with NAFLD than in those without liver involvement; (2) to assess the relationship between PFF and other fat depots, including liver fat, visceral (VAT) and subcutaneous adipose tissue (SAT); and (3) to explore the association between pancreatic fat accumulation and β-cell dysfunction and increased risk for prediabetes.

MATERIALS AND METHODS

Study subjects
This observational study included 158 overweight (BMI > 85th percentile and < 95th percentile) or obese (BMI ≥ 95th percentile according to age- and gender-specific percentiles of BMI) Caucasian children aged 10-18 years, who were recruited at the Hepatology outpatient Clinic of the Department of Pediatrics, Sapienza University of Rome, Italy. Eighty patients met the criteria for diagnosis of NAFLD (i.e., hepatic fat fraction (HFF) > 5% on MRI). Secondary causes of steatosis, including hepatic virus infections (hepatitis A-E and G, cytomegalovirus, and Epstein-Barr virus), autoimmune hepatitis, metabolic liver disease, α-1-antitrypsin deficiency, cystic fibrosis, Wilson's disease, hemochromatosis, and celiac disease were excluded using appropriate tests. The other 78 participants had normal levels of aminotransferases, HFF < 5%, and no evidence of other causes of chronic liver diseases (see above). The use of hepatotoxic drugs, as well as a history of type 1 or type 2 diabetes, renal disease, and smoking and chronic alcohol intake were exclusion criteria.

All study subjects had a complete physical examination, including weight measurements, standing height, BMI, waist circumference (WC), and systolic
blood pressure (BP) and diastolic BP, as reported in detail elsewhere\(^{21}\). Stage of development was assessed on the basis of breast development in girls and genital development in boys, according to Tanner’s criteria. All subjects were in Tanner stage II - V. The degree of obesity was quantified using Cole’s least mean-square method, which normalizes the skewed distribution of BMI and expresses BMI as standard deviation (SD) score\(^{19}\). They all underwent laboratory tests and phenotyping of abdominal visceral, pancreatic and liver fat by MRI.

The study protocol was reviewed and approved by the Ethics Committee of Policlinico Umberto I Hospital, Rome, Italy (identification number: 2464/24.05.2012). Written informed consent was obtained from the next of kin, caretakers, or guardians on behalf of the children enrolled in this study, in accordance with the principles of the Helsinki Declaration.

**Metabolic studies**

Blood samples were taken from each subject, after an overnight fast, to estimate glucose, insulin, C peptide, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and high-sensitivity C-reactive protein (HSCRP). An oral glucose tolerance test (OGTT) was performed for all obese children with the administration of glucose at 1.75 g/kg body weight (maximum dose 75 g); blood samples were obtained at 0 min and every 30 min thereafter for 120 min for determination of serum glucose and insulin. Estimates of insulin sensitivity were calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), defined by fasting insulin and fasting glucose, and whole-body insulin sensitivity index (WBISI), based on mean values of insulin and glucose obtained from OGTT and the corresponding fasting values\(^{22}\). Overweight/obese youths were considered to have prediabetes if they had either: (1) impaired fasting glucose (IFG), defined as a fasting glucose level ≥ 100 mg/dL to < 126 mg/dL; (2) impaired glucose tolerance (IGT), defined as a 2-h glucose concentration between ≥ 140 mg/dL and < 200 mg/dL; or (3) hemoglobin A1c value of ≥ 5.7 to < 6.5%\(^{23,24}\).

**Abdominal magnetic resonance imaging**

MR techniques are used to assess ectopic fat non-invasively. MR spectroscopy (MRS) is the most accurate non-invasive method\(^{25}\); however, others, including a modified Dixon in-and out-of-phase imaging, correlate well with both MRS and biopsy, with the advantage of assessing both liver and pancreatic fat from the same acquisition\(^{26,30}\). This technique was used to measure liver, as well as pancreatic, fat in this study.

All data were acquired on a 3.0 T MR scanner with a 50 mT/m maximum gradient length and 200 T/m per second maximum slew rate (Discovery MR 750; GE Medical Systems, Milwaukee, WI) using an eight-element body torso-array coil system. All subjects were supine and were carefully instructed to suspend respiration at the end of inspiration and to be consistent in their breath holds. To allow correct positioning, localizing sequences in the coronal, transverse and sagittal planes were performed during a breath hold. HFF and PFF were obtained by using an axial breath-hold low-flip angle T1-weighted two-dimensional triple-echo spoiled gradient-echo sequence\(^{26,30}\), with the following parameters: repetition time (TR) ms/echo time (TE) msec, 150/2.4 [first in-phase (IP1)], 3.69 ms (opposed phase), and 4.8 ms [second IP (IP2)]; flip-angle, 20°; section thickness, 6mm; intersection gap, 1 mm; field of view, 33 cm; and acquisition time, 15 s. Once data were acquired, they were transferred to a GE Advantage Workstation and analyzed using the software FuncTool 9.4.04b (GE Medical Systems, Milwaukee, WI, United States). To correct for T1* decay, the T1* time of each region of interest (ROI) was computed as previously described\(^{31,32}\). Measurement of HFF was performed by drawing three different ROIs (diameter 1-2 cm\(^2\); 2 in the right hepatic lobe and 1 in the left hepatic lobe) at three different sections of the liver (above, at the level of, and below the porta hepatis). These ROIs were carefully drawn to avoid vascular structures, motion or pulsation artifacts. To measure pancreatic fat, 1-2 ROIs of 1 cm\(^2\) in area were placed in the head, body and tail of the pancreas, making sure that the ROIs were surrounded by pancreatic tissue. The head of the pancreas was defined as the area of the pancreas to the anatomical right of the superior mesenteric vein. The body was defined as the anatomical right half of the remaining pancreatic tissue and the tail was defined as the anatomical left half of the remaining pancreatic tissue. The mean of all ROIs in each part of the pancreas was calculated to determine the average fat fraction in the head, body and tail, respectively, while the mean of all ROIs in the entire pancreas determined the overall PFF.

For the quantification of VAT and SAT, a T1-weighted LAVA-flex on axial plane was acquired with the IDEAL imaging and reconstruction method (TR, 4.2 ms; TE, 1.3 ms; flip-angle, 15°; matrix, 320 mm x 192 mm; section thickness, 5 mm, reconstructed 2.5 mm; intersection gap, 0). The fat-only dataset were transferred to personal computers for analysis, using a commercially available software (Slice-O-Matic; Tomovision Inc., Montreal, Canada), the procedures for which are described elsewhere\(^{31,34}\).

**Biochemical analyses**

All analyses were conducted by COBAS 6000 (Roche Diagnostics). Insulin and C peptide concentrations were measured on a Cobas e 601 module (Electrochemiluminescence Technology, Roche Diagnostics), while the remaining analytes were analyzed on a Cobas e 501
with respect to age, gender and BMI-SD score. The distribution in Tanner stage between the two groups was similar (Tanner II-V). Children with NAFLD had higher WC, systolic BP, VAT, as well as higher triglycerides, AST, ALT, GGT, and HSCRP, than those without liver involvement. Fasting insulin and C peptide levels, 2-h insulin concentrations, HOMA-IR, and WBISI differed significantly between the two groups. No differences were observed in fasting glucose concentrations, 2-h glucose levels and HbA1c.

The median PFF was significantly higher in NAFLD patients compared with subjects without liver involvement: 2.28 (IQR: 1.48-4.07) vs 1.77 (1.20-2.47); P < 0.05. The median fat content did not vary significantly between the head, body and tail of the pancreas, when analyzing the whole study population, as well as the two groups separately (data not shown).

### Results of Separate Logistic Regression

In univariate analysis, PFF was significantly associated with HFF (standardized \( \beta \) coefficient, 0.269; \( P = 0.016 \)), and VAT (0.250; \( P = 0.029 \)), while no statistically significant relationship was found between PFF and BMI-SD score (0.179; \( P = 0.11 \)), as well as between PFF and SAT (0.143; \( P = 0.22 \)). The association between PFF and HFF, however, was no longer significant after adjusting for age, gender, Tanner stage, BMI-SD score, and VAT (0.185; \( P > 0.05 \)).

We also found that PFF was significantly associated with fasting insulin (standardized \( \beta \) coefficient, 0.217; \( P = 0.045 \)), C peptide (0.241; \( P = 0.033 \)), HOMA-IR (0.266; \( P = 0.018 \)), WBISI (-0.269; \( P = 0.02 \)), and HSCRP (0.259; \( P = 0.021 \)). No significant association was observed between PFF and total cholesterol, HDL-C and triglycerides. To further analyze the role of PFF and other fat compartments in determining \( \beta \)-cell function, we performed multiple linear regression analysis with WBISI or HOMA-IR as the dependent variable, against the covariates age, gender, Tanner stage, BMI-SD score, and VAT. PFF was the only variable significantly associated with WBISI (standardized \( \beta \) coefficient, -0.398; \( P = 0.001 \)) as well as HOMA-IR (0.353; \( P = 0.003 \)).

### Prediabetes

We identified 18 children (14 with NAFLD, and four without NAFLD) as being prediabetic. Seven of the 18 prediabetic children had IGT; one patient had IFG; four patients had HbA1c ≥ 5.7; two patients had IGT + IFG; two patients had IFG + HbA1c ≥ 5.7; and the remaining two children had IGT+ IFG + HbA1c ≥ 5.7. Patients with prediabetes were older and had higher WC, HFF and PFF (Table 3) than those without prediabetes. Table 4 shows the effects of ectopic fat accumulation on the risk of prediabetes, based on the results of separate logistic regression. PFF and HFF were significantly associated with prediabetes after

### Statistical analysis

Statistical analyses were performed using the SPSS package (version 22.0), SPSS Inc., Chicago, IL, United States, were reviewed by Professor JF Osborn, Department of Infectious Diseases and Public Health Sciences, Sapienza University of Rome, Italy. Data are reported as means and standard deviations for normally distributed variables, or as median and interquartile range (IQR) for non-normally distributed variables. Differences between the two study groups were evaluated by a t-test or Mann-Whitney U-test, as appropriate. Proportions were compared by the \( \chi^2 \) test.

Pearson’s correlation and linear regression coefficients were used to examine the relationship between variables. Multivariate linear regression analysis was performed to investigate the association of PFF with WBISI, as well as HOMA-IR, after adjustment for age, gender, Tanner stage, BMI-SD score, VAT and HFF. To determine the primary fat depot predictors of prediabetes, multiple logistic regression analysis was employed using age, gender, Tanner stage, BMI-SD score, VAT, HFF and PFF as independent variables. A \( P \) value of less than 0.05 was considered statistically significant.

### RESULTS

#### Clinical, fat distribution, and metabolic characteristics of the two groups

Clinical and metabolic characteristics of participants by NAFLD status are shown in Tables 1 and 2, respectively. As expected from the study design, HFF values were significantly different between the two groups. Patients with and without NAFLD did not differ significantly

#### Table 1 Clinical characteristics of overweight/obese children with and without nonalcoholic fatty liver disease

| Characteristics                  | NAFLD (n = 80) | No NAFLD (n = 78) | \( P \) value |
|----------------------------------|----------------|-------------------|--------------|
| Age, yr                          | 12.0 (2.3)     | 12.0 (2.2)        | 0.91         |
| Male gender, n (%)               | 52 (65.0)      | 47 (60.2)         | 0.44         |
| BMI-SD score                     | 2.0 (0.41)     | 1.9 (0.37)        | 0.20         |
| Waist circumference, cm          | 94 (11)        | 89 (11)           | 0.013        |
| Systolic blood pressure, mmHg    | 115 (11)       | 110 (14)          | 0.025        |
| Diastolic blood pressure, mmHg   | 67 (9)         | 65 (9)            | 0.18         |
| Abdominal fat                    |                |                   |              |
| Visceral adipose tissue, cm²     | 392 (297-549)  | 299 (226-450)     | 0.002        |
| Subcutaneous adipose tissue, cm² | 1656 (999-1844)| 1369 (1101-2031) | 0.11         |
| Hepatic fat fraction, %          | 13.0 (7.2-24.3)| 1.3 (1.0-2.4)     | < 0.0001     |
| Pancreatic fat fraction, %       | 2.28 (1.48-4.07)| 1.77 (2.0-2.47)  | 0.035        |

Results are expressed as n (%), mean ± SD, or median (interquartile range). NAFLD: Nonalcoholic fatty liver disease; BMI-SD score: Body mass index-standard deviation score.

Clinical chemistry module (Photometric Technology), according to the instructions of the manufacturer.


**Table 2** Metabolic variables of overweight/obese children with and without nonalcoholic fatty liver disease

|                      | NAFLD (n = 80) | No NAFLD (n = 78) | P value |
|----------------------|---------------|-------------------|---------|
| Triglycerides, mg/dL | 101 (60)      | 84 (43)           | 0.045   |
| Total cholesterol, mg/dL | 152 (32)     | 158 (32)          | 0.220   |
| HDL-C, mg/dL         | 46 (14)       | 48 (13)           | 0.430   |
| Aspartate aminotransferase, U/L | 24 (21-30) | 22 (19-25)         | 0.007   |
| Alanine aminotransferase, U/L | 20 (14-44) | 17 (14-23)         | <0.0001 |
| γ-glutamyl transferase, U/L | 17 (13-22) | 13 (10-16)         | <0.0001 |
| Fasting glucose, mg/dL | 84 (8)        | 82 (5)            | 0.090   |
| 2-h glucose, mg/dL   | 94 (24)       | 90 (15)           | 0.310   |
| Fasting insulin, mU/mL | 19 (13)      | 13 (8)            | 0.001   |
| 2-h insulin, mU/mL   | 71 (67)       | 39 (30)           | <0.0001 |
| Fasting C peptide, pmoL/L | 1009 (358) | 769 (244)          | <0.0001 |
| HOMA-IR values       | 3.8 (2.7)     | 2.7 (1.7)         | 0.003   |
| WBFI                 | 4.45 (2.95)   | 6.45 (3.69)       | <0.0001 |
| HbA1c, %             | 5.2 (0.3)     | 5.1 (0.4)         | 0.750   |
| HSCRP, μg/L          | 2200 (900-3950) | 1300 (600-3150) | 0.002   |

HDLC: High-density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; HbA1c: Hemoglobin A1c; HSCRP: High-sensitivity C-reactive protein; NAFLD: Nonalcoholic fatty liver disease; WBFI: Whole-body insulin sensitivity index.

**Table 3** Comparison between children with and without prediabetes

|                      | Prediabetes (n = 18) | No prediabetes (n = 140) | P value |
|----------------------|----------------------|--------------------------|---------|
| Age, yr              | 13.4 (2.4)           | 11.8 (2.3)               | 0.009   |
| Male gender, n (%)   | 11 (61.1)            | 88 (62.8)                | 1.000   |
| BMI-SD score         | 2.07 (0.39)          | 1.91 (0.40)              | 0.130   |
| Waist circumference, cm | 102 (12)         | 90 (11)                  | <0.0001 |
| Visceral adipose tissue, cm² | 408 (300-602) | 316 (265-486)           | 0.066   |
| Subcutaneous adipose tissue, cm² | 1850 (1060-3017) | 1548 (1106-1969)       | 0.190   |
| Hepatic fat fraction | 18.0 (4.2-33.3)      | 4.6 (1.0-12.7)           | 0.024   |
| Pancreatic fat fraction | 3.60 (1.70-5.50)   | 1.90 (1.30-3.10)         | 0.045   |

BMI-SD score: Body mass index- standard deviation score.

**Table 4** Separate logistic regression analysis for fat depots associated with prediabetes

|                      | OR1 | 95%CI                  | P value |
|----------------------|-----|------------------------|---------|
| Visceral adipose tissue | 2.49 | 0.88-9.17              | 0.170   |
| Subcutaneous adipose tissue | 1.25 | 0.57-14.5              | 0.710   |
| Hepatic fat fraction | 1.90 | 1.16-3.10              | 0.010   |
| Pancreatic fat fraction | 3.50 | 1.03-11.9              | 0.045   |

1Adjusted for age, gender, Tanner stage, and body mass index- standard deviation score.

Fat accumulation in the liver and pancreas are in agreement with previous studies performed in adults and children. Using MRI to evaluate PFF, Rossi et al. evaluated the associations between pancreatic fat accumulation and other fat depots, including liver, SAT, and VAT, in a small sample of obese and lean adult individuals, and found that subjects with NAFLD had an approximately 2- to 3-fold increase in pancreatic fat accumulation compared with those without hepatic steatosis. However, when adjusted for age, gender, and VAT, the associations between pancreatic fat accumulation and liver fat content were no longer significant. In a study involving a cohort of 42 Caucasian obese adult subjects, Targher et al. showed that pancreatic fat accumulation, as evaluated by MRI, was positively associated with liver fat content and visceral fat, and that subjects with NAFLD had approximately a 2- to 3-fold increase in pancreatic fat compared with those without hepatic steatosis. However, when adjusted for age, gender, and VAT, the associations between pancreatic fat accumulation and liver fat content were no longer significant. An autopsy-based study of 80 patients showed a significant, positive association between the intrapancreatic fat content and the histological severity of NAFLD. In particular, significant relations were found between interlobular as well as total pancreatic fat and hepatic macrovescicular fat and NAFLD activity score. However, while correction for age or gender did not affect statistically the relation between pancreatic and hepatic fats, after adjustment for BMI, this association became not significant. In a cohort of 43 well-characterized adult patients with biopsy-proven NAFLD, Patel et al. demonstrated that MRI-determined pancreatic steatosis is common in patients with NAFLD, and that pancreatic fat content positively correlates with liver histology-determined steatosis grade. PFF did not significantly correlate with age, BMI or diabetes status. Finally, in a cohort of 50 children and adolescents, Cohen et al. demonstrated that MRI-estimated PFF correlates with HFF and percent body fat. However, after adjustment for gender, ethnicity and puberty, PFF was associated only with percent body fat.

It has been demonstrated convincingly that both obesity and NAFLD are closely associated with impaired glucose metabolism. The pathophysiological adjustment for age, gender, Tanner stage, and BMI-SD score. However, when all fat depots (i.e., VAT, SAT, HFF, and PFF) where included in the same model along with age, gender, Tanner stage and BMI-SD score, only HFF remained significantly associated with prediabetes [OR= 3.38 (95%CI: 1.10-10.4); P = 0.034].

**DISCUSSION**

This study showed that overweight/obese children with NAFLD have higher pancreatic fat accumulation than obese children without NAFLD, and that pancreatic fat is significantly associated with HFF, and VAT. However, the association between pancreatic and hepatic fat content was no longer significant after adjusting for age, gender, Tanner stage, BMI-SD score, and VAT. Our findings on the association between ectopic fat accumulation in the liver and pancreas are in agreement with previous studies performed in adults and children. Using MRI to evaluate PFF, Rossi et al. evaluated the associations between pancreatic fat accumulation and other fat depots, including liver, SAT, and VAT, in a small sample of obese and lean adult individuals, and found that subjects with NAFLD had an approximately 2- to 3-fold increase in pancreatic fat accumulation compared with those without hepatic steatosis. However, when adjusted for age, gender, and VAT, the associations between pancreatic fat accumulation and liver fat content were no longer significant. In a study involving a cohort of 42 Caucasian obese adult subjects, Targher et al. showed that pancreatic fat accumulation, as evaluated by MRI, was positively associated with liver fat content and visceral fat, and that subjects with NAFLD had approximately a 2- to 3-fold increase in pancreatic fat compared with those without hepatic steatosis. However, when adjusted for age, gender, and VAT, the associations between pancreatic fat accumulation and liver fat content were no longer significant. An autopsy-based study of 80 patients showed a significant, positive association between the intrapancreatic fat content and the histological severity of NAFLD. In particular, significant relations were found between interlobular as well as total pancreatic fat and hepatic macrovescicular fat and NAFLD activity score. However, while correction for age or gender did not affect statistically the relation between pancreatic and hepatic fats, after adjustment for BMI, this association became not significant. In a cohort of 43 well-characterized adult patients with biopsy-proven NAFLD, Patel et al. demonstrated that MRI-determined pancreatic steatosis is common in patients with NAFLD, and that pancreatic fat content positively correlates with liver histology-determined steatosis grade. PFF did not significantly correlate with age, BMI or diabetes status. Finally, in a cohort of 50 children and adolescents, Cohen et al. demonstrated that MRI-estimated PFF correlates with HFF and percent body fat. However, after adjustment for gender, ethnicity and puberty, PFF was associated only with percent body fat.

It has been demonstrated convincingly that both obesity and NAFLD are closely associated with impaired glucose metabolism. The pathophysiological...
mechanisms and clinical importance of fatty pancreas are, however, less clear. Previous studies have suggested that fatty infiltration of the pancreas may contribute to a loss of \( \beta \)-cell mass and function\[^{39,40} \], which may lead to the development of diabetes\[^{41} \].

In obese Zucker diabetic fatty rats, the triacylglycerol content of islets in prediabetic rats increased significantly and preceded the development of diabetes\[^{42} \]. However, in human studies, the data are inconsistent. Tushuizen et al\[^{43} \] demonstrated that in Caucasian men, pancreatic fat, as measured by MRS, was negatively associated with \( \beta \)-cell function parameters, including the insulinogenic index adjusted for insulin resistance, early glucose-stimulated insulin secretion, \( \beta \)-cell glucose sensitivity and rate sensitivity. However, these associations were significantly affected by the diabetic state, in that a significant association between pancreatic fat and \( \beta \)-cell dysfunction was only present in the nondiabetic group, suggesting that once diabetes occurs, factors additional to pancreatic fat may lead to further \( \beta \)-cell functional decline\[^{43} \].

Using MRI to evaluate PFF, Heni et al\[^{44} \] reported that among Caucasian adults with increased risk for type 2 diabetes, there was an inverse relationship between PFF and insulin secretion in those with IGT and/or IFG, but not in normoglycemic participants. The authors suggested that PFF may be a crucial pathogenic factor only when there is an altered glucose homeostasis and \( \beta \)-cell dysfunction. Lé et al\[^{45} \] found no significant correlations between PFF, as measured by MRI, and markers of insulin sensitivity or \( \beta \)-cell function in African American and Hispanics adolescents and young adults who were normoglycemic, despite being overweight.

van der Zijl et al\[^{46} \], using the gold standard hyperglycemic clamp, demonstrated that impairments in \( \beta \)-cell function in overweight Caucasian adults with IFG and IFG/IGT were accompanied by pancreatic fat accumulation, as determined by MRS. However, a direct relation between pancreatic fat accumulation and \( \beta \)-cell function could not be established. Finally, in a recent study using liver and pancreas sonography, Ou et al\[^{47} \] found in a large Chinese adult cohort that both fatty pancreas and NAFLD were associated with newly diagnosed diabetes, independent of age, gender, adiposity, and other cardiometabolic risk factors. In addition, fatty pancreas was independently related to prediabetes in male subjects, but not in females. It is likely that these discrepancies with regard to the relationship between pancreatic fat and \( \beta \)-cell dysfunction may reflect differences in the methods by which fatty infiltration of the pancreas was estimated, ethnicity, demographic features, and the glycemic status of the study subjects.

In the pediatric population reported by Cohen et al\[^{38} \], pancreatic fat accumulation was found in association with WBISI and \( \beta \)-cell function. When VAT, HFF, PFF were analyzed in the same regression model, pancreatic fat associations with WBISI tended to be significant, while the association with insulin secretion and \( \beta \)-cell function were not significant. In contrast, hepatic fat associations with the metabolic markers remained significant and were the strongest. Likewise, in our study PFF was correlated with outcome related to glucose or insulin, including WBISI, and HOMA-IR; however, in multiple linear regression analysis, HFF was the only variable significantly associated with these metabolic variables. Thus, our findings provide further evidence for a pathogenic role of hepatic fat in the development of glucometabolic disorders, because liver fat, and not fatty pancreas, was significantly associated with WBISI, and it was the only fat compartment associated with prediabetes.

In a group of overweight and obese African American and Latino adolescents, Toledo-Corral et al\[^{71} \] demonstrated that adolescents with prediabetes had increased ectopic fat storage in the liver and pancreas compared to those without prediabetes, independently of overall and visceral adiposity. They also found ethnic differences in ectopic fat distribution related to prediabetes status, such that pancreatic fat independently predicted prediabetes in African Americans patients, whereas in Latino adolescents, hepatic fat was the only predictor. Thus, ethnic differences in the deposition of ectopic fat in children and adolescents with prediabetes may differ, with pancreatic fat in African Americans, vs hepatic fat in Latino adolescents as well as in Caucasian children and adolescents, being associated with diabetes risk.

This study has a few potential limitations. First, because of the cross-sectional design, we could not establish temporal and causal relationships between NAFLD, pancreatic steatosis and visceral obesity. Second, there is a lack of direct measures of insulin secretion and action. Third, the small number of children with prediabetes may have limited the interpretation of the results, but could provide indications for further research.

In summary, this study demonstrated that in overweight/obese children and adolescents with NAFLD, pancreatic fat accumulation is increased compared with those without liver involvement. However, only the liver fat depot was independently related to prediabetes. Early intervention during childhood to recognize NAFLD might be a crucial step to avert an unfavorable metabolic phenotype.

**COMMENTS**

**Background**

Recent studies in children demonstrated that the prevalence of prediabetes increases with increasing hepatic fat content, and that fatty liver plays a central role in the impairment of liver, muscle, and adipose insulin sensitivity. Pancreatic fat has been identified as a novel obesity-related fat depot, which may contribute to the development of \( \beta \)-cell dysfunction.

**Research frontiers**

A better understanding of the relationship between pancreatic fat accumulation and liver fat may be clinically important because pancreatic fat might contribute to the development of metabolic disorders typically observed in patients with nonalcoholic fatty liver disease (NAFLD).
Innovations and breakthroughs
Pancreatic fat has been identified as a novel obesity-related fat depot, which may contribute to the development of β-cell dysfunction. In humans, pancreatic fat content is closely associated with increasing body mass index, visceral fat, insulin resistance, metabolic syndrome and hepatic fat content. To date, however, there have been few studies, especially in children, which addressed the association of pancreatic fat accumulation with either obesity or glucose intolerance. Moreover, there is currently a paucity of imaging data on the relationships of pancreatic fat with liver fat and other fat depots. This study demonstrated that in overweight/obese children with NAFLD, pancreatic fat is increased compared with those without NAFLD. However, only liver fat is independently related to prediabetes.

Applications
Early intervention during childhood to recognize NAFLD might be a crucial step in averting an unfavorable metabolic phenotype.

Terminology
NAFLD comprises a disease spectrum, ranging from simple fatty liver to nonalcoholic steatohepatitis, with varying degrees of inflammation and fibrosis, progressing to end-stage liver disease with cirrhosis and hepatocellular carcinoma. Prediabetes is an intermediate condition, identifying those with elevated blood sugars not yet in the diabetic range, which are at high risk of developing diabetes.

Peer-review
This well-designed cross-sectional, observational study of Pacifico et al aims to examine the associations of pancreatic fat with other fat depots, all evaluated by magnetic resonance imaging, and biochemical markers of β-cell function in 158 overweight/obese children (age: 10-18 years).

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