Original Article

Pancreatic Lipomatosis Is a Structural Marker in Nondiabetic Children With Mutations in Carboxyl-Ester Lipase

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Both pancreatic volume reduction and lipomatosis have been observed in subjects with diabetes. The underlying molecular and pathological mechanisms are, however, poorly known, and it has been speculated that both features are secondary to diabetes. We have recently described pancreatic atrophy and lipomatosis in diabetic subjects of two Norwegian families with a novel syndrome of diabetes and exocrine pancreatic dysfunction caused by heterozygous carboxyl-ester lipase (CEL) mutations. To explore the early pathological events in this syndrome, we performed radiological examinations of the pancreas in nondiabetic mutation carriers with signs of exocrine dysfunction. In a case series study at a tertiary hospital, we evaluated 11 nondiabetic and mutation-positive children with fecal elastase deficiency and 11 age- and sex-matched control subjects using ultrasound and magnetic resonance imaging (MRI) to estimate pancreatic fat content. The pancreata of nondiabetic mutation carriers exhibited increased reflectivity on ultrasound and had MRI findings indicative of lipomatosis. Apparently, carriers of heterozygous CEL mutations accumulate fat in their pancreas before the anticipated development of diabetes. Our findings suggest that lipomatosis of the pancreas reflects early events involved in the pathogenesis of diabetes and exocrine pancreatic dysfunction syndrome. Diabetes 56: 444–449, 2007

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MRI, magnetic resonance imaging; VIBE, volume interpolated breath-hold examination.

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suitable method to demonstrate lipomatosis in various tissues such as the liver and pancreas (16–18).

**RESEARCH DESIGN AND METHODS**

The regional committee for research ethics approved the study. We obtained written informed consent from all subjects or their parents. The non-diabetic mutation carriers were from two previously described families with CEL mutations (14) of Northern European descent and were recruited from the Norwegian Registry of Maturity-Onset Diabetes of the Young. To examine whether lipomatosis of the pancreas could be a general early structural marker of disease, we investigated three non-diabetic mutation carriers, who were <15 years of age. We used two control groups, one for the radiological studies and one for the glucose studies. Tanner staging 1–5 for puberty was used (P: pubes hair quantity, B: breast size).

For the radiological studies, 13 mutation carriers <20 years of age were identified. Two subjects were <5 years of age and considered too young for nonseparated MREI investigation. Of the 11 subjects available for the current studies, a 10-year-old male subject was Tanner P5. Three girls aged 10, 11, and 12 years of age were P1 and B2 with no menarche. All of the other subjects were prepubertal. The control group consisted of 11 healthy, sex- and age-matched subjects with no history of diabetes or pancreatic disease who were selected among relatives of the staff at our hospital. One 14-year-old boy was Tanner P3. One girl aged 12 years of age was P1 and B2 with no menarche. All of the other subjects were prepubertal. Because pancreatic size is influenced by the body surface area, we also calculated the pancreatic volume index, where we divided the estimated pancreatic volume by the body surface area (8).

For the glucose studies, we extracted control subjects from a previously published group (14) consisting of 11 (7 male, 4 female) adult family control subjects (without CEL single-base deletions) because we considered it difficult to perform intravenous glucose tolerance tests in the control group in addition to the radiological studies. We have previously described the acute insulin response to intravenous glucose and the glucose levels from a standard oral glucose tolerance test in a combined group of young and adult mutation carriers compared with family control subjects (14). We now describe the results for the mutation-carrying children that undertook the radiological investigations, using area under the curve estimation of C-peptide and insulin levels. Of the 11 mutation-carrying children, 8 had volunteered to measure glucose levels during oral glucose tolerance test and to measure insulin secretion by intravenous glucose tolerance test: 0.5 g/kg body wt, maximum 35 g, of glucose was given intravenously at time 0. Blood samples for the insulin measurement were drawn at −10, −5, 0, 1, 3, 5, and 10 min. The incremental trapezoidal area during the first 10 min of the test was calculated as a measure of first-phase insulin secretion (estimated as the area under the curve for insulin levels above the basal insulin level), and the values were compared with those of the family control subjects (14).

**Radiological studies.** For the ultrasound studies, abdominal ultrasound was performed using a curved array transducer (9–4 MHz, Philips iU22 Ultrasound System; Seattle, WA). One radiologist (I.S.H.) performed all ultrasound examinations. It was not possible for the radiologist to be blinded to the patient/control status because the subjects were investigated as a part of the clinical procedure. The patients were scanned in a supine position. Maximum anteroposterior and craniocaudal dimensions of the caput, corpus, and cauda of the pancreas were measured. Pancreatic texture and reflectivity relative to that of the liver were registered.

The magnetic resonance studies were performed on a 1.5-T Siemens Magnetom Symphony with a 20 mTm gradient system running Numaris 3.5 (Erlangen, Germany). For clinical and anatomical evaluation of the pancreas, we used a standard abdominal protocol, which can be found in online Appendix 1 (available at http://dx.doi.org/10.2337/db06-0859). Pancreatic volume was estimated using three-dimensional, fat-saturated, T1-weighted images (volume interpolated breath-hold examination [VIBE]) on which the same radiologist (I.S.H.) traced the contour of the pancreas on every slice, considering each encircled area to represent a volume of 3.5-mm thickness. The areas were added together to estimate the pancreatic volume. Signal intensities of the caput and corpus of pancreas relative to that of the liver on VIBE were measured in operator-defined regions of interest in an effort to avoid confounding anatomy. To quantify fat and water content of the pancreas, a three-point Dixon method (19,20) was applied (online Appendix 1). The signal intensities of the pancreas were measured in different regions of interest on the fat and water images. Fat-to-water ratios were calculated on a pixel-by-pixel basis by dividing the calculated signal on the fat image by the water image. For each patient, signal intensities were measured in three regions of interest in both the corpus and caput of the pancreas. The median value from each region was used for statistical calculations. The image analyses were performed on an Advantage Windows workstation from Gen-
eral Electric (Milwaukee, WI) and the Windows-based program nICE from NordicIceMedical (Bergen, Norway).

Definitions and statistical methods. We have used the term lipomatosis to describe any fat in the pancreatic parenchyma revealed by radiological investigations because these methods cannot differentiate between adipocytes within, or replacing, the exocrine tissue and intracellular fat (steatosis) in the pancreatic exocrine cells. Differences between case and control subjects in demographic characteristics; physiological values for glucose, insulin, and insulin C-peptide; and pancreatic dimensions and signal intensity ratios were analyzed using Student’s *t* test of independent groups assuming nonequal variance. A significance level of 5% was chosen. All data were analyzed using Stata 8.0 statistical software (Stata, College Station, TX).

RESULTS

Clinical characteristics of the subjects. General demographic and clinical characteristics for the 11 case and 11 radiological control subjects are given in Table 1 with *z* scores for BMI (21) and weight (22). All 11 mutation carriers had fecal elastase deficiency, indicating exocrine pancreatic dysfunction. Among the mutation carriers, two subjects had impaired glucose tolerance by World Health Organization criteria, and six subjects had normal glucose tolerance. For three presumably healthy mutation carriers, information on glucose tolerance was lacking. Levels of A1C and fasting glucose and C-peptide were within the nondiabetic range (Table 1). The results of intravenous and oral glucose tolerance tests are shown in Fig. 1.

Ultrasound and MRI studies. The pancreas in the mutation carriers had increased reflectivity and an inhomogeneous texture on ultrasound (Fig. 2). Neither ultrasound nor MRI revealed any signs of pseudocysts, peripancreatic edema, calcifications, or dilated ducts in the pancreata of the mutation carriers or the control subjects (Fig. 2 and Fig. 3A and B). Abdominal ultrasound showed decreased

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**FIG. 1.** Physiological studies in CEL mutation carriers compared with family control subjects. A: A significantly reduced first-phase insulin response during intravenous glucose tolerance testing, based on the estimation of the area under the curve (AUC) for insulin levels above the basal insulin level. B: The corresponding area under the curve values for C-peptides during intravenous glucose tolerance test. C: The fasting (0 h) and 120-min glucose levels (2 h) during oral glucose tolerance testing. *Significant difference (P < 0.05).*

**FIG. 2.** Abdominal ultrasound images of the pancreatic region in representative subjects. A and B: The pancreas of a control subject (A) and a mutation carrier (B) with inhomogeneous texture and increased reflectivity relative to the liver. The arrows indicate the boundaries of the pancreas. L, liver.
anteroposterior and craniocaudal diameters of the pancreas in mutation carriers (Table 2). The pancreatic volume estimated on MRI was significantly smaller among the mutation carriers \( (P = 0.021) \), but the pancreatic volume index (i.e., pancreatic volume adjusted for body surface area) was not significantly different from control subjects \( (P = 0.19) \) (Table 2). Using standard T1-weighted, fat-saturated MRI images (VIBE), we found that the pancreas was hypointense relative to liver in mutation carriers but slightly hyperintense or isointense relative to liver in the control subjects (Table 2 and Fig. 3A and B). Fat-to-water ratios (Dixon series) were significantly altered \( (P < 0.0001) \) in the mutation carriers, indicating lipomatosis of the pancreas (Table 2, Fig. 3C, D, and Fig. 4).

**DISCUSSION**

By radiological examination, we found structural changes suggesting that there is accumulation of fat in the pancreas in all examined nondiabetic children with mutations in the CEL gene. This extends our previous observation of lipomatosis in CEL-mediated disease to all examined mutation carriers >5 years old. Ultrasound and MRI of the pancreas seem to provide a noninvasive diagnostic option assessing pancreatic structural changes in CEL disease. This may be relevant for the phenotypic ascertainment of CEL mutation carriers in genetic studies and for the investigation of subjects with coexisting causes of fecal elastase deficiency (such as celiac disease).

The abdominal ultrasound demonstrated increased pancreatic reflectivity, indicating fibrosis or lipomatosis of the pancreatic parenchyma (23). The MRI-based Dixon method strongly indicated that lipomatosis, indeed, was the cause of the hyperreflectivity demonstrated on ultrasound. The three-point Dixon method has been shown to be highly reproducible and accurate for the estimation of true fat volume ratios (19,24). The method is useful to quantify fat in lean tissues such as liver (17), skeletal muscle, and pancreas (18). Further independent support of lipomatosis comes from the T1-weighted, fat-saturated MRI images (VIBE), which demonstrated altered signal intensities, with a hypointense pancreatic gland relative to liver in mutation carriers. On fat-saturated images, fatty tissue will normally appear hypointense, and a plausible explanation of the hypointensity of the pancreas is in-
TABLE 2
Ultrasound and magnetic resonance findings in the pancreas of nondiabetic mutation carriers and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nondiabetic CEL mutation carriers</th>
<th>Radiology study control subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial anteroposterior axis (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput</td>
<td>1.58 ± 0.26</td>
<td>2.21 ± 0.64</td>
<td>0.0009</td>
</tr>
<tr>
<td>Corpus</td>
<td>0.72 ± 0.24</td>
<td>1.24 ± 0.40</td>
<td>0.0018</td>
</tr>
<tr>
<td>Cauda</td>
<td>1.14 ± 0.33</td>
<td>1.45 ± 0.50</td>
<td>0.1246</td>
</tr>
<tr>
<td>Sagital craniocaudal (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput</td>
<td>2.92 ± 0.54</td>
<td>4.11 ± 1.13</td>
<td>0.0076</td>
</tr>
<tr>
<td>Corpus</td>
<td>2.38 ± 0.49</td>
<td>2.94 ± 0.21</td>
<td>0.0082</td>
</tr>
<tr>
<td>Cauda</td>
<td>2.20 ± 0.48</td>
<td>3.07 ± 0.63</td>
<td>0.0125</td>
</tr>
<tr>
<td>Subjects with hyperechogeneity compared with liver (n)</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Magnetic resonance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic volume (ml)</td>
<td>31 ± 13</td>
<td>43 ± 9</td>
<td>0.021</td>
</tr>
<tr>
<td>Pancreatic volume index (ml/m²)</td>
<td>31 ± 11</td>
<td>36 ± 6</td>
<td>0.19</td>
</tr>
<tr>
<td>Signal intensity ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas-to-liver ratio (fat-saturated, T1-weighted images)</td>
<td>0.62 ± 0.07</td>
<td>1.12 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat-to-water ratio (caput) (Dixon images)</td>
<td>0.80 ± 0.23</td>
<td>0.17 ± 0.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat-to-water ratio (corpus) (Dixon images)</td>
<td>0.92 ± 0.15</td>
<td>0.15 ± 0.08</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SD.

Increased fat content. Furthermore, reports by others have shown that pancreatic signal intensity less than that of the liver on T1-weighted, fat-saturated MRI images is highly correlated to pancreatic disease (25), supporting the view that this is a pathological finding in our patients.

Both ultrasound and MRI demonstrated a significant reduction of pancreatic size in mutation carriers. We found, however, that pancreatic volumes corrected for body surface area were not significantly altered in the mutation carriers. There was a tendency, however, toward reduced pancreatic volume indexes, and a better-powered study is necessary to investigate also whether pancreatic size reduction is an early event in the disease process and not only secondary to diabetes. By computerized tomography we have shown a significant reduction in the pancreatic volume indexes of adult diabetic mutation carriers, but not in four nondiabetic mutation carriers (14).

Pancreatic lipomatosis seems to precede the development of diabetes in subjects with a clearly defined molecular defect and appears to be an early structural marker of pancreatic exocrine disease in CEL mutation carriers. The subjects were not overweight, which excludes obesity as the cause of pancreatic lipomatosis (9). There was reduced first-phase insulin secretion in the nondiabetic children with CEL mutations, although insulin sensitivity was not formally assessed using hyperinsulinemic-euglycemic clamping. The control subjects were not obese but older, which could have explained an increased insulin secretion (26).

Notably, pancreatic lipomatosis has also been observed in other monogenic conditions with primary pancreatic exocrine dysfunction, namely cystic fibrosis (27), Shwachman-Diamond syndrome (27), and Johanson-Blizzard syndrome (27). Cystic fibrosis is associated with diabetes (28,29), and there are case reports documenting diabetes in Shwachman-Diamond syndrome (30) and Johanson-Blizzard syndrome (31) as well, providing further evidence for a potential role of lipomatosis in β-cell dysfunction. The lipomatosis has been described as adipose tissue mixed with the exocrine tissue for both cystic fibrosis (32) and Shwachman-Diamond syndrome (33) as well as for common forms of diabetes (9,10). Thus, it is possible that the lipomatosis in CEL mutation carriers represents a replacement of exocrine tissue with adipocytes and not intracellular acinar steatosis. How such ectopic adipose tissue may affect the β-cells and their function are important questions that must be answered in the future.

In conclusion, we found pancreatic lipomatosis to be a structural marker in nondiabetic subjects with mutations in the CEL gene. The lipomatosis of the pancreatic parenchyma in CEL mutation carriers could reflect a process in the early stages of the novel diabetes and exocrine pancreatic dysfunction syndrome MODY8.

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