Decreased Transcription of ChREBP-α/β Isoforms in Abdominal Subcutaneous Adipose Tissue of Obese Adolescents With Prediabetes or Early Type 2 Diabetes Associations With Insulin Resistance and Hyperglycemia

Romy Kursawe,† Sonia Caprio,† Cosimo Giannini,† Deepak Narayan,‡ Aiping Lin,§ Ebe D’Adamo,† Melissa Shaw,† Bridget Pierpont,† Samuel W. Cushman,¶ and Gerald I. Shulman∥∥∥

Insulin resistance associated with altered fat partitioning in liver and adipose tissues is a prediabetic condition in obese adolescents. We investigated interactions between glucose tolerance, insulin sensitivity, and the expression of lipogenic genes in abdominal subcutaneous adipose and liver tissue in 53 obese adolescents. Based on their 2-h glucose tests they were stratified in the following groups: group 1, 2-h glucose level <120 mg/dL; group 2, 2-h glucose level between 120 and 140 mg/dL; and group 3, 2-h glucose level >140 mg/dL. Liver and adipose tissue insulin sensitivity were greater in group 1 than in group 2 and group 3, and muscle insulin sensitivity progressively decreased from group 1 to group 3. The expression of the carbohydrate-responsive element-binding protein (ChREBP) was decreased in adipose tissue but increased in the liver (eight subjects) in adolescents with impaired glucose tolerance or type 2 diabetes. The expression of adipose ChREBPα and ChREBPβ was inversely related to 2-h glucose level and positively correlated to insulin sensitivity. Improvement of glucose tolerance in four subjects was associated with an increase of ChREBP/GLUT4 expression in the adipose tissue. In conclusion, early in the development of prediabetes/type 2 diabetes in youth, ChREBPβ expression in adipose tissue predicts insulin resistance and, therefore, might play a role in the regulation of glucose tolerance.

Emerging evidence suggests that the ability to retain fat in the subcutaneous adipose tissue (SAT) is beneficial in human obesity because of its association with reduced visceral fat, absence of fat deposition in the liver and muscle, and better insulin sensitivity (1,2). Recently, we showed that a reduced lipogenic/adipogenic expression and number of hypertrophic adipocytes may be linked to hepatic steatosis as well as to insulin resistance in obese adolescents (3). In that study, transcription of genes controlled by sterol-regulatory element-binding protein-1c (SREBP1c) were determined by RT-PCR, along with measures of adipocyte morphometry and cellularity (3). Although this early study indicated an important association between the insulin-stimulated SREBP1c pathway in SAT and ectopic fat accumulation/insulin resistance, it lacked any information about the role of the carbohydrate-responsive element-binding protein (ChREBP), a key determinant of systemic insulin sensitivity and glucose homeostasis (4). The role of adipose tissue in the development of the obesity-related insulin resistance is complex, encompassing increased release of fatty acids, altered adipokine secretion, and/or inflammation (5). Altered adipose tissue glucose metabolism also is a cause of insulin resistance, and adipose-tissue glucose transporter type 4 (GLUT4) plays a central role in systemic glucose metabolism (6,7). Of note, adipose-specific GLUT4 knockout mice have insulin resistance and type 2 diabetes (T2D) (6). Early in the development of type 2 diabetes, downregulation of human and mouse adipose tissue GLUT4 is present (8). More recent studies by Herman et al. (4) have greatly advanced the field by clearly demonstrating that ChREBP, a major regulator of de novo lipogenesis in adipose tissue, is highly regulated by GLUT4 in adipose tissue and that ChREBP in adipose tissue is required for the improved glucose homoeostasis resulting from increased adipose GLUT4 expression. Although significant progress has been achieved toward the understanding of the physiological role and expression of ChREBP in adipose and liver tissue of rodents, much less is known about its regulation in humans, particularly in obese adolescents. In the current study, we expand on our previous observation regarding the role of dysregulation of lipogenesis in the pathogenesis of insulin resistance by determining the dynamic changes in the expression of ChREBP and its two recently discovered isoforms, along with GLUT4, patatin-like phospholipase domain-containing protein 3 (PNPLA3), fatty acid synthase (FASN), and acetyl-CoA carboxylase (ACC) in adipocytes obtained from the abdominal subcutaneous depot of obese adolescents with varying degree of glucose tolerance but with similar level of obesity. The expression of ChREBP and its lipogenic target genes also were measured in liver tissue obtained in a subset of subjects in whom a liver biopsy was performed for diagnosis of non-alcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH). We hypothesized that adolescents with prediabetes or early T2D, independent of obesity, would display reduced transcription of ChREBP in SAT that would correlate with the expression of GLUT4 and insulin resistance and, more importantly, with the level of hyperglycemia. The opposite would be present in the liver, where an...
increased expression of ChREBP would be associated with hepatic steatosis and insulin resistance.

RESEARCH DESIGN AND METHODS

Subjects. The Yale Pathophysiology of T2D in Obese Youth Study is a long-term project aimed at examining early alterations in glucose metabolism in relation to fat patterning in obese adolescents. As part of this study, subjects underwent a detailed assessment of glucose tolerance status, a hyperinsulinemic-euglycemic clamp, a dual-energy X-ray absorptiometry scan, and an abdominal magnetic resonance imaging (MRI). Fifty-three obese adolescents agreed to undergo a subcutaneous periumbilical adipose tissue biopsy and were divided into three groups based on their 2-h glucose level: 2-h glucose level < 120 mg/dL (27 with normal glucose tolerance [NGT]), between 120 and 140 mg/dL (16 with NGT at risk for impaired glucose tolerance [IGT]), and > 140 mg/dL (5 with IGT and 5 with T2D). A liver biopsy was performed in eight subjects because of sustained elevation in ALT. None of the subjects was using medications known to affect glucose and lipid metabolism. The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The study was approved by the ethics committees of the Yale University Hospital. Data on insulin sensitivity, adipocyte cell size, and genes regulating adipogenesis from 38 subjects were reported in a previous report (5).

Metabolic studies. The oral glucose tolerance test. All subjects were invited to the Yale Center for Clinical Investigation for an oral glucose tolerance test (OGTT) at 8:00 a.m. after an overnight fast. An OGTT was then performed as previously described (9). The composite whole-body insulin sensitivity index was calculated using the formula described by Matsuda et al. (10).

The hyperinsulinemic-euglycemic clamp. After a 10-h overnight fast, at 7:00 a.m., two intravenous catheters, one for blood sampling and one for infusion of glucose, insulin, and tracers, were inserted in the antecubital vein of each arm (27 with normal glucose tolerance [NGT]), between 120 and 140 mg/dL (16 with NGT at risk for impaired glucose tolerance [IGT]), and > 140 mg/dL (5 with IGT and 5 with T2D). A liver biopsy was performed in eight subjects because of sustained elevation in ALT. None of the subjects was using medications known to affect glucose and lipid metabolism. The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The study was approved by the ethics committees of the Yale University Hospital. Data on insulin sensitivity, adipocyte cell size, and genes regulating adipogenesis from 38 subjects were reported in a previous report (5).

Liver fat assessment: fast MRI. Hepatic fat fraction, an estimate of the percentage of fat in the liver, was measured by fast-gradient MRI (12). The sampling arm was kept in a heated box for arterIALIZATION of blood. Whole-body insulin sensitivity was measured by two-step euglycemic clamp by infusing insulin as a primed continuous infusion at 4 μU·m−2·min−1 and 80 μU·m−2·min−1. Each step lasted 2 h. A primed continuous infusion of 6,6-deuterium glucose and of 2H5-glycerol were used to quantify effects of insulin on glucose and glycerol turnover. To maintain the plasma enrichment of 2H-glucose constant throughout the clamp, we used the Hot Glucose infusion (GINF) method (11).

Dual-energy X-ray absorptiometry and MRI. Whole-body composition was measured by dual-energy X-ray absorptiometry with a Hologic scanner (Boston, MA). Abdominal MRI studies were performed on a GE or Siemens Sonata 1.5-T system (12) as previously reported (13).

Liver fat assessment: fast MRI. Hepatic fat fraction, an estimate of the percentage of fat in the liver, was measured by fast-gradient MRI (12). This method strongly correlated with 1H-MRS (r = 0.95; P < 0.001) (14), as well as with macrovesicular steatosis determined by liver histology in children (r = 0.86; P < 0.0001) (15).

Subcutaneous adipose biopsies. After local lidocaine administration, a 1-cm scalpel incision was made inferior to the umbilicus, from which 1 g of SAT was obtained. The tissue was washed immediately for osmium fixation, two 50 mg samples were used immediately for in vitro lipogenesis test, and the rest was flash-frozen for RNA extraction. In vitro lipogenesis was tested as previously described (3) (Supplementary Data). RNA was extracted and quantification of several differentially expressed genes by real-time RT-PCR was performed using an ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) (Supplementary Data). RNA expression was studied in 35 subjects (26 in group 1 and 9 in group 3) using Affymetrix Human Gene 1.0 ST arrays (Supplementary Data).

Liver biopsies. Liver biopsies were performed in eight subjects referred to a pediatric hepatologist because of elevated ALT levels. Half of the specimen was sent to pathology, and the other half was used for RNA extraction and quantitative PCR (Supplementary Data).

Analytical methods. Plasma glucose levels were measured using the YSI 2700 STAT Analyzer (Yellow Springs Instruments) and lipid levels by an Autoanalyzer (model 747–200; Roche-Hitachi). Plasma insulin, adiponectin, and leptin were measured with a radioimmunoassay (Linco, St. Charles, MO). Analysis of enrichments of 2H-glucose and 2H5-glycerol in plasma was performed as described elsewhere (11). The glucose disposal rate was calculated during the last 30 min (steady state) of each step of the clamp and was expressed milligrams (mg) per minute per kilogram of lean body mass (LBM) (mg/kg LBM·min). Endogenous hepatic glucose production and glycerol turnover at baseline and during the two steps of the insulin clamp, along with the clamped glucose disposal rates, were calculated as previously reported (11).

Statistical analysis. Univariate general linear model and χ2 test (for categorical variables) were used to compare the clinical and laboratory characteristics among the three groups. Potential confounders (sex, age, BMI, and ethnicity) were entered in an ANOVA model in which the metabolic and cell size parameters, as well as age as expression as quantitated by RT-PCR, were dependent variables and the group was the primary grouping variable; normally distributed parameters were log-transformed before entry into the model. Spearman correlation was used to assess associations between variables of interest. All analyses were performed using IBM SPSS 19 (SPSS, Chicago, IL). For all analyses, a P < 0.05 was considered statistically significant.

RESULTS

Body fat patterning and metabolic characteristics by 2-h glucose levels. Age, gender, percent total fat, and BMI were similar across the three groups (Table 1). However, the groups differed slightly by ethnicity, with the number of African Americans being lower in the second group. Significant increases in ratios of visceral to visceral and subcutaneous (P = 0.005) and hepatic fat content (P%) were observed in the groups with higher 2-h glucose levels (P = 0.022). With the increase in 2-h glucose levels, we observed an increase in fasting glucose (P < 0.001) and insulin levels (P = 0.006), as well as a decrease in insulin sensitivity measured by the OGTT (P = 0.001) and the clamp (P = 0.005). The low insulin infusion caused a greater suppression of hepatic glucose production in group 1 (P = 0.046), whereas during the high-insulin infusion step suppression of hepatic glucose production was identical in all three groups. Basal glycerol turnover did not differ among the groups. In group 3, suppression of lipolysis was less during the low-dose insulin infusion (P = 0.013), whereas during the high-dose it was similar in all three groups (Table 1).

Subcutaneous adipose tissue biopsies. Affymetrix gene arrays were used to evaluate potential differences in the gene expression profiles of the subcutaneous fat biopsy specimen. The genechip analysis demonstrated that 46 genes were upregulated in group 3 compared with group 1, and 85 genes were downregulated in group 3 compared with group 1. When applying pathway analyses, the transcription ChREBP regulation pathway emerged as one of the most significantly different pathways between group 1 and group 3 (Supplementary Table 1A).

Expression of both isoforms of ChREBP (α and β) was significantly different across the groups (ANOVA P < 0.02; Fig. 1B). Because ChREBP expression is regulated by GLUT4 in adipose tissue (4), we measured the expression of GLUT4 across the groups and found a significant decrease in GLUT4 expression with increasing 2-h glucose level (P = 0.041; Fig. 1A). ChREBP regulates the transcription of several genes involved in fatty acid synthesis (FASN, ACC), and the expression of genes (thioredoxin-interacting protein, Rgs16) that
TABLE 1
Metabolic characteristics of the obese adolescents undergoing fat biopsy (n = 53)

<table>
<thead>
<tr>
<th></th>
<th>2-h glucose &lt;120 (n = 27)</th>
<th>2-h glucose 120–140 (n = 16)</th>
<th>2-h glucose &gt;140 (n = 10)</th>
<th>P*</th>
<th>P adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.1 ± 3.4</td>
<td>14.3 ± 2.7</td>
<td>15.7 ± 2.4</td>
<td>0.471</td>
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</tr>
<tr>
<td>Gender (female/male)</td>
<td>18/9</td>
<td>9/7</td>
<td>5/5</td>
<td>0.603‡</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (C/HAA)</td>
<td>6/6/15</td>
<td>9/5/2</td>
<td>3/2/5</td>
<td>0.044‡</td>
<td></td>
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<tr>
<td>Anthropometrics</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.9 ± 8.8</td>
<td>37.4 ± 6.9</td>
<td>38.5 ± 6.1</td>
<td>0.936</td>
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<tr>
<td>Fat (%)</td>
<td>40.6 ± 5.6</td>
<td>40.8 ± 6.5</td>
<td>39.8 ± 5.1</td>
<td>0.926</td>
<td>0.774</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>55.0 ± 13.2</td>
<td>54.0 ± 8.4</td>
<td>61.1 ± 10.6</td>
<td>0.391</td>
<td>0.997</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>114.9 ± 12.0</td>
<td>119.9 ± 10.5</td>
<td>127.1 ± 9.0</td>
<td>0.017</td>
<td>0.028</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>46.6 ± 8.8</td>
<td>39.4 ± 8.5</td>
<td>40.3 ± 9.9</td>
<td>0.029</td>
<td>0.259</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>102.4 ± 11.8</td>
<td>130.2 ± 5.0</td>
<td>190.2 ± 42.7</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>Matsuda index (whole-body insulin sensitivity index)</td>
<td>2.6 ± 1.7</td>
<td>1.5 ± 0.8</td>
<td>0.8 ± 0.4</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>Glucose disposal rate (mg/kg LBM · min; n = 43)</td>
<td>11.5 ± 6.0</td>
<td>7.4 ± 2.3</td>
<td>5.8 ± 1.1</td>
<td>0.005</td>
<td>0.016</td>
</tr>
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<td>Hepatic glucose production</td>
<td>33 ± 35</td>
<td>3 ± 30</td>
<td>5 ± 21</td>
<td>0.046</td>
<td>0.251</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>27.2 ± 15.3</td>
<td>37.8 ± 18.2</td>
<td>54.9 ± 39.9</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>VAT/VAT+SAT ratio</td>
<td>0.089 ± 0.032</td>
<td>0.119 ± 0.029</td>
<td>0.124 ± 0.033</td>
<td>0.003</td>
<td></td>
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<tr>
<td>Hepatic fat content (%)</td>
<td>1.5 ± 3.8</td>
<td>11.6 ± 13.6</td>
<td>13.4 ± 11.0</td>
<td>0.001</td>
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<tr>
<td>Abdominal fat distribution</td>
<td></td>
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<tr>
<td>Visceral fat (cm²)</td>
<td>63.4 ± 35.9</td>
<td>78.4 ± 30.5</td>
<td>79.5 ± 24.0</td>
<td>0.263</td>
<td>0.241</td>
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<tr>
<td>Subcutaneous fat (cm²)</td>
<td>621.1 ± 248.2</td>
<td>586.5 ± 190.3</td>
<td>580.9 ± 195.2</td>
<td>0.846</td>
<td>0.501</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>46.6 ± 8.8</td>
<td>39.4 ± 8.5</td>
<td>40.3 ± 9.9</td>
<td>0.029</td>
<td>0.259</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>72.9 ± 41.8</td>
<td>141.0 ± 77.3</td>
<td>165.0 ± 105.5</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>FFA (µmol/L)</td>
<td>531.8 ± 168.0</td>
<td>621.5 ± 147.5</td>
<td>651.1 ± 247.7</td>
<td>0.128</td>
<td>0.077</td>
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<tr>
<td>Adipocytokines</td>
<td></td>
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<tr>
<td>Adiponectin (µg/mL)</td>
<td>10.9 ± 5.6</td>
<td>6.8 ± 3.8</td>
<td>4.9 ± 1.6</td>
<td>0.006</td>
<td>0.018</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>35.5 ± 14.5</td>
<td>34.1 ± 17.4</td>
<td>33.6 ± 12.4</td>
<td>0.949</td>
<td>0.454</td>
</tr>
</tbody>
</table>

Average ± SD. C, caucasian; H, Hispanic; AA, African American; BP, blood pressure; TG, triglycerides; FFA, free fatty acid. *One-way ANOVA P < 0.05 in boldface; †ANCOVA adjusted for age, gender, ethnicity, BMI; ‡P < 0.05 in boldface.

are not known to be regulated by other lipogenic transcription factors. Significant differences in the expression of SREBP1c (P = 0.008), FASN (P = 0.006), and ACC (P = 0.028) across the three groups were found with increasing 2-h glucose level, whereas thioredoxin-interacting protein (TXNIP) showing the same trend missed significance (Fig. 1A). Because ACC and FASN are regulated primarily at the level of transcription, it is expected that decreases in the activities of these enzymes are reflected by decreases in their mRNA.

Expression of adipose ChREBP correlates with expression of GLUT4 (r = 0.457; P = 0.001), PNPLA3 (r = 0.480; P = 0.0004), FASN (r = 0.444; P = 0.001), ACC (r = 0.360; P = 0.009), TXNIP (r = 0.410; P = 0.005), 2-h glucose level (r = −0.556; P = 0.010) and insulin sensitivity measured by the clamp (r = 0.368; P = 0.018; n = 43), as well as the OGTT (r = 0.417; P = 0.002; n = 53). Furthermore, the expression of both isoforms of ChREBP (α and β) was significantly correlated with 2-h glucose level (ChREBPα: r = −0.433 and P = 0.002; ChREBPβ: r = −0.307 and P = 0.038) and insulin sensitivity measured by the clamp (ChREBPα: r = 0.329 and P = 0.036; ChREBPβ: r = 0.345 and P = 0.039). Multiple linear regression analysis for ChREBPα and ChREBPβ as predictors of insulin sensitivity measured by the clamp (M/LBM), demonstrated that expression of ChREBPβ, but not ChREBPα, predicts insulin sensitivity (Table 2). Insulin sensitivity measured by the clamp is a better predictor for ChREBPβ expression in SAT (r² = 0.285; P = 0.004), whereas 2-h glucose level seems to be a better predictor for ChREBPα expression (r² = 0.120; P = 0.047).

To determine whether improvement of glucose tolerance status will reverse the downregulation of GLUT4 and ChREBP, we repeated SAT biopsies in four subjects who had improvement from IGT to NGT (Supplementary Table 2; converter), and as a control we repeated biopsies in five subjects who continued to have NGT (Supplementary Table 2; nonconverter). All subjects were recruited from our longitudinal pathophysiology cohort undergoing repeated OGTTs and were treated neither pharmacologically nor through lifestyle interventions. Quantitative PCR on the repeated SAT biopsies showed an increase in the expression of GLUT4, ChREBP, PNPLA3, SREBP1c, FASN, and ACC after improvement in 2-h glucose level (Fig. 2A), whereas no difference in expression was found in the nonconverters (Fig. 2C). The same could be observed for...
the two ChREBP isoforms, α and β (Fig. 2B, D). Statistical analysis of all nine repeated biopsies showed a significant correlation between change in 2-h glucose level and change in ChREBP expression ($r = -0.317; P = 0.007; n = 9$), as well as change in insulin sensitivity (M/LBM) and change in ChREBP expression ($r = -0.357; P = 0.085; n = 8$).

To assess if the decrease in lipogenic gene expression is associated with a decrease in insulin sensitivity of the adipose tissue, we measured the insulin-stimulated incorporation of glucose into triglycerides in the fresh adipose tissue biopsy specimen using C14-labeled glucose. Insulin-stimulated de novo lipogenesis (nmol glucose incorporated per hour per gram of adipose tissue) was significantly reduced in 5 subjects from group 3 compared with 14 subjects from group 1 ($P = 0.047; \text{Fig. 3}$).

**Liver biopsies.** Although hepatic steatosis, defined as percentage of hepatic fat fraction $>5.5\%$, was seen in 50% of the entire cohort, liver tissue specimen were obtained only from eight subjects after they underwent a liver biopsy because of persistent elevation in ALT levels. Three subjects had NGT (2-h glucose level $<120$ mg/dL), three subjects had IGT (2-h glucose level $120–140$ mg/dL), and two had T2D (2-h glucose level $>140$ mg/dL) (Supplementary Table 3). We analyzed gene expression by quantitative PCR in NGT (group 1; 2-h glucose level $<120$ mg/dL) compared with IGT/T2D (group 2; 2-h glucose level $>140$ mg/dL). In contrast to what was seen in the SAT, the expression of PNPLA3, ChREBP, SREBP1c, FASN, and ACC was significantly increased in the liver specimen of IGT/T2D subjects compared with those with NGT (Fig. 4A). The same could be observed for the two ChREBP isoforms, α and β (Fig. 4B).

**DISCUSSION**

In the current study, we expand on our previous observation regarding the role of dysregulation of lipogenesis in the pathogenesis of insulin resistance by investigating the interactions between glucose tolerance, insulin sensitivity, and the expression of lipogenic genes in abdominal SAT and liver tissue in equally obese adolescents across the spectrum of glucose tolerance. Notably, we found that independent of overall degree of obesity, the lipogenic expression as determined by the measurement of ChREBP,
SREBP1c, PNPLA3, ACC, and FASN mRNA was decreased in the SAT of IGT/T2D adolescents, in marked contrast to the increased liver expression of these genes. Importantly, the expression of ChREBP in the SAT was inversely related with 2-h glucose level and increased after conversion from IGT to NGT.

For the first time, we report a potential role of ChREBP in the development of glucose dysregulation in obese adolescents. The opposite regulation of ChREBP in the liver and adipose tissue in the current study was found to be related to both insulin resistance and the 2-h glucose levels, with ChREBPβ being the major isoform predicting insulin sensitivity (Table 2). In contrast to the SAT, in the liver we found an increased expression of lipogenic genes (ChREBP, SREBP1c, PNPLA3, FASN, ACC) in IGT/T2D adolescents. The increased expression of these lipogenic genes correlated positively with insulin resistance, which is in contrast to recently published data by Benhamed et al. (21), showing a negative correlation of ChREBP expression and HOMA-IR. Of note, our patients were in a higher range of HOMA-IR (mean HOMA-IR, 13.5) compared with Benhamed’s adult cohort with a mean HOMA-IR of 5.6. Whereas our patients were equally obese, it is known from literature that compared with lean adult subjects, obese subjects have a decreased expression of lipogenic genes in adipose tissue but increased hepatic

**FIG. 2.** Changes in subcutaneous lipogenic gene expression in converters (A, B) or nonconverters (C, D). A and C: Subcutaneous expression of ChREBP, PNPLA3, GLUT4, SREBP1c, FASN, and ACC normalized to the expression of 18S ribosomal RNA and based on the expression of a human control adipose tissue (2^ΔΔCt). B and D: Subcutaneous expression of both isoforms of ChREBP (α and β), normalized to the expression of 18S ribosomal RNA and based on the expression of a human control adipose tissue (2^ΔΔCt). Expression values of biopsy 1 (white bars) were set to 1 and values for biopsy 2 (black bars) are expressed as fold-changes compared with biopsy 1 (mean ± SD, n = 4 for A, B and n = 5 for C, D).
lipogenesis (22). In adult obese subjects ChREBP expression and protein levels in the liver were higher compared with lean subjects, whereas the expression was decreased in adipose tissues (23). The increased hepatic lipogenesis is consistent with the effects of high insulin levels on liver lipogenic genes demonstrated in vitro (24–26). Moreover, the hepatic overexpression of ChREBP was seen in the context of liver steatosis as measured not only by MRI but also more specifically by liver biopsy, which revealed the presence of NASH in five out of eight patients (representative images for NGT, IGT, and T2D; Fig. 4C). The upregulation of lipogenesis in the liver and the presence of hepatic steatosis translated into a lack of insulin suppression of hepatic glucose production during the first step of the insulin clamp, indicating severe hepatic insulin resistance with increasing 2-h glucose level (group 3). Consistent with our data, downregulation of ChREBP in liver of ob/ob mice has been shown to improve hepatic steatosis as well as insulin sensitivity and glucose tolerance (27), whereas newer data from the same group show that overexpression of ChREBP in the liver of C57Bl/6J mice leads to hepatic steatosis without causing insulin resistance (21). Although some transgenic mouse studies have disassociated hepatic steatosis from hepatic insulin resistance (21,28), other studies (29–33) of rodent models of NAFLD have demonstrated that diacylglycerol activation of protein kinase C epsilon (PKCe) is the key trigger in the pathogenesis of NAFLD-associated hepatic insulin resistance. Moreover, recent studies strongly suggest that hepatic DAG content in lipid droplets is the best predictor of insulin resistance in humans, supporting the hypothesis that NAFLD-associated hepatic insulin resistance is caused by an increase in hepatic DAG content, which results in activation of protein kinase C (34). Nevertheless, it is also conceivable that other factors associated with steatosis, such as inflammation, circulating adipokines, and endoplasmic reticulum stress, affect insulin sensitivity without necessarily being directly related with hepatic lipid accumulation (35).

The cause of the downregulation in ChREBP in the SAT of obese adolescents with high 2-h glucose levels is not entirely clear but might be related to the effects of alteration in GLUT4 expression, as recently shown by Herman et al. (4). Adipose tissue GLUT4 regulates the expression of ChREBP, a major determinant of adipose tissue fatty acid synthesis and systemic insulin sensitivity (4). Glucose-mediated activation of the ChREBPα isoform induces expression of the ChREBPβ isoform and therefore increases fatty acid synthesis in adipose tissue, which improves systemic insulin sensitivity (4). Multivariate regression analyses showed that ChREBPβ might be the major isoform predicting insulin sensitivity, but our results are based only on mRNA levels. It is plausible that activation of ChREBPα, for example, by posttranslational modifications, is highly important in insulin sensitivity, and this is likely to be the dominant factor that induces the expression of ChREBPβ. In our obese adolescent cohort, expression of adipose ChREBP strongly correlates with GLUT4 expression and insulin sensitivity. Consistent with adipose–GLUT4 KO mice (6), we found profound hepatic and peripheral insulin resistance with the increasing 2-h glucose levels in our obese adolescents.

Although GLUT4 also is significantly decreased in the adipose tissue of obese nondiabetic subjects (36), PNPLA3 is reduced in the adipose tissue of T2D subjects, but not in obese subjects, and increases with insulin treatment (16), suggesting that PNPLA3 dysregulation in adipose tissue may be associated with impaired insulin sensitivity and glucose intolerance in T2D subjects, but not in obese patients. In our cohort, adipose PNPLA3 expression positively correlated with insulin sensitivity and negatively with fasting glucose both in obese and nonobese subjects (37). PNPLA3 gene exhibits features of lipogenic genes (38) and is regulated by SREBP1c and ChREBP (16,39,40). Glucose-dependent binding of ChREBP to a carbohydrate-response element in the PNPLA3 promoter recently has been demonstrated by chromatin immunoprecipitation, and silencing of ChREBP in human hepatocytes abolished induction of PNPLA3 mRNA by glucose (17).

Reversal of the expression in GLUT4 and ChREBP as well as in the expression of its lipogenic target genes in the adipose tissue after normalization of the glucose tolerance status in four obese subjects, with two subjects losing weight and two subjects gaining weight, supports the importance of GLUT4 and ChREBP in the regulation of this complex lipogenic machinery. Two of the subjects with conversion to NGT not only had improvement in their hyperglycemia but also had improvement in their insulin sensitivity and showed the highest increase in GLUT4 and ChREBP expression. Furthermore, we found that glucose incorporation into triglycerides in vitro in adipose tissue specimens from IGT/T2D adolescents was impaired in response to insulin stimulation (Fig. 3). Our findings are consistent with those reported for adipocytes from obese adults with T2D (41).

Although our study is mainly cross-sectional and does not prove causality, the direct correlation between adipose ChREBP expression and insulin sensitivity, as well as the indirect correlation with 2-h glucose levels, suggests that adipose ChREBP may be involved in regulating whole-body insulin action and glucose homeostasis by upregulation of adipose tissue de novo lipogenesis. The phenotype of impaired glucose tolerance and decreased adipose ChREBP expression seem to coexist with a phenotype of hepatic steatosis and increased liver ChREBP expression. Limitations of the study are attributable to the fact that we measured only expression and not protein content, which was mainly the result of limited amount of tissue obtained.
from these adolescents. Furthermore, we sampled only SAT and the results could be different in visceral adipose tissue, as shown for other mRNA levels (42). Because of ethical reasons, we also were not able to include a normal control group for the liver biopsies. Our longitudinal data are from a small number of adolescents; therefore, presented data for converters from IGT to NGT are very preliminary and further studies are needed in this area.

Several strengths need to be highlighted in this study, including: the detailed metabolic characterization of three obese groups of adolescents who differed in their glucose tolerance status; the use of state-of-the-art measurements of insulin sensitivity; imaging techniques for assessment of lipid content in liver and abdominal fat distribution; and measurements of the expression of genes regulating lipogenesis in SAT and liver.

In conclusion, early in the development of prediabetes/T2D in youth, ChREBPβ expression in adipose tissue predicts insulin resistance and therefore might play a role in the regulation of glucose tolerance.

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R.K. and S.C. wrote the manuscript. R.K. performed the statistical analyses. S.C. initiated the concept of the study and designed it together with R.K., S.W.C., and G.I.S. D.N. was the surgeon for the biopsies. A.L. performed the statistical analyses for the Affymetrix genechip data. C.G. and E.D. performed the hyperinsulinemic-euglycemic clamps. M.S. and B.P. were responsible for recruiting the subjects and performing the MRI analysis. All authors contributed to the interpretation of the data. S.C. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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