Lower Total Adipocyte Number but No Evidence for Small Adipocyte Depletion in Patients With Type 2 Diabetes

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OBJECTIVE — We hypothesized that, compared with obese subjects, patients with type 2 diabetes have a lower total adipocyte number with fewer small adipocytes.

RESEARCH DESIGN AND METHODS — Abdominal subcutaneous adipose tissue was obtained from lean and obese subjects with or without type 2 diabetes matched for BMI. Adipocyte size was measured by osmium fixation and sizing/counting in a Coulter counter. Adipocyte size and number subdistributions (small, medium, large, and very large) were determined.

RESULTS — Compared with obese subjects, type 2 diabetic patients had larger mean adipocyte size and 67% bigger very large adipocytes; the total adipocyte number was lower, but the fraction of small adipocytes was increased by 27%.

CONCLUSIONS — Total adipocyte cellularity is lower in type 2 diabetic subjects than in obese subjects. We found no evidence for depletion of small adipocytes in patients with type 2 diabetes. This suggests the presence of a defect in early maturation of adipocytes in patients with type 2 diabetes.

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There is a growing appreciation that adipose tissue dysfunction in type 2 diabetes is due in part to adipocyte hypertrophy. Mean adipocyte size is inversely correlated with insulin sensitivity (1). A greater number of small adipocytes is associated with insulin sensitivity (2,3). Danforth (4) hypothesized that adipocytes are hypertrophic due to defects in preadipocyte differentiation to adipocytes, leading to an inability to buffer excess dietary fat.

In this study, we aimed to identify the differences in adipocyte size and number subdistributions in abdominal subcutaneous adipose tissue (SAT) of lean and obese subjects with or without type 2 diabetes. We hypothesized that, compared

with obese subjects, type 2 diabetic patients have a lower total adipocyte number with fewer small adipocytes.

RESEARCH DESIGN AND METHODS

The present study used baseline data collected from 260 patients participating in a variety of clinical studies at the Pennington Biomedical Research Center. Patients were excluded if they had significant renal, cardiac, liver, lung, or neurological disease. Hypertension was acceptable if blood pressure was less than 140/90 mmHg on medications. Patients were excluded for prior use of thiazolidinediones or drugs known to affect lipid metabolism, energy metabolism, or

body weight or drug abuse and smoking. The study included three groups: lean (BMI <25 kg/m²), obese (BMI >25 kg/m²), and obese with type 2 diabetes (BMI >25 kg/m²). Protocols were approved by the Institutional Review Board of the Pennington Biomedical Research Center. All volunteers gave written informed consent.

Body fat mass was measured by dual-energy X-ray absorptiometry. Visceral and subcutaneous fat mass was measured using a GE High-Speed CT scanner. From the eight cross-sectional areas, visceral adipose tissue (VAT) volume was calculated and converted to VAT mass (kilograms) using the conversion factor 0.9193 kg/l for adipose tissue.

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated by multiplying the fasting glucose level (millimoles per liter) by the fasting insulin level (microunits per milliliter) and dividing the product by 22.5 (5).

Adipocyte sizing was performed as previously described (2). Briefly, abdominal SAT samples were fixed in osmium tetroxide and digested with 8 mol/l urea in 154 mol/l NaCl solution. Cells were filtered over a 10 μ m nylon screen, collected into a Triton X-100 solution, and analyzed on a Coulter counter. The diameter of each osmium-fixed triglyceride droplet was used to calculate cell volume. Using statistical analysis (details can be found in an online appendix, available at <http://care.diabetesjournals.org/cgi/content/full/dc08-2240/DC1>), four adipocyte subdistributions were identified as follows: small, medium, large, and very large. This method is highly reproducible ($R^2 = 0.785$) (supplemental Figure A3). The subdistribution fraction is the percentage of adipocytes in a specific subdistribution. Adipocyte number was determined by dividing the subcutaneous abdominal fat mass (multislice computed tomography) by the abdominal subcutaneous mean size (6).

Comparison between lean, obese, and type 2 diabetic subjects was performed using an ANCOVA test with sex, race, and age as covariates. Statistical significance is defined relative to a nominal two-sided 5% type 1 error rate. Tukey-Kramer ad-

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Figure 1—Adipocyte subdistributions (size and number) in lean, obese, and type 2 diabetic subjects. Abdominal SAT samples were fixed, digested, and analyzed on a Coulter counter. The diameter of each osmium-fixed triglyceride droplet was used to calculate cell volume. For each participant, four subdistributions were determined: small (S), medium (M), large (L), and very large (VL) adipocytes. There were 27 participants in the lean group, 192 in the obese group, and 41 with type 2 diabetes. Comparison was performed using ANCOVA tests with sex, race, and age as covariates. Tukey-Kramer adjustment ($\alpha < 0.05$) was used as a post hoc test. A: There is no significant difference in small, medium, large, and very large adipocyte size subdistribution in obese compared with lean patients. Interestingly, compared with the BMI-matched obese subjects, patients with type 2 diabetes have smaller small and medium adipocytes and bigger very large adipocytes. Data are means $\pm$ SD. * $P < 0.05$. B: Adipocyte number was determined by dividing the subcutaneous abdominal fat mass by the adipocyte mean size. Each subdistribution fraction represents percentage of adipocytes in a specific subdistribution from the total number of adipocytes analyzed. Boxes represent the mean of each subdistribution absolute number. Means $\pm$ SD of the adipocyte fractions expressed as percentage from the total adipocyte number are presented in supplemental Table 2. Total adipocyte number is smallest in lean and bigger in obese patients and those with 2 diabetes ($P < 0.05$). Interestingly, patients with type 2 diabetes have less adipocytes than BMI-matched obese subjects ($P < 0.05$). However, from all the subdistributions, the small fraction (percent from the total adipocyte number [%]) was significantly greater in patients with type 2 diabetes than in BMI-matched obese subjects ($P < 0.05$).

justment ($\alpha < 0.05$) was used as a post hoc test.

RESULTS— The obese and type 2 diabetic patients were matched for BMI and abdominal SAT; however, the latter had more VAT. The characteristics of the study populations are presented in supplemental Table 1.

Mean $\pm$ SD adipocyte size was larger in patients with type 2 diabetes than in obese subjects (1.0 ± 0.05 vs. 0.79 ± 0.04 μ l; $P < 0.05$). Compared with BMI-matched obese subjects, patients with type 2 diabetes had 67% bigger very large adipocytes and 20% smaller small adipocyte size (Fig. 1A). Importantly, total adipocyte number was lower in patients with type 2 diabetes ($P < 0.05$) while the fraction of small adipocytes was 27% greater ($P < 0.05$) (Fig. 1B and supplemental Table 2).

In lean and obese subjects, BMI was positively correlated with adipocyte mean size, large size, and very large size ($R = 0.57$, $R = 0.36$, and $R = 0.25$, respectively; $P < 0.05$) and negatively with small adipocyte size ($R = -0.39$; $P < 0.05$). HOMA-IR, a marker of insulin re-

sistance, was positively correlated with adipocyte mean size, large size, and very large size ($R = 0.54$, $R = 0.27$, and $R = 0.41$, respectively; $P < 0.05$) and negatively with small size ($R = -0.35$; $P < 0.05$). Adipocyte number was positively correlated with HOMA-IR ($R = 0.32$; $P < 0.05$).

There are no significant correlations between adipocyte size and number with BMI in patients with type 2 diabetes.

CONCLUSIONS— In this large sample of adipose tissue biopsies, we show for the first time that patients with type 2 diabetes have fewer subcutaneous adipocytes compared with BMI-matched obese subjects. This suggests that in individuals with type 2 diabetes, the number of adipocytes fails to increase as body fat increases. As suggested by Danforth, this may lead to fat accumulation in tissues such as VAT, muscle, and liver—all known to contribute to insulin resistance. We found that patients with type 2 diabetes have increased visceral tissue, supporting the concept that a failure of SAT to store

energy leads to an increase in VAT and possibly other sites of ectopic fat. However, it is possible that these individuals have a low number of adipocytes in childhood; consequently, if this number is fixed, as some studies suggest, they might be at risk for developing diabetes as adults.

Compared with BMI-matched obese subjects, patients with type 2 diabetes had greater mean adipocyte size mainly driven by an increased size of the very large adipocyte, suggesting that type 2 diabetes is accompanied by hypertrophy rather than hyperplasia. Contrary to our hypothesis, patients with type 2 diabetes have greater fraction of small adipocytes. The increased size of the largest adipocyte (very large) in patients with type 2 diabetes might stimulate recruitment and proliferation of an adipocyte precursor, which leads to greater small adipocytes fraction (7). However, the size of small adipocytes is lower, suggesting that these new adipocytes cannot further accumulate lipid.

These observations suggest impairment of the complete maturation of adipocytes with no effect on late fatty acid

storage in patients with type 2 diabetes. This hypothesis is in accordance with recent data showing that insulin resistance per se causes impairment in adipogenesis (8,9). Future studies should test this novel hypothesis, i.e., that there might be a defect in the complete maturation of small adipocytes in patients with type 2 diabetes (supplemental Figure 4).

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