Lower total adipocyte number, but no evidence for small adipocyte depletion in patients with type 2 diabetes

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Objectives: We hypothesized that compared to obese, patients with type 2 diabetes have lower total adipocyte number with less small adipocytes.

Research design and methods: Abdominal subcutaneous adipose tissue was obtained from lean and obese 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 sub-distributions [small, medium, large and very-large] were determined.

Results: Compared to obese, type 2 diabetes patients had greater mean adipocyte size and 67% bigger very-large adipocytes; total adipocyte number was lower, but the fraction of small adipocytes was increased by 27%.

Conclusions: Total adipocyte cellularity is lower in type 2 diabetes vs. obese. 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 hypertrophia. Mean adipocyte size is inversely correlated with insulin sensitivity (1). Greater number of small adipocytes is associated with insulin sensitivity (2; 3). Danforth hypothesized that adipocytes are hypertrophic due to defects in pre-adipocyte differentiation to adipocytes, leading to an inability to buffer excess dietary fat (4).

In this study, we aim to identify the differences in adipocyte size and number sub-distributions in abdominal subcutaneous adipose tissue of lean and obese with or without type 2 diabetes. We hypothesized that: compared to obese, type 2 diabetes patients have lower total adipocyte number with less small adipocytes.

**RESEARCH DESIGN AND METHODS**

The present study used baseline data collected from 260 patients participating in a variety of clinical studies at Pennington Biomedical Research Center [PBRC]. 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; drug abuse and smoking. They were 3 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 PBRC. All volunteers gave written informed consent.

Body fat mass was measured by Dual Energy X-Ray Absorptiometer. Visceral and subcutaneous fat mass was measured using a GE High Speed CT scanner. From the 8 cross-sectional areas, visceral adipose tissue volume was calculated and converted to VAT mass (kg) using the conversion factor 0.9193 kg/L for adipose tissue.

The Homeostasis Model Assessment-Insulin Resistant index [HOMA-IR] was calculated by multiplying the fasting glucose (mM) by the fasting insulin (µU/mL) and dividing the product by 22.5 (5).

Adipocyte sizing was performed as previously described (2). Briefly, abdominal subcutaneous adipose tissue samples were fixed in osmium tetroxide and digested with 8M urea/NaCl. Cells were filtered over a 10 µm nylon screen and collected into a Triton X-100 solution and analyzed on a Coulter Counter. The diameter of each osmium-fixed TG droplet was used to calculate cell volume. Using a statistical analysis [details in the online appendix available at http://care.diabetesjournals.org] four adipocyte sub-distributions were identified: small, medium, large and very-large. This method is highly reproducible [R²=0.785; see supplemental Figure A3 in the online appendix]. The sub-distribution fraction is the percentage of adipocytes in a specific sub-distribution. Adipocyte number was determined by dividing the subcutaneous abdominal fat mass [multislice CT] to the abdominal subcutaneous mean size (6).

Comparison between lean, obese and type 2 diabetes was performed using ANCOVA test with gender, race and age as covariates. Statistical significance is defined relative to a nominal two-sided 5% type 1 error rate. Tukey-Kramer...
adjustment [$\alpha < 0.05$] was used as a post-hoc test.

**RESULTS**

The obese and patients with type 2 diabetes were matched for BMI and abdominal subcutaneous adipose tissue, however the latter had more visceral adipose tissue. The characteristics of the study populations are presented in supplemental Table A1 in the online appendix.

Mean adipocyte size was larger in patients with type 2 diabetes compared to obese [1.0+0.05 vs. 0.79+0.04, p<0.05; µm$^2$; Mean ± SD]. Compared to BMI-matched obese, patients with type 2 diabetes had 67% bigger very-large adipocytes and 20% smaller small adipocyte size [Figure 1A]. Importantly, total adipocytes number was lower in patients with type 2 diabetes [p<0.05] while the fraction of small adipocytes was 27% greater [p<0.05] [Figure A1B and supplemental Table 2 in the online appendix].

In lean and obese, BMI was positively correlated with adipocyte mean size, large and very-large size [R=0.57, R=0.36, R=0.25, p < 0.05], and negatively to small adipocyte size [R=-0.39, p < 0.05]. HOMA-IR, a marker of insulin resistance, was positively correlated with adipocyte mean size, large and very-large size [R=0.54, R=0.27, R=0.41, p < 0.05], and negatively to 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 as compared with BMI-matched obese. This suggests that individuals with type 2 diabetes fail to increase the number of adipocytes 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 subcutaneous adipose tissue 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 to BMI-matched obese, patients with type 2 diabetes had greater mean adipocyte size, and this mainly driven by an increased size of the very-large adipocyte, suggesting that type 2 diabetes is accompanied by hypertrophia rather than hyperplastia. 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 adipocyte precursor, which lead 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 A4].

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REFERENCES
FIGURE LEGEND: Adipocyte sub-distributions [size and number] in lean, obese and patients with type 2 diabetes

Subcutaneous abdominal adipose tissue samples were fixed, digested then analyzed on a Coulter Counter. The diameter of each osmium-fixed TG droplet was used to calculate cell volume. For each participant, 4 sub-distributions were determined: small [S], medium [M], large [L] and very large adipocyte [VL]. There were 27 participants in the lean group, 192 in the obese group and 41 patients with type 2 diabetes. Comparison was performed using ANCOVA tests with gender, race and age as covariates. Tukey-Kramer adjustment \( \alpha < 0.05 \) was used as a post-hoc test.

[A] Adipocyte size: There is no significant difference in small, medium, large and very-large adipocytes size sub-distribution in obese vs. lean patients. Interestingly, compared to the BMI matched obese, patients with type 2 diabetes have smaller small and medium adipocytes and bigger very-large adipocytes. Mean ± Standard deviation. *, p-value < 0.05.

[B] Adipocyte number: Adipocyte number was determined by dividing the subcutaneous abdominal fat mass by the adipocyte mean size. Each sub-distribution fraction represents percentage of adipocytes in a specific sub-distribution from the total number of adipocytes analyzed. Boxes represent the mean of each sub-distribution absolute number. Mean and standard deviation of the adipocyte fractions expressed as percentage from the total adipocyte number are presented in supplemental Table A2 in the online appendix.

Total adipocyte number is smallest in lean and bigger obese and patients with type 2 diabetes [p<0.05]. Interestingly, patients with type 2 diabetes have less adipocytes compared to BMI matched obese [p<0.05]. However, from all the sub-distributions, the small fraction [% from the total adipocyte number, %] was significantly greater in patients with type 2 diabetes compared with the BMI matched obese [p<0.05].