methods

Determination of adipocyte size by computer image analysis

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Abstract **Methods that allow rapid and accurate determination of adipocyte size are important to studies of energy and glucose metabolism. The direct measurement of adipocyte size by microscopy is widely used, although the method is tedious and time consuming. Computer-assisted image analysis can overcome most of the disadvantages associated with this technique. We report a new method for determining adipocyte size by measuring the cross-sectional area of adipocytes with computer image analysis. This method allows a large number of adipocytes to be measured rapidly with computer hardware and software that are readily available.**—Chen, H. C., and R. V. Farese, Jr. **Determination of adipocyte size by computer image analysis, *J. Lipid Res.* 2002.** 43: **986–989.**

Supplementary key words adipocyte surface area • quantification • obesity

Adipocytes play an important role in energy and glucose metabolism. In addition to serving as a site for energy storage (in the form of triglycerides), adipocytes act as endocrine cells, secreting molecules that regulate energy expenditure, food intake, and glucose metabolism (1). Recent findings suggest that the size of adipocytes is a major modulator of their endocrine function. For example, hypertrophic adipocytes secrete greater amounts of tumor necrosis factor α and free fatty acids than normal adipocytes, and this excess secretion has been hypothesized to cause insulin resistance (2). Methods that allow quick and accurate determination of adipocyte size, therefore, are important to studies of energy and glucose metabolism.

Several methods for determining adipocyte size have been described, each with its advantages and disadvantages. Because none of these methods directly quantifies cell volume, which is the most accurate measurement for cell size, no "gold standard" exists for adipocyte size measurement. The measurement of adipocyte cross-sectional surface area by manual tracing of adipocytes has been used in several recent studies (2, 3) and appears to provide accurate and reproducible results. However, this method is tedious and time consuming. Here we report a new method for determining adipocyte size by measuring the cross-sectional area of adipocytes with computer image analysis. This method allows a large number of adipocytes to be measured rapidly and accurately with computer hardware and software that are readily available.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice from the Jackson Laboratory (Bar Harbor, ME) were housed in a pathogen-free barrier facility (12 h light/12 h dark cycle) and fed rodent chow (Ralston Purina, St. Louis, MO). For high-fat diet experiments, mice were fed a Western-type diet containing 21% fat by weight (TD88137, Harlan Teklad, Madison, WI). All experiments were approved by the Committee on Animal Research, University of California, San Francisco.

Histology

Samples of adipose tissue were obtained from reproductive fat pads of 14-week-old male mice for chow experiments and 16-week-old mice for high-fat experiments. The same region of the fat pad was used for all animals to minimize cell size variation due to differences in anatomical location (4). The samples were fixed in paraformaldehyde, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin.

Image analysis

The histology sections were viewed at 10× magnification, and images were obtained with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI). The images were converted into a binary format with Adobe PhotoShop 5.0.1 (Adobe Systems, San Jose, CA) and Image Processing Tool Kit (Reindeer Games, Gainesville, FL). The following commands were used for the conversion: Gray Mode, Threshold, and Watershed. The binary black and white images were compared with the original images to ensure an accurate conversion. Minor adjustments, if needed, were made with the following commands: Erode and Paintbrush. The total number and cross-sectional areas of adipocytes were calculated with the command Measure All. Cross-sectional areas were expressed by the computer as mm^2. Results were directly loaded into a spreadsheet program (Excel, Microsoft Inc., Redmond, WA) for analysis. Because each millimeter of the digital image equaled ~50 μm, the calculated areas

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were multiplied by a conversion factor of 2,500 (50^2) to determine the cross-sectional area of the adipocytes in μm^2. Values less than 100 μm^2 were assumed to represent artifacts from the image-conversion process and were excluded from analysis.

Triglyceride and DNA measurement

Triglycerides of an entire reproductive fat pad were measured with a kit (A320, Sigma Chemical Co., St. Louis, MO) as described (5). DNA was extracted with phenol: chloroform:isoamyl alcohol (Sigma), precipitated with ethanol, and quantified with a spectrophotometer at 260 nm.

Statistical methods

Data are shown as mean ± SD. Measurements of mean adipocyte surface area were compared with Mann-Whitney rank-sum test.

RESULTS

To test the validity of our method, we used it to study a well-characterized phenomenon, the increase in adipocyte size in response to a high-fat diet (2, 4). Adipocytes from the reproductive fat pads of male mice fed a high-fat diet for 12 weeks were larger than those of mice fed a chow diet (**Fig. 1A** and **B**). The image conversion process produced accurate binary representations of the histological images (Fig. 1C and D).

These binary images were analyzed to determine the cross-sectional surface area of each adipocyte. Mice fed a high-fat diet had a greater number of large adipocytes than mice fed a chow diet (**Fig. 2A** and **B**). This difference in frequency distribution was reflected in a ~2.5-fold increase in mean surface area of adipocytes from mice fed a high-fat diet (Fig. 2C). Similar findings were observed in female mice (not shown).

We compared our method with a technique commonly used to quantify adipocyte size, the calculation of the triglyceride-DNA ratio of a sample of adipose tissue. Reproductive fat pads from mice fed a high-fat diet had a mean triglyceride-DNA ratio that was ~1.9-fold greater than that of fat pads from mice fed a chow diet (Fig. 2D). These findings suggest that mice fed a high-fat diet had larger adipocytes. Thus, the results obtained with computer image analysis were consistent with those obtained with triglyceride-DNA measurement.

DISCUSSION

This study demonstrates the use of computer image analysis for quick and accurate determination of adipocyte size. In agreement with published results (2, 4), our method showed an increase in mean adipocyte size as well as a shift in size distribution toward larger adipocytes in mice fed a high-fat diet. The results obtained with computer image analysis also correlated with those obtained with a method frequently used to measure adipocyte size, the calculation of triglyceride-DNA ratio.

Several methods for determining adipocyte size have been described, each with its advantages and disadvantages. A relatively quick method involves the measurement of triglyceride and DNA contents of an adipose tissue, with the estimated adipocyte size expressed as triglyceride content per DNA content. This method most likely underestimates adipocyte size, because the DNA measurement also reflects the presence of pre-adipocytes and stromal-vascular cells, which may proliferate in response to high-fat feeding. This underestimation may account for the smaller magnitude of increase in adipocyte size detected by triglyceride and DNA measurement than by computer image analysis in the current study. Adipocytes can be separated from stromal-vascular cells with collagenase treatment, but this usually results in the breakage of large adipocytes (6). The calculation of triglyceride-DNA ratio also does not provide information regarding the size distribution of cells. Because computer image analysis measures the size of individual adipocytes, this method has the advantage of determining cell size distribution, as well as mean cell size.

One widely used technique for measuring adipocyte size was originally described by Hirsch and Gallian (6) and subsequently modified by others (7–10). In this method, adipocytes are fixed in osmium tetroxide and counted electronically. The mean adipocyte size is then calculated by dividing the lipid weight of a comparable sample by the electronically determined cell number. A major advantage of this method is that it provides a direct measurement of adipocyte number and, depending on the counter used, adipocyte size. Disadvantages include

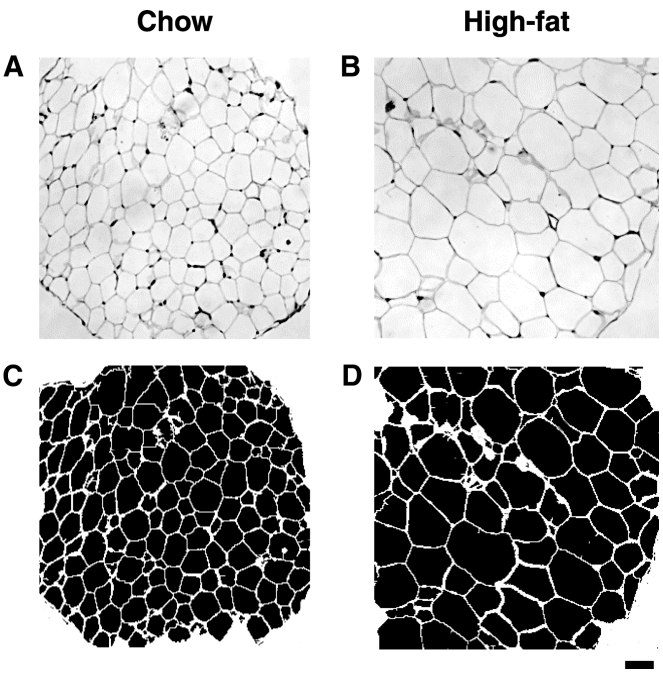

Fig. 1. Conversion of histological images into binary representations. A and B: Hematoxylin and eosin-stained sections of adipose tissue. Images captured at 10× magnification. C and D: Computer-generated binary images of A and B. Each image was generated in less than 5 min. Bar represents 50 μm.

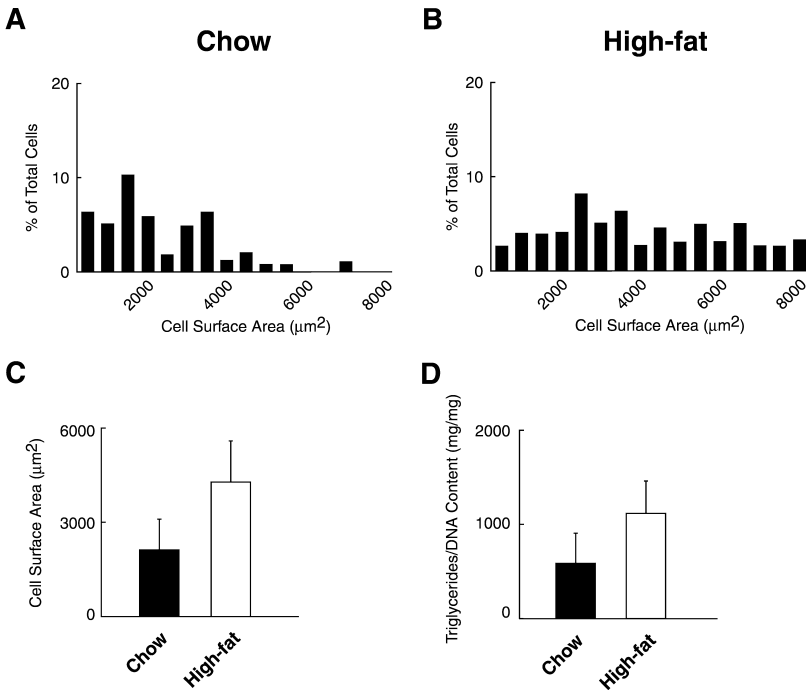

Fig. 2. Increased adipocyte size in mice fed a high-fat diet. A: Frequency distribution of adipocyte cell surface area from mice fed a chow diet. B: Frequency distribution of adipocyte cell surface area from mice fed a high fat diet. C: Mean surface area of adipocytes. n = two per group. >150 cells were measured for each mouse. D: Triglyceride-DNA ratio of adipocytes. n = three per group.

the expense of the electronic counters and the toxicity of osmium tetroxide. Osmium may also cause cell swelling and result in an overestimation of adipocyte size (6).

Direct measurement of adipocyte diameter by microscopic examination is another frequently reported method (4, 11, 12). With this technique, fixed histological sections of adipose tissue can be used to determine adipocyte size. This method is inexpensive and minimizes cell distortion caused by methods described previously. However, it is tedious. Furthermore, the volume of an irregularly shaped adipocyte cannot be easily or accurately calculated from its diameter. The measurement of adipocyte cross-sectional surface area provides a better estimation of cell volume and has been used in several recently published studies (2, 3). This method appears to provide accurate results. However, because it involves the manual tracing of adipocytes, the method is time consuming and may produce results that are operator-dependent. Our method overcomes most of the problems associated with the measurement of adipocyte diameter or surface area by microscopy. It directly measures cell surface area, which is a better representation of adipocyte size than cell diameter. More importantly, because the method is computerized, a large number of adipocytes can be quickly and reliably measured. The distribution and mean values of adipocyte surface area obtained with our method correlate well with those derived from the manual tracing of adipocytes (2).

An alternative to examining fixed histological sections is to measure the size of freshly prepared adipocytes isolated by collagenase digestion. Although collagenase treatment produces adipocytes that are circular and uniform in shape, such treatment distorts measurement results by causing the lysis of large adipocytes, as reported in the literature (6) and observed in our personal experience. Moreover, results obtained by the examination of unfixed, free-floating adipocytes cannot be easily reproduced or verified. Thus, we believe that measuring adipocyte surface area of paraffin-embedded tissues may be more advantageous and more convenient than examining the surface area of collagenase-treated adipocytes.

In addition to determining adipocyte size, our method can measure the number of adipocytes in a fixed histological section of adipose tissue. However, it may not be a practical or accurate method to determine the total number of adipocytes in a fat pad. Such a determination would require either histological examination of the entire tissue or mathematical corrections that likely will introduce errors and distort the results.

Determination of adipocyte size by computer image analysis has several limitations. One limitation, common to all methods that rely on examination of histological images, is that poor fixation of samples will distort measurements of adipocyte size. Another potential distortion, crush artifacts introduced during tissue processing, can be minimized by measuring a large number of samples, which can be easily achieved by this rapid procedure. In addition, the image conversion process, although fairly accurate, can introduce artifacts, especially around the borders. In theory, all artifacts can be removed before data analysis. However, this may be tedious and time consuming, potentially eliminating one main advantage of this method. To minimize the distortion caused by artifacts, we excluded values less than 100 μm^2 in our analysis. Such a threshold should eliminate most of the values generated by artifacts without significantly affecting the measurement of actual adipocytes.

In summary, we report a new method for determining adipocyte size by measuring the cross-sectional area of adipocytes with computer image analysis. This method al-

lows a large number of adipocytes to be measured rapidly and accurately with computer hardware and software that are readily available. Computer image analysis should provide another useful tool for studying the biology of adipocytes.

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