Adipocyte 1:4, 242–245; October/November/December 2012; © 2012 Landes Bioscience

Implications of ^{2}H-labeling of DNA protocol to measure in vivo cell turnover in adipose tissue

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Adipose tissue expansion in obesity involves a series of cycles of adipocyte hyperplasia, hypertrophy and hypoplasia due to alterations in adipogenesis, adipocyte cellular metabolism and cell death, respectively. Increased frequency of these cycles may lead to deterioration of adipocyte function and viability, accelerated exhaustion of the adipocyte progenitor pool and extensive adipose tissue remodeling, all leading to impaired expandability of subcutaneous adipose tissue, ectopic lipid accumulation and insulin resistance. Understanding the mechanisms that contribute to adipocyte turnover is thus important. We have recently refined and published an existing method to assess in vivo adipogenesis using incorporation of the stable isotope deuterium into the DNA of isolated adipocytes and adipocyte progenitors from adipose tissue. In this commentary, we highlight further implications of this method to determine the rate of adipocyte hypertrophy and adipocyte death that will enhance our understanding of adipocyte cell turnover and cellular mechanisms that control regional adipose tissue growth.

Keywords: adipose tissue, adipocyte, adipogenesis, apoptosis, cellular turnover, obesity, fat distribution, stable isotope, deuterium

Abbreviations: ^{2}H, deuterium; SV, stromal vascular; f_{AD}, fractional replacement rate of DNA from adipocytes or fractions of newly formed and enlarged adipocytes; f_{aSV}, fractional replacement rate of DNA from plastic adherent stromal vascular cells or fractions of newly replicated adipocyte progenitors plus newly differentiated small adipocytes

Submitted: 02/09/12
Revised: 05/18/12
Accepted: 05/21/12
http://dx.doi.org/10.4161/adip.20817

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Commentary to: Tchoukalova YD, Fitch M, Rogers PM, Covington JD, Henagan TM, Ye J, et al. In vivo adipogenesis in rats measured by cell kinetics in adipocytes and plastic-adherent stroma-vascular cells in response to high-fat diet and thiazolidinedione. Diabetes 2012; 61: 137–44; PMID:22124466; http://dx.doi.org/10.2337/db10-1768

In obesity, adipose tissue expands as a result of overall energy surplus through increase in adipocyte size (hypertrophy) and number (hyperplasia), but individuals vary by the predominant cellular mechanisms involved. Arner et al. have introduced the adipocyte morphology value, defined as the difference between the measured adipocyte size and the expected size given the curve-linear fit for a given body fat mass, to reflect the primary mechanisms involved in adipose tissue expansion. They show that relatively greater subcutaneous abdominal adipocyte hypertrophy is linked to insulin resistance and lower total adipocyte number, independently of sex and adiposity levels.[1] Analysis of the cellular mechanisms that are involved in adipocyte turnover is, therefore, necessary to enhance our understanding of the significance of the adipocyte morphology for metabolic health.

An established approach to study the contribution of adipocyte hypertrophy and hyperplasia to adipose tissue growth is to determine mean adipocyte volume or mean lipid content and calculate the fat cell number from the ratio of fat depot volume to mean adipocyte volume or total lipid content of the adipose tissue depot to the mean lipid content per fat cell. Recently, more sophisticated analyses of the fat cell size distribution in human cross-sectional and longitudinal studies have been used simultaneously with expression analysis of adipogenic genes to imply adipogenesis.[2,3] Furthermore, in animal models, adipocyte size-distribution snapshots taken at different time points during high-fat dietary treatment using a cross-sectional design[4] or obtained longitudinally by serial micro-biopsies of inguinal adipose tissue depots[5] were able to show the dynamics of adipocyte cellularity with weight gain. Interestingly, both studies report an oscillatory pattern of adipocyte cellularity, comprised of consecutive hyperplastic, hypertrophic and hypoplastic phases, presumably due to alterations in adipogenesis (replication of adipocyte progenitors and preadipocytes-to-adipocyte differentiation), lipid metabolism in mature adipocytes and

adipocyte death, respectively. Though these approaches yield valuable information, they are not applicable to study human subjects due to the ethical consideration of their invasiveness.

We have refined a novel method to assess in vivo adipogenesis using the incorporation of the stable isotope deuterium (^{2}H) into the DNA of isolated adipocytes and stromal vascular (SV) fractions from adipose tissue[6] that was originally developed by Dr Marc Hellerstein at UC Berkeley.[7] Specifically, we have purified the isolated adipocytes from potential other cellular contaminants via a negative immunoselection and have determined the ^{2}H-enrichment of their DNA relative to the ^{2}H-enrichment of the DNA of bone marrow cells, which are used as a reference for complete cell turnover. The ratio of the ^{2}H-enrichment of the DNA from immunopurified adipocytes to that from bone marrow cells indicates the fractional replacement rate of the DNA from adipocytes or the fraction of new terminally differentiated (mature) adipocytes (f_{AD}). In addition, we have enriched the isolated SV cells, which are used with adipocyte progenitors and small adipocytes, which have not reached sufficient buoyancy and have remained in the SV fraction, by a short-time culturing to take advantage of their property to attach quickly to plastic surfaces. Measurement of the fractional replacement rate of DNA from the plastic-adherent SV subfractions (f_{aSV}) thus reflects the proliferation of adipocyte progenitors plus early differentiation of adipocytes, which are yet small in size. Overall, this method provides an advantage over the in vitro techniques for assessment of adipogenesis, as it provides an integrative evaluation of adipogenesis within the natural microenvironment of the adipose tissue.

We have observed that f_{aSV} cells have higher ^{2}H-incorporation than f_{AD} and correlates with weight gain, suggesting that it is a feasible and accurate reflection of the hyperplastic phase of the adipose tissue expansion cycle. Consequently, the measurement of the f_{AD}, although theoretically valuable for the assessment of the rate of terminal differentiation, may require long labeling periods that limits its value for assessment of the dynamics of adipocyte hypertrophy. Yet, it has been previously shown that the rate of enlargement of adipocytes is proportional to the lipid flux available for storage, i.e., the difference between the lipid load and the storage capacity of the adipocytes.[5] Therefore, measurement of the storage and removal of triglycerides can reliably substitute the f_{AD}. The ^2H_2O-labeling method has been utilized to study the triglyceride synthesis, de novo lipogenesis and lipolysis in humans by measuring the ^{2}H-enrichment of the triglyceride-glycerol and individual fatty acids followed by mass isotopomer distribution analysis (MIDA).[7,8] Thus, combining measurements of the f_{aSV} and lipid turnover will yield information regarding the hyperplastic and hypertrophic aspects of adipose tissue growth.

Recently, Spalding et al. introduced an in vivo method to study fat cell and lipid turnover in humans by measuring the incorporation of atmospheric ^{14}C derived from above ground nuclear bomb tests in adipocyte DNA.[9,10] It is noteworthy that the lipid turnover rates calculated by the ^2H_2O- and the ^{14}C-labeling methods are comparable, verifying the reliability of both approaches. While ^{14}C-labeling method allows for retrospective analysis over a long period of time (years), the ^{2}H-labeling of DNA from adipocytes can provide measurement of adipocyte turnover for a shorter duration of time (months). Hence, the latter approach is advantageous, in that it may be utilized in a variety of interventional studies, i.e., diet, exercise and drugs.

In addition to adipogenesis, the elimination of old and/or distressed cells via programmed cell death (apoptosis) and/or necrosis is an equally important aspect of cell turnover to regulate adipocyte number. Strissel et al. demonstrate that increased death of large adipocytes is the last event in the adipocyte morphological cycle of adipose tissue expansion in mice following adipocyte hypertrophy.[4] There is limited evidence to show that adipocyte apoptosis is a form of adipose tissue remodeling in response to increased energy intake (high fat diet) and an inflammatory microenvironment that is associated with limited adipose tissue expandability, which is linked to metabolic complications.[4,11,12] Adipocyte death is increased in obese rodents and humans and modulates obesity-associated infiltration of macrophages.[13,14] Overall, the role of preadipocyte/adipocyte loss in obesity and metabolic complications, as well as the analysis of molecular mechanisms that control adipose tissue apoptosis in human obesity requires further studies. Hence, an in vivo method to assess the disappearance rate of adipocytes and preadipocytes will share all the aforementioned benefits, as compared with the current in vitro approaches used to assess adipose tissue apoptosis, including immunohistochemistry, preadipocyte cultures and gene expression. The atmospheric ^{14}C-labeling method[9] reports a 10% turnover of adipocytes per year and an invariable fat cell number in obese individuals with early onset obesity, suggesting that adipocyte death matches the generation of adipocytes. However, assessment of adipocyte death in vivo in subjects with onset of obesity later in life has not been performed. Analyzing the loss of ^{2}H-enrichment in the DNA of adipose cells after discontinuing the ^2H_2O-labeling protocol could occur exclusively by cell loss and thus represents an alternative valuable tool in understanding the adipocyte cell death in vivo. Overall, the combined measurement of in vivo adipogenesis, lipid turnover and adipocyte/preadipocyte loss using the ^2H_2O-labeling protocol in humans will yield novel information regarding adipose tissue cell turnover and the characteristics of adipocyte morphological cycles during adipose tissue expansion in vivo.

An important unfavorable consequence of adipose tissue remodeling with advancing of the obesity is the inhibition of preadipocyte differentiation by hypertrophic adipocytes, local inflammation and hypoxia,[15-17] combined with enhanced proliferation of preadipocytes.[18,19] Therefore, the adipocyte hyperplasia in each consecutive round of the cellular cycle of adipocyte morphology would be achieved with incremental less efficient preadipocyte differentiation, coupled with increased requirements for proliferation. Frequent cycling will thus promote replicative senescence of adipocyte progenitor cells with gradual impairment of function.

Indeed, decreased replicative potential, premature cellular senescence and loss of the multi-lineage differentiation potential of adipose tissue-derived mesenchymal cells from patients with morbid obesity compared with lean people were recently reported.[20] The detrimental result of these events is inadequate expandability of adipose tissue. Thus, analyzing the patterns of the adipocyte morphological cycles will provide insight to determine the functional capacity of human subcutaneous adipose tissue to store excess lipids.

The ^2H_2O-labeling method also has significant future implications that will enhance our understanding of cellular mechanisms involved in human regional adipose tissue growth and development of distinct fat distribution phenotypes. Spalding's calculation of cell turnover in vivo used samples from only the subcutaneous abdominal depot.[9] However, cross-sectional comparisons of adipocyte cellularitybetween depots suggest depot-specific variation in the involvement of adipocyte hypertrophy and hyperplasia. For example, in obese women, hyperplasia occurs in the order: subcutaneous femoral > subcutaneous abdominal > omental depots.[21,22] In response to high fat diet, the subcutaneous abdominal adipose depot of males and females expands by adipocyte hypertrophy, whereas the growth of the subcutaneous femoral depot is due to adipocyte hyperplasia.[23] Furthermore, the adipocyte morphology differs between mouse strains[24] and between men and women.[22] Given the oscillatory nature of the dynamics of adipocyte morphology with adipose tissue growth, these data may represent separate facets of this process. In addition, depot and sex differences in the rates of adipose tissue remodeling and adipocyte death have also been observed in diet-induced and genetic models of obesity in rodents.[4,25,26] It is also well documented that differences in regional fat distribution affect metabolic parameters in humans.[27-30] We are currently utilizing this in vivo method in a human study to analyze the adipogenic properties and cellular mechanisms that contribute to depot-specific differences in subcutaneous adipose tissue development and their relation to ectopic lipid accumulation and insulin sensitivity. Analysis of the depot- and sex-differences in the magnitude and frequencies of the adipocyte morphological cycles will provide insight to the mechanisms that control the fat distribution phenotypes in obesity and the link to obesity-related metabolic complications.

Acknowledgments

This work is supported by grant DK 090607.

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