MEASURING ADIPOCYTE PROLIFERATION IN VIVO USING $^2\text{H}_2\text{O}$
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Abstract
The current working hypothesis of fat cell development posits that obesity reflects an increase in fat cell size, number, or both, however, loss of fat is thought to occur only through a decrease of fat cell size, not cell number. To begin to investigate this model, we have developed a technique for measuring adipocyte proliferation and turnover in vivo. Male Sprague-Dawley rats were primed to 2% $^2\text{H}_2\text{O}$ in body water via injection and given 4% $^2\text{H}_2\text{O}$ in drinking water. Deoxyadenosine was isolated from DNeasy spin columns and derivatized to deoxyribose-aldonitrile-triacetate for GC-MS analysis. After five weeks of labeling, mature adipocytes were 23.8% new (i.e., had undergone 5 phase during $^2\text{H}_2\text{O}$ exposure) and preadipocytes were 52.4% new. In conclusion, proliferation of adipocytes and preadipocytes can be directly measured in vivo in adult mammals without use of radioactivity, and may be useful for non-toxic characterization of adipocyte development in humans in vivo.

Background
• Variations in body fat mass can be due to differences in fat cell size (hypertrophy) or fat cell number (hyperplasia).
• The occurrence of adipocyte proliferation in adult humans has remained controversial.
• Identification of preadipocytes in the stromal-vascular fraction of adipose tissue in adult humans provides a potential source of new fat cells.
• No technique has been available for directly measuring proliferation (cell division) of adipocytes in humans, however.
• Toxins preclude use of DNA labeling agents ($^3\text{H}$-thymidine or bromodeoxyuridine, particularly for long-term labeling (as is necessary for slow turnover cells).
• We recently described a non-radioactive (stable) isotopic method for measuring cell proliferation, in vivo, by labeling the deoxyribonucleotide (dR) moiety of DNA through the de novo synthesis pathway from $^2\text{H}$-thymidine (Fig 1; Ref 1).
• Here we describe a modification of this technique using $^2\text{H}_2\text{O}$ to label dR (Fig 1), to measure proliferation of adipocytes in vivo.

Methods

Preparation of DNA for Gas Chromatography-Mass Spectrometric (GC-MS) analysis
• Epimidal and retroperitoneal fat pads were resected at necropsy.
• Mature adipocytes and vascular-stromal cells were dissociated with collagenase via agitation at 37°C for 45-60 minutes (Ref 2, 5).
• Separation occurred by flotation of less dense mature adipocytes (Fig 4).
• Bone marrow cells were isolated as index of fully-replaced (100% turned-over) tissue (Ref 4).
• DNA was isolated from the cell fractions using DNeasy tissue kit (Qiagen) and enzymatically hydrolyzed to yield deoxynucleobases (Ref 4).
• Deoxyadenosine was isolated using LC-18 SPE columns (Supelco) and derivatized to deoxyribose-aldonitrile-triacetate for GCMS analysis (m/z 198, 199, 200).

Preparation of fatty acids to measure mass isotope pattern
• Initial effluent from DNeasy spin columns were frozen for sampling of solidified lipids.
• Lipids were extracted by Folch method and TG were isolated using TLC.
• TG were transensterified for GC-MS analysis of palmitate-methyl esters (m/z 270, 271, 272).
• Enrichment of precursor pool ($^2\text{H}_2\text{O}$ in body water) was calculated by MIDA (Ref 1, 3).

Calculation
1) Fraction of newly divided cells in adipose tissue

$\text{dR enrichment (EM1)} = \frac{x - 0.0011 \times 0.0053 \times 0.0219 c}{0.0883 - 0.0178}$

where x represents EM1/EM2 of palmitate

Results
• Adipocyte proliferation can be measured in vivo by administration of $^2\text{H}_2\text{O}$. Differences exist in proliferation of mature adipocyte and vascular-stromal cells in fat depots, consistent with a precursor-product relationship.
• Differences exist in cell proliferation and de novo lipogenesis between epimidal and retroperitoneal fat deposits.
• This approach can easily be used for measurement of adipocyte proliferation, and de novo lipogenesis, in human fat depots.

Conclusions

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References
2) Swierczewski E. 1983. Measurement of cell proliferation by labeling of DNA with $^3\text{H}$-thymidine (Ref 1).