Animal Science 2005, **80:** 239-240
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Animal Science

Topical Note

Adipocytes may not be a terminally differentiated cell type: implications for animal production

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Mature adipocytes may not be a terminally differentiated cell form, as previously thought by about everyone in science. Instead, these adipocytes may be capable of re-entering the cell cycle and forming proliferative-competent precursor cells like pre-adipocytes, adipofibroblasts, or even other forms of cells. Why is this possibility important to animal science? The main reason is in directing the cellularity of adipocyte depots in meat animals. For example, we know that the fat characteristics of beef animals are a direct function of the 'cellularity' of cells committed to form adipocytes. Beef steers given a high-concentrate diet commonly possess fewer adipocytes in any given fat depot, but most of the cells are completely filled with lipid to capacity. Alternatively, steers on a high-roughage diet seem to possess more fat cells, but fewer of them are filled to capacity with storage lipid. A variety of systemic and local regulators have been implicated in altering the cellularity of any given fat depot. Major regulators appear to be growth hormone, any number of repartitioning agents, as well as adipocytokines produced by the cells within the fat depot themselves. The specific mechanisms involved in directing cellularity of any specific fat depot, however, are poorly understood.

Adipogenesis refers to the process of development of a fat cell. Therefore, adipogenesis reflects a process of development whereby cells of the adipose lineage proliferate to expand the cell population, stop proliferating and become committed to expressing cellular and molecular markers for the mature fat cell phenotype. Subsequently these cells are capable of metabolizing lipid (lipogenesis and lipolysis; Fernyhough *et al.* (2005)). A huge research effort has been expended on determining the regulation of different stages of adipogenesis, in order to learn about cellular regulation, determine mechanisms of energy partitioning and facilitate development of lipostatic agents. Cells used for these types of studies commonly are cell lines (e.g. 3T3-L1 cells) and stromal vascular cells (reviewed in Fernyhough *et al.* (2005)). Whereas the regulators, cellular/molecular markers and patterns of adipogenesis are well understood, less is currently known about whether the process of adipogenesis is reversible.

Adipocytes provide energy through lipolysis during low energy intake. However, is it possible for mature adipocytes to enter lipolysis, whereby lipid is mobilized for some other metabolic function, reinitiate the mechanisms involved in proliferation, and then actually divide? Studies, concluded nearly 30 years ago, suggested that mature adipocytes could be induced to undergo lipolysis, reinitiate proliferative mechanisms and begin to proliferate in vitro (Adebonojo (1975a and b) reviewed in Fernyhough *et al.* (2005)). Studies on reverse adipogenesis were conducted sporadically over the next 10 to 20 years (Sugihara *et al.*, 1986, 1987 and 1989; Justesen *et al.*, 2004) but few total studies were published on this mechanism because of the lack of available efficient culturing methodology. Ceiling culture techniques were devised in the 1980s, which allowed mature fat cells to be cultured with some repeatability (Sugihara *et al.*, 1986, 1987 and 1989). Using this method, we have observed mature adipocytes initiating the proliferation programme in cultures derived from bovine adipose tissue (see Figure 1).

The method of obtaining mature adipocytes was as follows: subcutaneous adipose tissue was excised and transported to the cell culture laboratory where it was chopped, enzymatically digested for 1 h, filtered to remove tissue debris, and centrifuged for 10 min (Beckman TJ-6 centrifuge with a TH-4 horizontal rotor) at 1400 × g. The underlying pellet and enzyme solution were removed and the remaining fatty layer (containing mature adipocytes) was rinsed and centrifuged. This process was repeated an additional two times to liberate and pellet the stromal vascular cells (all cells other than mature adipocytes). After the final rinse, the upper layer was placed in ceiling culture, whereby mature (lipid filled) adipocytes floated to the surface and were adhered to the surface. This process took 5 days. After this time, the cells were liberated from the flask and differential plating was performed further to purify the culture as well as provide the clonal densities needed to record cell division. After inversion of the flask (approx. 1 day after plating) the basal medium was changed to DMEM + 100 ml/l foetal bovine serum (FBS) and the adipocytes were evaluated with photomicroscopy for an additional 4 days. The cell in Figure 1 was captured in the process of cell division. Each of the daughter cells contain approximately half of the original cell's compliment of lipid. This process of symmetric cell division, as well as asymmetric cell division (a process whereby one of the daughter cells retains all or almost all of the original cell's complement of lipid), has been observed and recorded on numerous occasions in our laboratory (Fernyhough *et al.*, 2005; M.E. Fernyhough unpublished observations) as well as by other laboratories (Justesen *et al.*, 2004; Cancello *et al.*, 2005).

If mature adipocytes are capable of undergoing lipolysis to a stage at which re-entry to cell cycle does occur, what might be the physiological relevance of this process? Determining the molecular cues that induce adipocytes to enter lipolysis and reinitiate the proliferative cellular programme is an area of research with large potential benefits for animal science. More ingenious methods might be defined to help producers regulate desirable fat depots whereas other, more undesirable, fat depots may not become filled at all. Thus, it may be possible to direct the cellularity of specific adipose depots in order to generate more desirable meat products for consumers. Presently, we do not know if there are differences in the propensity of cells to dedifferentiate, based on developmental age, sex, nutritional status, or depot location.

Another important consideration that we presently do not understand is that different populations of cells may be contained within the adipose lineage. For example, could fibroblast-type cells undergo lipogenesis (storing at least some lipid, as most cell types do, including muscle, hepatocytes and steroidogenic cells) and lipolysis while retaining their general fibroblast phenotype? Such a cell has been proposed and termed an adipofibroblast. In other words, loss of lipid and resumption of proliferation may actually occur only in cells that are not terminally differentiated. Other cells within fat tissue may actually represent what we would normally consider terminally differentiated adipocytes that are capable of entering a programme of marked lipolysis but without the capacity to re-enter the cell cycle.

Our preliminary observations provide support for the conduct of cloning studies to evaluate the specific characteristics of individual cells within any given adipose depot. We are presently examining the regulation of lipogenesis in progeny cells (cells derived from the proliferation of once-mature adipocytes) *in vitro*. We are also comparing the capability of progeny cells to proliferate and differentiate to that of traditionally isolated stromal vascular cells. Of some interest to us is the fate of the storage triglyceride within the mature adipocyte. If lipid is 'lost' from the cell in order to assume a proliferative state, then the cell may be using the lipid for synthesis of new cell membrane phospholipids, oxidation of fatty acid for ATP generation, or mobilizing and secreting fatty acids into the media (i.e. exporting energy to adjacent cells). While a specific application of this research may take some time in order to become applied, a greater understanding is needed of the factors that induce adipocytes to enter lipolysis and that might subsequently re-initiate adipogenesis.

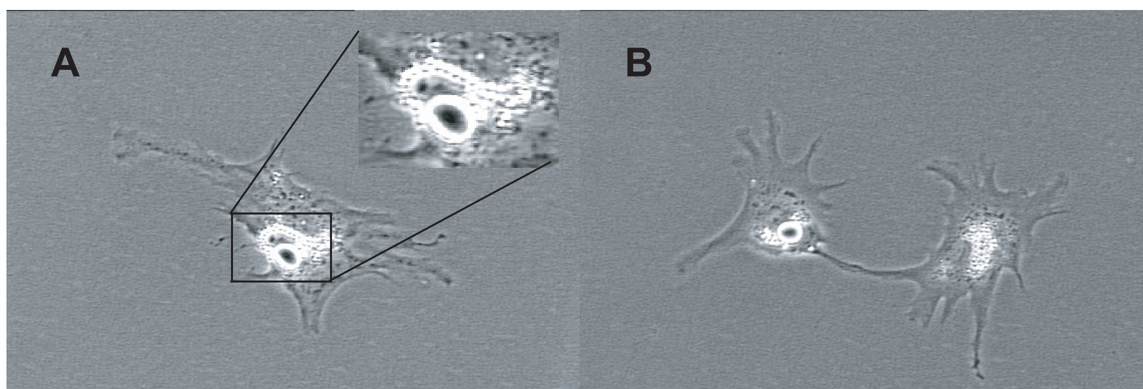

Figure 1 Proliferation of mature adipocytes *in vitro*. The photomicrographs were captured at 200 × magnification with a NIKON inverted Diaphot microscope, equipped with Sony RGB (0·6 in chip) camera and OPTIX image analysis system. Panel A: the morphology of a lipid-containing mature adipocyte after 3 days on DMEM + 100 ml/l FBS (200 × magnification). The inset is an enlarged nuclear area to show detail. Panel B: after 4 days of exposure to DMEM + 100 ml/l FBS, the one original adipocyte had divided to produce daughter cells. Each daughter cell possessed roughly half the complement of lipid of the parental cell although one cell retains the large lipid drop whereas the other cell received many small droplets (200 × magnification).

References

Adebonojo, F.O. 1975a. Studies on human adipose cells in culture: Relation of cell size and cell multiplication to donor age. *The Yale Journal of Biology and Medicine* **48:** 9-16.

Adebonojo, F.O. 1975b. Monolayer cultures of disaggregated human adipocytes. *In Vitro* **11:** 50-54.

Cancello, R., Pietri-Rouxel, F. and Clement, K. 2005. Spontaneous lipid accumulation in primary cultures of dedifferentiated human adipocytes. *Adipocytes* **1:** 73-78.

Fernyhough, M.E., Bucci, L.R., Hausman, G.J., Antonio, J., Vierk, J.L. and Dodson, M.V. 2005. Gaining a solid grip on adipogenesis. *Tissue and Cell* In press.

Fernyhough, M.E., Helterline, D.I., Vierck, J.L., Hausman, G.J., Hill, R.A. and Dodson, M.V. 2005. Dedifferentiation of mature adipocytes to form adipofibroblasts: More than a possibility. *Adipocytes* **1:** 17-24.

Justesen, J., Pedersen, S.B., Stenderup, K. and Kassem, M. 2004. Subcutaneous adipocytes can differentiate into bone-forming cells *in vitro* and *in vivo. Tissue Engineering* **10:** 381-391.

Sugihara, H., Funatsumaru, S., Yonemitsu, N., Miyabara, S., Toda, S. and Hikichi, Y. 1989. A simple culture of fat cells from mature fat tissue fragments. *Journal of Lipid Research* **30:** 1987-1995.

Sugihara, H., Yonemitsu, N., Miyabara, S. and Toda, S. 1987. Proliferation of unilocular fat cells in primary culture. *Journal of Lipid Research* **28:** 1038-1045.

Sugihara, H., Yonemitsu, N., Miyabara, S. and Yun, K. 1986. Primary cultures of unilocular fat cells: Characteristics of growth *in vitro* and changes in differentiation properties. *Differentiation* **31:** 42-49.