RELATIONSHIP BETWEEN ADIPOSE CELL SIZE AND LIPOGENESIS IN OVINE ADIPOSE TISSUE

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INTRODUCTION

Adipose tissue has the capacity to store excess calories in the form of triacylglycerols. However, in animals which are killed for meat, this specialized function may result in carcasses with deposits of excess fat. This fat often has to be manually trimmed from certain cuts of meat and is of economic concern to both producers and processors of meat-producing animals.

Adiposity can occur through an increase in adipose cell number (hyperplasia), cell size (hypertrophy) or both. In the sheep, adipose cell size and number increase for the first 11 months, whereas beyond this age further accumulation of fat is due to filling of existing cells with lipid (Hood and Thornton, 1979). The general conclusion of two reviews (Allen, 1976; Hood 1977) is that adiposity in extramuscular fat depots of sheep, cattle and pigs results primarily from hypertrophy rather than hyperplasia of adipose cells.

Using adipose cell populations isolated from animals of different age or weight, relationships have been drawn between adipose cell volume and the rate of lipid synthesis (Smith, 1971; Hansen et al., 1974; Glumann and Vinten, 1974; Hood and Allen, 1975). In these studies it is difficult to attribute changes in lipid synthesis solely to the effects of adipose cell size, as other variables (e.g. age, adiposity, nutritional status) compound the experiment. True effects of adipose cell size can only be determined on single heterogeneous populations of adipose cells from a single adipose tissue.

This paper discusses the use of a technique (Hood and Thornton, 1980) to determine the relative incorporation of radioactive acetate into lipid in adipose cells of varying sizes within a population of cells isolated from a single sample of ovine adipose tissue.

METHODS

Sheep were selected with liveweights of either 33 or 49 kg (designated as groups A and B respectively) from a line of large framed Merino wethers. Three sheep in each group were fed lucerne chaff at less than _ad libitum_ for 15 days, then combined in a single pen and offered the same diet of lucerne chaff _ad libitum_ for approximately 40 days, that is, until slaughter.

The order of slaughter on a particular day was randomized. The sheep were stunned with a captive bolt pistol and the blood vessels of the neck severed. The pelt was removed from around the base of the tail and over the shoulder and the abdominal cavity and brisket opened to permit immediate sampling of adipose tissue from the subcutaneous (rump and shoulder), omental (adjacent to the pyloris), perirenal and brisket depots.

Tissue slices (<1 mm thick) of adipose tissue (200-300 mg) were rinsed in isotonic NaCl at 37°C to remove free lipid released from adipose cells which had ruptured during slicing. The slices of adipose tissue were dried on filter paper, weighed and incubated with 10 μCi of acetate-1-^{14}C (Hood et al., 1972). After 2 hours each tissue slice was washed with 0.154 M NaCl to remove surface ^{14}C-acetate and the adipose tissue slice fixed with 3% osmium tetroxide (Hirsch and Gallian, 1968). After a minimum of 72 hours fixation, the adipose cells were isolated (Hirsch and Gallian, 1968).

Adipose cells were serially separated into groups according to their diameters using plastic Nalgene filter units (No. 245/0045) after removal of the plastic bases and the Millipore filters. The modified filters can be fitted together, such that a nylon screen, supported by the grid on the lower unit, can be held in place by the upper unit (Fig. 1). Ten nylon screens (Swiss Screen Pty. Ltd., Sydney) were placed, in order of decreasing pore size (250,223,202,183,153,130,102,80, 53 and 25 μ), on the grids of each filter unit. The arrangement of filter units is shown in Figure 1. The isolated population of adipose cells was washed onto the upper screen (250 μ) and eluted through the 10 screens with distilled water to separate the cells into groups on the basis of diameter. The adipose cells from each screen were washed into weighed scintillation counting vials with distilled water and the fixed cells allowed to settle. Most of the water was removed with a Pasteur pipette and the last traces removed by drying over anhydrous silica gel in a dessicator for 24 hours. When the nylon screens were properly seated on the plastic grids of the filter units and the cells are rinsed with copious amounts of distilled water the distribution of adipose cells calculated from the weight of cells on each screen was similar to that obtained using data from a Coulter electronic counter (Hood and Thornton, 1980).

The number of cells on each screen was calculated from the weight of osmium-fixed adipose cells (assumed density of 1.1 g/ml) and the calculated average volume of cells collected between successive screens. After decolorising the osmium-treated cells with hydrogen peroxide (Etherton et al., 1977), the radioactivity in each counting vial, that is for a known number of cells, was measured using a Packard 2660 liquid scintillation counting system.

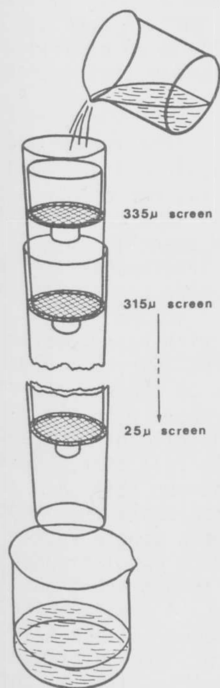

Figure 1. Schematic diagram of the arrangement of the ten filter units used in the separation of osmium tetroxide-fixed adipose cells.

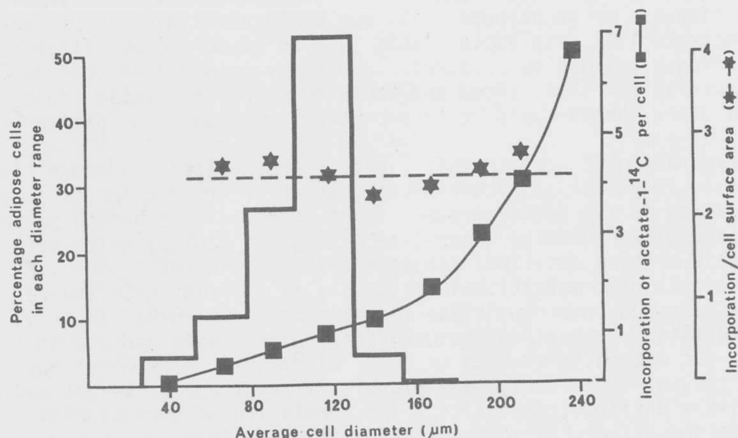

Figure 2. The distribution of cells in a heterogeneous population of adipose cells and the incorporation of acetate-1-^{14}C into lipid, expressed on a per cell basis, in ovine omental adipose tissue.

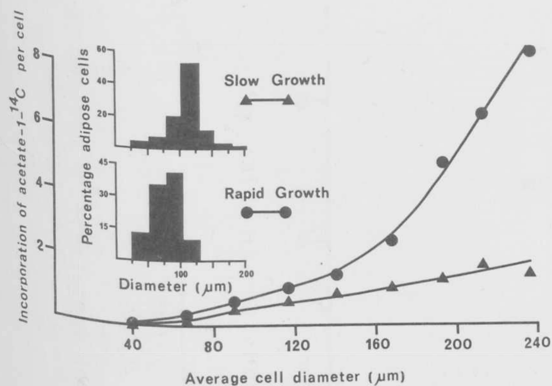

Figure 3. The percent of adipose cells in each diameter range and their incorporation of acetate-1-^{14}C into lipid in perirenal adipose tissue from sheep with different growth rates.

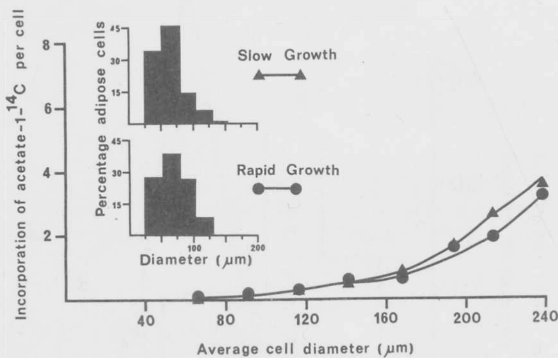

Figure 4. The percent of adipose cells in each diameter range and their incorporation of acetate-1-^{14}C into lipid in brisket adipose tissue from sheep with different growth rates.

RESULTS AND DISCUSSION

The selection and subsequent feeding of these sheep was designed to enable the isolation of adipose cells of different sizes which were lipogenically active. Liveweight gains during the 40 day preslaughter period were 200 and 115 g/day and the final average weights of the sheep in groups A and B were 42.8 and 58.3 kg respectively.

A typical distribution of adipose cells from ovine omental adipose tissue is shown in Figure 2. The average volume of the cells in this population is 0.648 nl. Although less than 1% of the adipose cells in omental adipose tissue were greater than 153 μ in diameter, these large cells were very active in converting acetate - 1-^{14}C to lipid (Fig. 2). In ruminant adipose tissue most of the radioactivity was in the form of long chain fatty acids when acetate was used as substrate (Hood et al., 1972; Hood and Thornton, 1980).

Lipogenesis, expressed on a per cell basis, increased with increasing cell size in omental adipose tissue (Fig. 2). However when the incorporation of radioactivity per cell was adjusted for surface area by dividing the incorporation values by the surface area of a cell of average size which was withheld on a particular screen, the corrected activity was similar for cells of all sizes (Fig. 2). This suggests that increased activity in large adipose cells is a function of the surface area of the cell.

These data provide no information on the reason why some cells are larger than others. The reason may not be due to activated biochemical pathways in particular cells but possibly due to the position of the cell in the adipose tissue. Large adipose cells may be located in areas of least physical pressure, hence have a greater opportunity to fill with lipid. This is also supported by differences in adipose cell size between sites. Larger adipose cells have been reported in the internal adipose depots (perirenal and omental) than in intramuscular sites for sheep (Hood and Thornton, 1979) or cattle (Hood and Allen, 1973). Presumably in these internal sites, there would be less physical resistance to prevent the filling of adipose cells with lipid.

The incorporation of acetate into lipid in the perirenal adipose tissue of the sheep with rapid growth (group A) was greater than that in slower growing sheep of group B for adipose cells of all sizes (Fig. 3). This greater incorporation in the faster growing sheep occurred even though the adipose cells were on the average smaller than in the slower growing sheep (see histograms Fig. 3) which were heavier than the sheep with the faster growth rate. These data indicate that cell size is not an absolute determinant of the rate of lipogenesis. Clearly growth rate-nutritional status has an overriding influence, when compared with cell size. However, within a heterogeneous population of cells from one animal there is a relationship between cell size and fatty acid synthesis. Comparisons between adipose cell size and biochemical parameters, emphasises the importance of carrying out metabolic studies on cells of specific size which have been isolated from a single section of adipose tissue. Previously, animals of different age or weight had been used to prepare populations of adipose cells with a different mean size. Interpretation of such studies is difficult as it is often not possible to attribute the effects to adipose cell size or differences in growth rate or adiposity. A similar effect of growth rate on lipogenesis to that in Figure 3 was observed in perirenal and subcutaneous adipose tissue from the rump and shoulder (data not shown). However in the adipose tissue from the brisket, growth rate did not effect lipogenesis since incorporation per cell was similar for cells of similar size (Fig. 4). In the brisket, the distribution of adipose cells in each diameter range was similar for the sheep in groups A and B (see histograms, Fig. 4). In the group of sheep which underwent rapid growth the brisket was less active than adipose tissue from the other sites in these sheep.

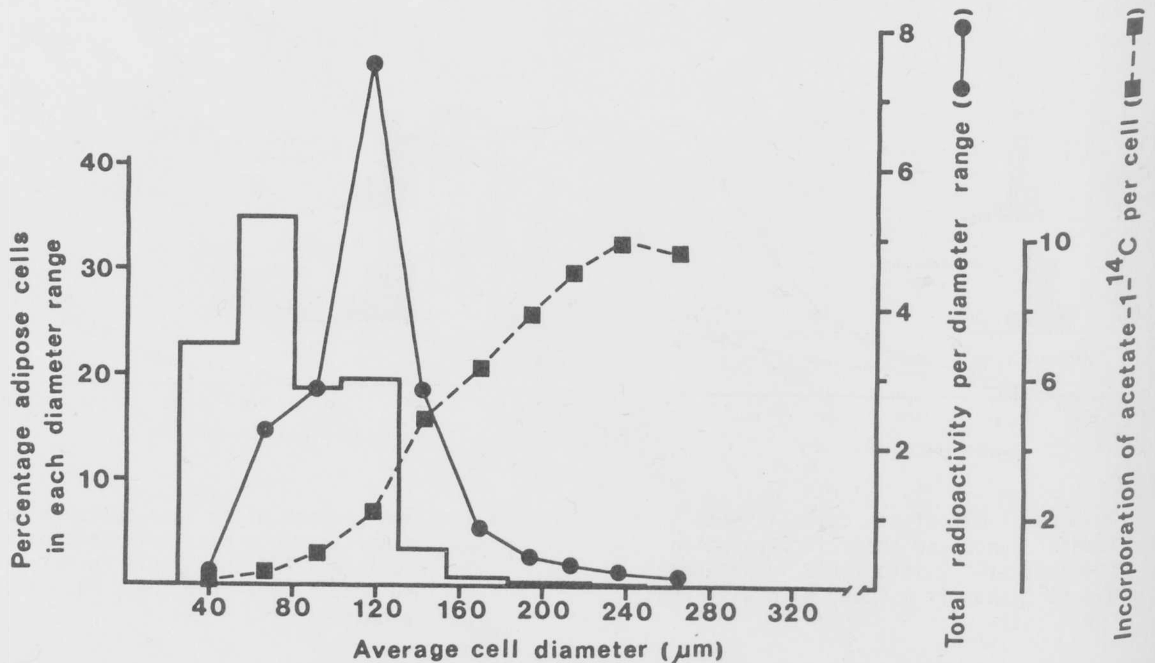

Figure 5. The incorporation of acetate-1-^{14}C into lipid in intramuscular adipose tissue from the longissimus dorsi muscle (Hood and Thornton, 1980).

A preliminary study of the adipose cell separation technique using adipose tissue from Dorset Horn x Merino sheep with large fat depots (58% of carcass meat as fat) found that lipogenesis increased with cell size (Hood and Thornton (1980); Fig. 5). In the very largest cells (260 μ for intramuscular and 300-335 μ for subcutaneous adipose tissue) there was a decline in activity, however this decline may have been artefactual due to grouping of smaller cells. This finding was not observed in any of the tissues studied in the present experiment in which the adipose cells were less than 240 μ in diameter.

When the number of cells per diameter range was taken into account, intramuscular cells in the range 100-130 μ were the most active in lipogenesis (Fig. 5). In all tissues where small adipose cells (25-50 μ) were plentiful (Fig. 4 and 5), these cells contribute very little to the incorporation of substrate to fatty acids.

The technique described in this communication is suitable to study the relationship between adipose cell size and lipogenesis, since the method utilises individual cells within a population of cells rather than comparing data from cells of different mean size, which may have been isolated from different animals. Rapid growth of sheep is reflected in increased incorporation of acetate into lipid in all cells in a heterogeneous population of adipose cells. Large adipose cells are more active than small cells within a single population, and this may be related to the greater surface area of large cells than small cells.

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