ADIPOSE TISSUE GROWTH IN LIVESTOCK SPECIES

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SUMMARY

The growth and development of adipose tissue from livestock species share common features across species. Prenatal growth involves proliferative division of presumptive adipocytes (preadipocytes) located in the stromal vascular cells of the prospective adipose tissue, followed by differentiation of the preadipocytes into immature adipocytes (adipoblasts). Postnatal growth involves marked hypertrophy of the adipoblasts and also appears to include a period of apparent hyperplasia of preadipocytes or recruitment (lipid-filling) of adipoblasts. Documenting the events associated with this period of secondary hyperplasia in livestock species will be made more precise with the adaption of several new technologies, such as recombinant DNA probes for genes encoding lipid-metabolising enzymes and specific monoclonal antibodies to cell-surface antigens.

INTRODUCTION

The United States beef industry produced almost five billion pounds of excess fat in 1985; contributions by the pork and lamb industries increased this total substantially. One reason for the production of excess fat in feedlot steers is that feeding grain and increasing time on feed improve the palatability and acceptability of meat from their carcasses (Zinn et al. 1970; Tatum et al. 1980; Crouse et al. 1984). Several investigations from this institution have demonstrated the positive relationship between the abundance of marbling adipose tissue and beef palatability (Smith et al 1983; Savell et al. 1987; Smith et al. 1987).

Before an animal is slaughtered, fat is more correctly referred to as adipose tissue to reflect its properties as a heterogeneous population of cells, organised into a dynamic tissue. It is located beneath the skin, between muscles and muscle fibres, in the abdominal cavity and in the bone marrow. Adipose tissue increases in weight and in proportion to body weight as an animal grows. Growth of adipose tissue occurs in normal animals when energy intake exceeds energy requirements for growth of other tissues (e.g. muscle) and maintenance of normal bodily function. The increase in adipose tissue mass associated with its growth is due primarily to the deposition of triglycerides within the cytosol of cells called adipocytes. When requirements for energy exceed energy intake, triglycerides are broken down to provide supplemental energy, and adipose tissue mass decreases. In this way, adipose tissue serves as a site for energy storage. Adipose tissue also functions to insulate the body core from the cold and as a cushion to vital organs. Because adipose tissue has these functions in the living animal, a minimum amount of adipose tissue is necessary for normal bodily function. However, this minimum amount of adipose tissue is far less than the amount found on typical slaughter livestock in the US.

To circumvent this obvious waste of resources and resultant inefficiency in production incurred in the over-fattening of livestock, it has been the goal of animal scientists to modify the manner in which animals deposit adipose tissue, either by selective breeding or through the use of exogenous growth modifiers. The effectiveness of both processes would be enhanced significantly through a better understanding of the processes involved in the growth and development of adipose tissue.

Fetal and early postnatal adipose tissue growth

The question of the origin of adipocyte precursor cells (preadipocytes) has attracted much attention in recent years, although studies have been done in this area since the early 1970s. During this period of time, several investigators were able to isolate fibroblast-type cells from the stromal-vascular fraction of human (Poznanski et al. 1973; Van et al. 1976) and rat (Bjorntorp et al. 1978; Goldstein and Johnson, 1982) adipose tissue. These cells were observed to accumulate lipid, as well as take on other characteristics of mature adipocytes (such as increased activities of lipoprotein lipase and various lipogenic enzymes) during their development. When mature, these culture-derived adipocytes closely resembled isolated mature fat cells from the same adipose tissue source. These early studies were the fist to conclusively demonstrate the presence of adipocyte precursors in adult adipose tissue, as well as show that this precursor cell type could develop in culture. Subsequent studies described the differentiation of isolated adipocyte precursors and the extent of acquisition, after these cells were grown to confluence, of their lipid-metabolising enzymes.

The initial event following differentiation to a lipid-accumulating cell type is the visible accumulation of lipid around the nucleus. There is at this time an induction of, or increased activity of, those enzymes involved in fatty biosynthesis and esterification. Enzymes of the former type include ATP-citrate lyase, pyruvate carboxylase, acetyl-CoA carboxylase and fatty acid synthetase; enzymes of the latter type include glycerol-phosphate acyltransferase, phosphatide phosphohydrolase, and diacylglycerol transferase. More lipid droplets accumulate and lead to a displacement of the nucleus to the periphery of the cell. Eventually, the lipid droplets coalesce to form one large central droplet. The recent cloning of specific recombinant DNA (rDNA) probes has provided scientists with extremely sensitive tools for documenting these processes. The use of rDNA probes to investigate adipocyte differentiation assumes that increases in enzyme activity concurrent with cell differentiation are the results of increased transcription of the genes encoding these proteins. This can be measured precisely by hybridization of the rDNA probes to the mRNA transcripts corresponding to the genes of interest.

In adipose tissue, one of the most promising indexed of cellular differentiation is fatty acid-binding protein (FABP). The FABPs are intracellular proteins that are intimately involved in the compartmentation of the metabolism of fatty acids. We have quantified FABP activity in bovine (Smith et al. 1985), porcine (St. John et al. 1988) and ovine (Coleman et al. 1988) subcutaneous

adipose tissue. Although the discrete function(s) of the various FABPs are not known with certainty, previous research in our laboratory (Smith et al. 1985) and unpublished observations) and others (e.g. see Glatz and Veerkamp, 1985), has established that FABP activity is related to the degree of fatty acid uptake and/or utilisation in a tissue. In addition, the observation of Bernlohr et al (1985) that adipose tissue FABP mRNA is one of the first tissue-specific mRNAs to be expressed during the adipose differentiation of mouse 3T3-L1 preadipocytes suggest that regulation of adipose FABP synthesis may be an important factor in the development of adipose tissue. The transcription of the FABP gene increases 10-to-20 fold during the same period (Cook et al. 1985). These studies have demonstrated clearly that the differentiation of preadipocytes into adipoblasts involves the initiation of transcription of the genes encoding the lipid-metabolising enzymes.

While rDNA probes provide information about cell differentiation, they have yet proven useful in investigations of adipocyte proliferation. Other recent studies, utilising in situ autoradiography, have elucidated the morphological and biochemical sequence of events that occurs during the proliferation of adipocytes. Hausman and Kaufman (1986) described the mitotic activity that occurs in the developing adipose tissue of fetal and postnatal pigs, measured by the amount of tritiated thymidine, mitotic activities appeared to differ throughout the developmental period observed. Highest activity occurred after 45 d gestation, and almost all cells labelled at this point were stromal in nature (Hausman and Kaufman, 1986), which is in agreement with previous observations that stromal cells incorporate this label before the label is seen in adipocytes (Housman et al. 1980). Mitotic activity of the adipose tissue stromal fraction decreased beyond this point in time, which corresponded to the formation and lipid-filling of the adipoblasts.

The greatly enhanced mitotic activity in early gestation described by Hausman and Kaufman (1986) tentatively can be assumed to have resulted in the formation of a large "bed" of preadipocytes. There was, however, no evidence that mature adipocytes were undergoing proliferation; if they were, it would have been detected by the incorporation of the tritiated thymidine into these cells as well as into the fibroblastic cells. Not only did this not occur, but mitotic structures and labelled cells never were seen in or around the lobules of adipoblasts after lipid accumulation had been apparent (Hausman and Kaufman, 1986). To date, then, no evidence has been presented to seriously challenge the concept that, in adipose tissue developing normally in the growing animal, proliferation of mature adipocytes is not a major factor in cellular hyperplasia.

Postnatal adipose tissue growth and secondary hyperplasia

It is now accepted that adipose tissue grows during the early growth/puberty period by a combination of hyperplasia ("secondary hyperlasia") and hypertrophy. It has been observed that the hyperplasia of perirenal and subcutaneous is completed by approximately the first year of age in cattle (Hood and Allen, 1973; Garbutt et al. 1979; Cianzio et al. 1985). Any increases in adipose tissue weight beyond this stage of growth therefore were attributed to cell hypertrophy (increased cell size) alone.

It is possible that adipocyte proliferation occurs even later postnatally, but in cattle at least this may depend on the relative maturity of the adipose tissue depot. Cattle deposit adipose tissue in different depots at varying rates. Animals fed high-energy diets deposit fat initially in the abdominal cavity and intermuscular fat depots. This early accretion is followed by deposition into the later maturing subcutaneous and interfascicular adipose tissue depots (Berg et al. 1979). Moody and Cassens (1968), utilising classical histological techniques, demonstrated that interfascicular adipocytes in bovine longissimus muscle increased both in size and number per depot as the amount of visible marbling fat increased, suggesting adipocyte proliferation in the relatively immature interfascicular adipose tissue depot. Hodd and Allen (1973) and more recently, Cianzio et al (1985), provided evidence that, while hyperplasia was virtually indetectable in subcutaneous and perirenal adipose issue after 12 months of age, it was occurring in interfascicular adipose tissue in cattle that were 13 months old. Both investigations provided compelling, yet indirect evidence that hyperplasia was occurring within the interfascicular adipose tissue depot in relatively mature animals.

Some of the strongest evidence that secondary hyperplasia was occurring in cattle comes from a study using your Charolais and Friesian bulls (Robelin, 1981). In this study, a marked alteration occurred in adipocyte mean diameter distributions. The adipocyte diameter distributions changed from monophasic (having only one peak cell diameter) to biphasic (in which two peaks were apparent). This occurred for both perirenal and subcutaneous adipocytes, indicating that new populations of smaller cells had come into existence (Robeline, 1981). A recent study from this laboratory (Smith et al. 1987) demonstrated a biphasic distribution for ovine subcutaneous adipose tissue, although this was observed only in very young lambs.

This phenomenon has been explained by evidence that cell numbers reach a plateau early in life, while cell hypertrophy continues until, as some investigators believe, a "critical" fat cell size is reached. At that point in time a signal is generated which leads to the recruitment of new fat cells. Addition of these newer, smaller cells to the existing population leads to a decrease in the mean adipocyte size. These new cells can also, in turn, reach the critical size and initiate another recruitment phase (Johnson and Francendese, 1985).

If there is indeed a critical size which, when reached, induces secondary hyperplasia, then this size seems to vary among species, and even within a species. In young Charolais and Friesian bulls, the point at which secondary hyperplasia occurred was at 44-55% of their mature weight, which corresponded to a mean subcutaneous adipocyte diameter of approximately 100 μm (Robelin, 1981). In another experiment with cattle, however, when 1-to-2% of the total adipocyte population of obese animals (35% of body weight as fat) reached 250-to-260 μm in diameter, and the mean cell diameter was 160μm,

then the number of cells less than 100 μm increased, resulting in a biphasic adipocyte size distribution (Lee et al. 1983).

In many cases, it has been difficult to demonstrate bimodal adipocyte distributions. We have observed only monophasic fat cell distributions in mature sheep (Smith and Prior, 1986; Smith et al. 1987; Coleman et al. 1988), and have been singularly unsuccessful in demonstrating a biphasic adipocyte distribution in adipocytes from cattle at several stages of maturity (Smith et al. 1984; Smith and Crouse, 1984; Miller et al. 1988). However, manipulation of animal growth with the β-adrenergic agonist, clenbuterol has provided indirect evidence for secondary hyperplasia in growing lambs and cattle. Coleman et al (1988) reported that clenbuterol decreased fat accretion in lambs fed a finishing diet by blocking secondary hyperplasia rather than by inhibiting cell hypertrophy. In heifers (Miller et al. 1988) and steers (Schiavetta et al. 1988), clenbuterol restricted adipocyte hypertrophy and dramatically altered the cellularity of the 9-1-11 th rib section. In heifers fed clenbuterol during the last 50 days of the finishing period, clenbuterol decreased 9-10-11 th rib adipocyte number more than doubled, relative to untreated animals. Thus, secondary hyperplasia appears to occur in livestock, and is especially sensitive to manipulation.

Identifying adipocyte progenitor cells - directions for future research

The origin of the new adipocytes that appear during secondary hyperplasia is of great interest. What currently is unknown is whether these new adipocytes 1) are a result of cellular proliferation of preadipocytes, of 2) come from a pool of adipoblasts, cells that had differentiated from preadipocytes during the pre-adult period but had not yet begun to accumulate lipid, and hence were undetectable as adipocytes by standard methods of measurement. One of the most promising methods for detecting preadipocytes and/or non-lipid filled adipoblasts is through the use of recently developed monoclonal antibodies to cell-surface adipocytes antigens (Wright and Hausman, 1988; Killefer et al. 1988). With these, it was possible to detect adipoblasts (and possibly preadipocytes) prior to the appearance of lipid droplets. Coupled with the use of specific rDNAs, such as that corresponding FABP (which will detect cells only after differentiation), it will in the future be possible to distinguish among preadipocytes, non-lipid-filled adipoblasts, and fibroblasts that are not destined to become adipocytes. Once this capability has been established, direct documentation of the cellular phenomena responsible for the accumulation of adipose tissue from mature animals finally will be possible.

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