PHYSIOLOGICAL MATURITY OF MUSCLE AND ADIPOSE CELLS IN MEAT ANIMALS*

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INTRODUCTION

Physiological maturity is a term used to refer to the relative stage of development of body processes, functions or composition. Interest in this topic ranges from growth and metabolic aspects of the animal to the application of meat grading standards. This paper will deal exclusively with developmental aspects of muscle and adipose cells. The influence of hyperplasia and hypertrophy on skeletal muscle and adipose tissue will be considered in detail because differences in these cellular aspects are reflected in the "mature" size of an animal, the nature of the growth curve for protein and fat in the animal, the ability of a carcass to make a specific grade and most importantly, the efficiency of producing meat. Currently, the discussions dealing with proposed changes in U.S.D.A. beef carcass grades are in part related to an apparently higher proportion of beef cattle which do not grade U.S.D.A. Choice because at the traditional market weights, they are, on the average, physiologically younger.

Early studies with cattle (Haecker, 1920; Moulton et al., 1922; Callow, 1947) sheep (Hammond, 1921; Joubert, 1956) and swine (McMeekan, 1940a,b) established that muscle and bone mature earlier than fat. Furthermore, Brody (1945) demonstrated that the body weight growth curve for all farm animals has the same general shape. This suggested that the cellular processes contributing to growth must have much in common among species.

MUSCLE-RELATED ASPECTS

It is generally accepted that muscle cell hyperplasia is completed during the fetal period in meat animals and that growth of muscle after birth is due to muscle cell hypertrophy (Staun, 1963). Initial reports in this regard were made by Waters (1909) and are supported by work of McMeekan (1940b), Joubert (1956) and others. Some hyperplasia occurs during the first seven to fourteen days after birth in the muscles of rodents (Chiakulas and Pauly, 1965). This species difference may have important effects on skeletal muscle development if the animal undergoes stress, especially stress due to poor nutrition, immediately after birth. Recent reviews (Hegarty, 1971; Ashmore and Addis, 1972) have highlighted some of the technical problems as well as more recent advances in understanding muscle growth and development. Robinson


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(1971) emphasized that there are teleological advantages to the animal if hyperplasic growth is completed in utero when the maternal reserves can serve to buffer nutritional stresses which can cause irreversible damage during hyperplasia. Thus, Robinson (1971) postulated that a stress during hyperplasic growth may cause irreversible stunting, whereas stress during postnatal growth caused by muscle hypertrophy would be followed by compensatory growth. After reviewing the limited literature on pigs, Pond (1973) concluded that protein deprivation during the gestation period appeared to have a stunting effect on postnatal weight gain but the effect on routine carcass measures was negligible and the effect on mature size was unknown. However, McCance and Widdowson (1974) have reported that the runt pig, which is the result of insufficient nutrients in utero, is born smaller than its control littermate and remains smaller even at 36 months of age. Furthermore, it has been shown (Widdowson, 1971) that the muscles of the runt pigs weighed less than their large littermate controls. A recent study in our laboratory with runt and large-littermate control pigs has shown that at similar live weights, runts tended to have lighter weight muscles than controls. Of additional interest is the fact that the average diameter of muscle fibers in the runt pigs were larger than in the control pigs. Figure 1 illustrates these differences in a fiber diameter frequency distribution for the psoas major muscle of runt and large-littermate control pigs studied at about 78 kg carcass weight. Since the psoas major muscle was similar in length for both groups, weighed less in the runts and had fibers significantly (*P < .01*) larger in diameter in the runts, one can tentatively conclude that there must have been fewer muscle fibers in the psoas major muscle of the runt pigs compared to large-littermate controls. It should also be noted that the runts required 26 days longer than controls to reach 78 kg and that the fiber diameter frequency distribution (figure 1) for the psoas major of the runt pigs included a higher percentage of fibers larger than 90μ in diameter. Thus, the larger average fiber diameter in the runts suggests that the growth of muscle in the runt pigs was physiologically more mature than in the control pigs. This greater physiological maturity of the muscle cells in the runt pigs may have been a major factor contributing to their slower growth rate when compared to littermate controls.

Hedrick (1968) has reviewed a number of reports on compensatory growth and there seems to be little question that this can occur postnataally and can be followed by changes in muscle cell hypertrophy. This effect on muscle cell diameter has been demonstrated in beef (Waters, 1909), swine (McMeekan, 1940b) and sheep (Joubert, 1956). Furthermore, McMeekan (1940b) stated that even though the pigs in his studies were the same chronological age, the different nutritional regimens produced animals that were widely different in physiological age and were retarded in development.

Thus, what are the developmental variables which ultimately determine the accretion of muscle protein and fat in an animal? For both muscle and adipose tissue, there is a period of growth which is predominantly due to hyperplasia or hypertrophy. However, in studying the hypertrophic
Figure 1. Frequency distribution for muscle fiber diameter in control and runt pigs studied at 78 kg live weight.
growth phase of muscle, one should also take into account the possible changes in fiber types and increases in muscle cell length, whereas in the adipocyte one need only be concerned about changes in cell diameter.

Data in figure 2 serve to illustrate that two different types of pigs can have the same muscle weights, but developmentally the muscles can be at different stages of maturity (Hegarty et al., 1973). For this comparison, Hormel Miniature (HM) and Minnesota 3x1 (M3xl) pigs were studied at different body weights and can be directly compared at 28 and 54 kg. At these body weights there were no significant (P > .05) differences in the fat-free weight of the biceps brachii, psoas major or semitendinosus muscles. Additionally, there were no significant (P > .05) differences in the length of the tibia, fibula and femur bones which provides evidence that the skeletal dimensions were similar and that muscle lengths must have been similar. At 28 and 54 kg, however, there were significant differences in muscle fiber diameters between the HM and M3xl pigs for each muscle. For example, in the biceps brachii muscle of the 28 kg pigs, the fiber diameters were about 45 μ (M3xl) and 70 μ (HM). A similar difference in fiber diameter persisted at 54 kg for the biceps brachii as well as in the other muscles. Although fiber number was not determined directly, it can be deduced that since muscle weights and lengths were similar, the large differences in muscle fiber diameter must be offset by differences in fiber number. If one accepts that muscle fiber diameter has a maximum limit (Brody, 1945), then one would classify the muscles from the HM as being more physiologically mature than those in the M3xl. The shape and displacement of the fiber diameter curve (figure 2) for the biceps brachii muscle supports this concept. For example, in the biceps brachii muscle, there was a definite plateauing of fiber diameter which occurred between 28 and 54 kg in the HM but not until 83 to 109 kg in the M3xl. However, in both breeds, the plateau in fiber diameter for the biceps brachii occurred between about 75 and 85 μ. Thus, the developmental differences in muscle fiber diameter between these two strains of pigs was in keeping with differences in their growth rates and mature sizes. For example, at 54 kg body weight, the average age of the HM was 200 days, whereas the M3xl pigs were 99 days old. In addition, the HM pigs at 54 kg and the M3xl pigs at 83 kg had approximately 40% extramuscular carcass fat (Hood and Allen, 1974). Thus, both muscle cell diameter and extramuscular fat content indicate that the HM pigs were physiologically more mature than conventional M3xl pigs at the same body weight.

An aspect of developmental maturity of muscle which was not considered in the above study has to do with the relative distribution of the muscle fiber types. As reported by Cooper et al. (1970), the fiber types in porcine muscle undergo major changes during the first few months of life which results in an increased proportion of anaerobic (white) fibers. In addition, there can be genetic differences in fiber type which are related to differences in muscling and meat quality traits (Dilley et al., 1970; Holmes and Ashmore, 1972; Ashmore, 1974; and West, 1974). Anaerobic fibers are, in general, larger in
Figure 2. Muscle weight, fiber diameter and sarcomere length changes in muscles as associated with increasing live weight of Minnesota 3 X Minnesota 1 (Minn. 3 X 1) and Hormel miniature (HM) pigs. From Hegarty et al., 1973 (17).
diameter than aerobic (red) fibers. Therefore, anaerobic fibers may conceivably reach their full growth potential later in life or at a heavier body weight and thereby delay the onset of the "fattening phase" of growth as schematically illustrated in figure 3. It is apparent from the relationship illustrated in figure 3 that with a fixed number of fibers, a larger muscle mass can be produced with a higher proportion of anaerobic fibers. This is the situation when athletes become more muscular as the result of training for forceful exercises such as sprinting, weight lifting, etc. The domestication, selection for increased muscling, higher lean/fat ratios, and increasing confinement of meat animals are factors which could bring about a higher proportion of the larger diameter, anaerobic type fibers. This is supported by the work of Dildey et al. (1970) who reported that muscular pigs with an increased tendency to produce PSE pork have larger anaerobic fibers and a higher ratio of anaerobic/aerobic fibers than more poorly muscled pigs with a lower incidence of PSE pork. An extreme example of muscular hypertrophy is the "double muscling" condition in cattle. Holmes and Ashmore (1972) reported that this condition is in part due to a higher conversion of aerobic fibers to anaerobic fibers than in the normal animal. In addition, the anaerobic fibers were also larger than normal. Thus, these cattle are extremely muscular because of muscle fiber hyperplasia and an increased proportion of large diameter anaerobic fibers (Holmes and Ashmore, 1972; Swatland and Kieffer, 1974, and West, 1974). It should also be noted that these cattle have minimal fat deposits (Hendricks et al., 1973) which, as will be discussed later, may be related to the degree of development in the muscle as theorized in figure 3.

ADIPOSE TISSUE-RELATED ASPECTS

With regard to adipose tissue, a limited number of studies have been done in meat animals to elucidate the factors controlling the prenatal and postnatal maturation of the fat cell and concomitant increase in adipose tissue mass. However, during the last few years a cooperative research project in the North Central Region has provided some information on adipose tissue cellularity in swine, cattle and sheep. Some of these studies were reviewed by Anderson (1972) and will also provide a basis for much of the discussion in this section.

Early studies (Bell, 1909; Waters, 1909) with cattle suggested that fattening was due both to hyperplasia and hypertrophy of adipocytes. Waters (1909) reported that the diameter of fat cells in cattle ranged from 10-250\mu depending upon the length and nature of the feeding program. More recently, Hood and Allen (1973) reported that in beef cattle the intramuscular adipocytes were possibly still undergoing hyperplasia at 14 months of age, whereas hyperplasia was apparently completed in subcutaneous and perirenal depots between 8 and 14 months of age (figure 4). Work with barrows also indicates that in contrast to muscle, adipocyte hyperplasia was not completed until between 83-109 kg live weight in conventional pigs and 37-45 kg live weight in HM barrows (figure 5).
Figure 3. Theoretical relationship between muscle cell type and accretion of fat in the animal body.
Figure 4. Number frequency distribution of adipose cells from bovine adipose tissue. HxA, Hereford X Angus animals, 14 months old and 470 kg live weight; Hereford animals, 8 months old and 215 kg live weight; LD, SM, PF, and TR refer to adipose cells from the longissimus dorsi, semimembranosus, pectoralis profundus and trapezius muscles, respectively. SQ and PERI refer to subcutaneous and perirenal adipose tissue, respectively. From Hood and Allen, 1973 (20).

Figure 5. Increase in the number of extramuscular adipose cells during the growth of conventional lean (HxY), conventional obese (M3x1) and miniature (HM) types of swine. From Hood and Allen (21).
TABLE 1. CARCASS CHARACTERISTICS OF PIGS WITH WIDELY DIFFERENT BODY TYPES¹,²,³

<table>
<thead>
<tr>
<th>Traits</th>
<th>Breeding Groups</th>
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<tbody>
<tr>
<td></td>
<td>York x Duroc</td>
</tr>
<tr>
<td>Slaughter weight, kg.</td>
<td>109.0⁴</td>
</tr>
<tr>
<td>Cold carcass weight, kg.</td>
<td>79.7⁵</td>
</tr>
<tr>
<td>Backfat thickness, cm.</td>
<td>3.15³</td>
</tr>
<tr>
<td>Loineye area, sq. cm.</td>
<td>38.0⁴</td>
</tr>
<tr>
<td>Ham and loin, %</td>
<td>43.4³</td>
</tr>
<tr>
<td>Carcass dissectable muscle, %</td>
<td>54.2⁴</td>
</tr>
<tr>
<td>Extracted carcass lipid, %³</td>
<td>27.02²</td>
</tr>
<tr>
<td>Muscle/bone ratio⁵</td>
<td>8.03³</td>
</tr>
<tr>
<td>Muscle/fat ratio⁶</td>
<td>2.01³</td>
</tr>
</tbody>
</table>

¹ N = 6 barrows/group.
² All pigs were fed ad libitum a corn-soybean diet.
³ Means in the same row with different superscripts are significantly different at P < .05.
⁴ Determined from a composite sample of the entire carcass minus leaf fat.
⁵ Weight of the semimembranosus, biceps femoris, semitendinosus and quadriceps muscles divided by weight of tibia, fibula and femur.
⁶ Weight of carcass dissectable muscle divided by weight of extracted carcass lipid.

The conventional pigs consisted of Hampshire x Yorkshire (HxY) barrows and M3xl barrows, the latter of which had a much lower muscle/fat ratio (table 1). These conventional and miniature pigs were 5-6 months of age, which is in close agreement with work (figure 6) by Anderson and Kauffman (1973), who reported that adipocyte hyperplasia was completed at about 5 months of age in Chester White barrows.

Figure 7 illustrates the relative importance of increases in adipocyte number and size when the HxY and M3xl pigs were compared during growth from 28-109 kg or at similar percentages of carcass extramuscular fat. When compared over the same period of growth (28-109 kg), it was apparent that the major difference between both groups was the much greater percent increase in adipocyte cell volume for the
Figure 6. Changes in the accumulated weight of carcass adipose tissue (solid line), the volume ($\mu = \text{microns}$) if adipose cells from the middle subcutaneous backfat region (dashed line), and extrapolated total adipose cell number (dotted line) as determined by Coulter counter measurements of the osmium-fixed cells.
From Anderson and Kauffman, 1973 (2).

Figure 7. Percent increase in adipocyte hyperplasia and hypertrophy of the middle subcutaneous (MSQ) depot of pigs between 28 and 109 kg live weight. HxY (109 kg) and M3x1 (54 kg) pigs had similar percentages of extramuscular carcass fat.
M3xl. This is indicative that the adipocytes of the M3xl pigs were more mature than adipocytes in the HxY pigs at 109 kg. However, the percent increase in adipocyte volume was very similar, and the percent increase in cell number was much greater in the HxY pigs when these two groups of pigs were compared at approximately equal percentages of carcass extramuscular fat (31.7%, 109 kg HxY; 30.6%, 54 kg M3xl). The much greater increase in cell number for the HxY pigs can be attributed to their older chronological age and much heavier weight. However, the similar percent increase in adipocyte cell volume in the latter comparison is indicative of a similar degree of maturity in the adipocytes of the 109 kg HxY and 54 kg M3xl pigs. This is also supported by the fact that the average cell volumes for both groups in this latter comparison did not differ greatly.

At a given live weight, the leaner, more well-muscled HxY pigs had a greater number of extramuscular adipocytes than the more obese, less well-muscled M3xl pigs (figure 5). Lee et al. (1973) also reported that even though Poland China barrows were fatter than gilts, the barrows had fewer adipocytes than the gilts at a constant body weight. A possible explanation of these observations was not apparent until extramuscular adipocyte number was examined in relation to true body size (fat-free carcass weight). The relationship between these two variables is shown in figure 8). It is apparent from these results that the number of extramuscular or perirenal adipocytes was closely related to true body size in these two widely different types of conventional pigs. The possible reason for this relationship may be related to the fact that pigs with a larger true body size require a larger number of adipocytes in order to be able to reach the same degree of fatness as a pig with a smaller true body size. In addition to data shown in figure 8, this is supported by the fact that when the HxY (109 kg), M3xl (54 kg) and HM (37 kg) pigs had similar percentages (30.6-31.7) of extramuscular fat, the ratio of extramuscular adipocyte number to true body size was similar (1.13-1.24). Furthermore, the diameters of the outer subcutaneous (83.4-84.7 μ) and middle subcutaneous (86.7-93.5 μ) adipocytes were similar for all three groups of pigs at these live weights. Thus, one might conclude that on this basis these three diverse types of pigs were at similar levels of physiological maturity at live weights of 109 kg (HxY), 54 kg (M3xl) and 37 kg (HM).

Lee et al. (1973) reported that underfeeding of pigs during the suckling period had no appreciable influence on adipose cell number and size of the subcutaneous, viscera and bone fat of underfed and control pigs fed to a constant body weight (figure 9). Thus, even though the underfed pigs were chronologically older, adipocyte number and development was similar for both groups of pigs. Lister and McCance (1967) have also reported that altering the nutrient level early in the postnatal growth period cannot change the adipose cell population for subcutaneous and viscera fat. In contrast to these fat depots, however, Lee et al. (1973) found that postnatal nutritive restriction resulted in less intramuscular lipid (figure 10) due to fewer and smaller adipocytes (figure 9). This led the authors to postulate that differentiation and
Figure 8. Relationship between true body size and number of extramuscular or perirenal adipocytes among three body types of swine. From Hood and Allen (21).
Figure 9. Number of adipose cells in the body and their distribution among body parts: weight constant group.
From Lee, Kauffman and Grummer, 1973 (23).

Figure 10. Amount of fat in the animal and its distribution among carcass components: weight constant group.
From Lee, Kauffman and Grummer, 1973 (23).
growth of intramuscular adipocytes are influenced by early postnatal nutritive restriction. Since this effect was unlike that found in the subcutaneous, viscera and bone fat, it is another indication that the cellular development of the intramuscular depot is physiologically less mature. Hood and Allen (1973) have also reported that intramuscular adipocytes from beef cattle are less mature than subcutaneous or perirenal adipocytes (figure 4).

In work with pigs and cattle, we have noted that the largest fat cell that we have measured was about 220 microns (cattle). This is in the same range as maximum adipocyte cell size which was reported by Anderson and Kauffman (1973). Waters (1909) reported that cattle fed for fat stock shows could have fat cells as large as 250µ, but more typically they were less than 200µ. With these results in mind, it is pertinent to ask, "How fat can a fat cell become?" Some studies have been initiated to answer this question and to study adipose tissue cellularity in animals beyond normal market weight or animals that are excessively fat. Data in figure 11 were compiled from the known history E×Y barrows used in figures 5 and 7, and from market weight barrows and heavy weight sows of unknown history selected for thick (> 5 cm) backfat deposits. In figure 11 the percent distributions of adipocyte volume for the middle subcutaneous adipose tissue are shown on the left-hand side and the percent distributions for adipocyte cell number are shown on the right-hand side of figure 11. It is obvious that while percent distribution for cell volume give an accurate description of the significance of different diameter adipocytes to the mass of adipose tissue, percent distributions of adipocyte volume are not useful in depicting changes in the population of adipocytes. This is due to the fact that adipocytes of small diameter make an insignificant contribution to adipose tissue volume in comparison to adipocytes of large diameter. Therefore, the frequency distributions for fat cell diameters on the right-hand side of figure 11 are of particular interest. From the initial observations in figure 11, it appears that as backfat thickness increased in these three groups of pigs there was an increasing tendency for a biphasic distribution of adipocyte diameters. For both the unknown history barrows and sows in figure 11, there were a large number of adipocytes between 25-50µ, fewer adipocytes between 50 and 75-100µ, and a second population of adipocytes between 100-200µ. The biphasic nature of adipocyte diameter distributions in these two groups of fat pigs is of interest to understanding the cellularity and growth of adipose tissue. The adipocytes of less than approximately 50µ in the biphasic distributions could result from either a reinitiation of hyperplasia, or the fact that certain adipocytes, although present, fail to enlarge and reach the minimum size for detection until the adipose tissue is more mature. In this technique using osmium tetroxide fixation of the tissue, the minimum size for detection was 22µ, even though adipocytes can be distinguished by histological examination at 10-15µ. Therefore, the actual reason for the biphasic distribution is not known, but nevertheless its presence indicates that in these pigs there were two distinct populations of adipocytes at different stages of maturity. It is also important to note that in relation to maximum fat cell size, the fattest pigs studied did not contain fat cells larger than 203µ.
Figure 11. Adipocyte volume and diameter distributions for pigs with different backfat thicknesses. HxY = 109 kg Hampshire x Yorkshire barrows. BF = Average backfat thickness (cm). Market = unknown history market weight carcasses. Sows = unknown history heavy sow carcasses.
Finally, how might we relate the growth curve for protein and fat to some of the developmental aspects of muscle and adipose cell? Bergen (1974) has prepared an idealized log-log plot of body weight in relation to the accretion of fat and protein. As shown in figure 12, the accretion of protein decreases as body weight increases, and at some point, dependent upon the physiological maturity of the animal, the accretion of fat exceeds that of protein. As reviewed by Bergen (1974), the increased accretion of fat is more closely related to body weight than age, and in general, the longer the fattening phase of growth can be delayed, the more efficient the animal will be in converting foodstuffs into lean meat. This would seemingly be related to the potential for muscle growth as determined by fiber number, metabolic type of fiber and nearness to reaching maximum muscle cell size (physiological maturity).

Thus, figure 3 provides a basis for some theoretical considerations relating muscle fiber type and developmental maturity to fat accretion in the animal’s body. As discussed previously, because of fewer physiological restrictions conceivably related to transport of oxygen and carbon dioxide, the anaerobic muscle fiber is known to have a greater potential for hypertrophy than the aerobic fiber. Thus, with a constant number of muscle cells, the potential for muscle growth is greater with an increased proportion of anaerobic fibers. Figure 3 depicts relative differences in the maximum size of these two muscle cell types and suggests that fat accretion would exceed protein accretion (muscle growth) at a heavier body weight in an animal with the same total number of muscle fibers but a higher proportion of anaerobic fibers. Thus, in relation to maturity of muscle cell hypertrophy and fat deposition, the animal with a higher proportion of anaerobic fibers would be physiologically less mature than an animal of the same body weight with a higher proportion of aerobic fibers. However, as emphasized by Ashmore (1974), the consequences of a major increase in anaerobic fibers may bring about desirable changes in the quantitative aspects of meat production, but detrimental changes in meat quality and response of the animal to environmental stress. This appears to be the case in muscular pigs that exhibit porcine stress syndrome and cattle with genetic muscular hypertrophy.

In the U.S. and many other countries as well, the genetic selection programs which have been effective in altering the muscle/fat ratios of meat animals have usually been based on one or more parameters for both muscle and adipose tissue. This is true also for the U.S.D.A. grading standards that apply to beef, pork and lamb. The data in table 1 serve to illustrate genetic differences in muscling and adiposity of three conventional-size types of swine. These differences among breeding groups are representative of different degrees of genetic change in the proportion of muscle and adipose tissue in the U.S. swine population during the last 15-20 years. Likewise, such genetic differences are the summation of events controlling the cellular and metabolic aspects of muscle and adipose tissue. Although, additional information is still needed to elucidate the mechanisms controlling the growth and development of muscle or adipose tissue, there is also a need for studies where the cellular and metabolic aspects of muscle and adipose tissue are investigated concurrently.
LITERATURE CITED


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Mike Dikeman: I was wondering if the projectionist could perhaps shrink the slides just slightly, because part of the labeling is off of the screen and it might help to have a little less light around the stage so we can see the pointer. So if the projectionist could do those two things we'd appreciate it.

Our next speaker, Dr. J. J. Guenther from Oklahoma State University, has been conducting a very interesting study on physiological maturity of different body types of cattle and we felt that this study fit in very well with our overall theme of physiological maturity. So J. J., I'll just turn it over to you.