Changes in fatty acid synthesis associated with growth and fattening

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During growth and fattening, differences exist in the way nutrients are partitioned for deposition as fat and muscle protein. Knowledge of the partitioning of energy is of practical significance in the production of lean animals. In this review, emphasis will be placed on changes in fatty acid synthesis which occur during the growth and fattening of animals grown for meat, and on factors affecting these changes.

Growth and body composition

Many studies have indicated that body composition of animals, within the same breed, is related to body-weight and is largely independent of age and nutrition (Zucker & Zucker, 1963; Lee *et al.* 1973; Searle, 1977). Therefore, it is very important in studies relating metabolic activity to body composition that control and experimental animals should be compared at similar weights rather than at similar ages. In this context, there exist sex and breed differences which need to be considered. At the same live weight, females are fatter than males (Searle & Griffiths, 1976*a*), whereas at any given live weight smaller breeds are fatter and enter the fattening phase of growth at a lower live weight than larger breeds (Fig. 1) (Searle & Griffiths, 1976*b*; Searle, 1977).

In the early stages of growth, fat deposition is related to both hyperplasia and hypertrophy of adipocytes, whereas in the fattening stage of animals which have reached physiological maturity, hypertrophy is solely responsible for fat deposition (Anderson & Kauffman, 1973; Hood & Allen, 1973*a*; Haugebak *et al.* 1974; Hood & Thornton, 1979). The live weight at which animals change to a fattening stage of growth varies and is characteristic of a breed within a species (Fig. 1). In each stage of growth, adipose tissue is the major site for fatty acid synthesis in meat animals, whereas the liver is the major site for fatty acid synthesis in birds.

The rate of deposition of lipid in adipose tissue depends on four metabolic processes: (1) uptake of fatty acids from blood; (2) *de novo* fatty acid synthesis; (3) lipolysis and return of free fatty acids to the circulatory system, and (4) fatty acid oxidation *in situ*. Work has recently been reviewed in this area (Vernon, 1980; Christie, 1981; Hood, 1982) and no attempt will be made in this presentation to review the subject completely.

Genetic differences

The tendency to deposit fat in an animal can be decreased by genetic selection. At any given body-weight, the fattest animals will have the smallest mature size

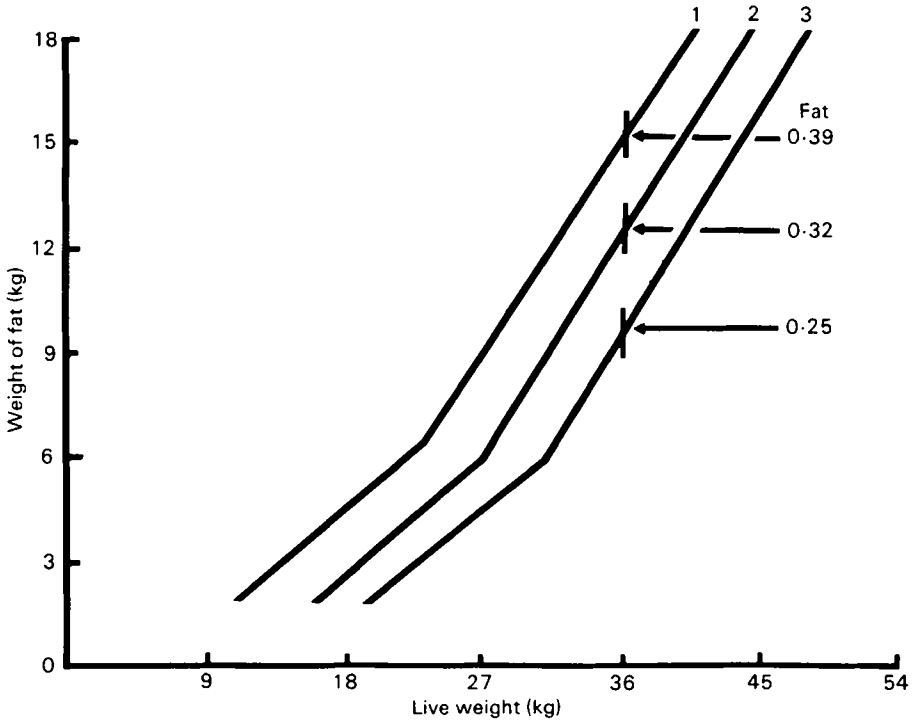

Fig. 1. Relationship between body fat (kg) and live weight (kg) for (1) Camden Park Merino, (2) Medium Peppin Merino and (3) Border Leicester × Merino sheep (Searle & Griffiths, 1976*b*; Searle, 1977). The proportion of fat in sheep of each breed at 36 kg live weight is indicated.

and the leanest the largest mature size. Although this statement is generally true, fatness can be also influenced by appetite, dietary regimen, physical activity and genetic factors. Pietran pigs have smaller appetites than Large Whites which prevent them from being fatter at commercial slaughter weights than would be expected from their smaller mature size (Wood, 1980). Selection for small body size in mice favours those individuals that have a high rate of fat turnover (Eisen *et al.* 1982). This genetic difference in fat turnover among lines has accrued as a correlated response to selection for growth rate. Selection for improved food conversion efficiency in broiler chickens results in a marked reduction in total body fat, whereas selection for increased food consumption has the opposite effect (Pym & Solvyns, 1979). In a related study, differences in in vitro fatty acid synthesis and malate dehydrogenase ($NADP^+$) activity among genetic lines could not be attributed to differences in food consumption or growth rate (Hood & Pym, 1982).

The endocrine regulation of energy metabolism (Geelen *et al.* 1980; Trenkle, 1981) and the efficiency of energy retention in genetically obese animals (Romsos, 1981) have recently been reviewed.

Fatty acid synthesis during growth

The rate of fatty acid synthesis was extremely low in adipose tissue of suckling pigs, but increased rapidly when they began consuming a dry diet (Allee *et al.*

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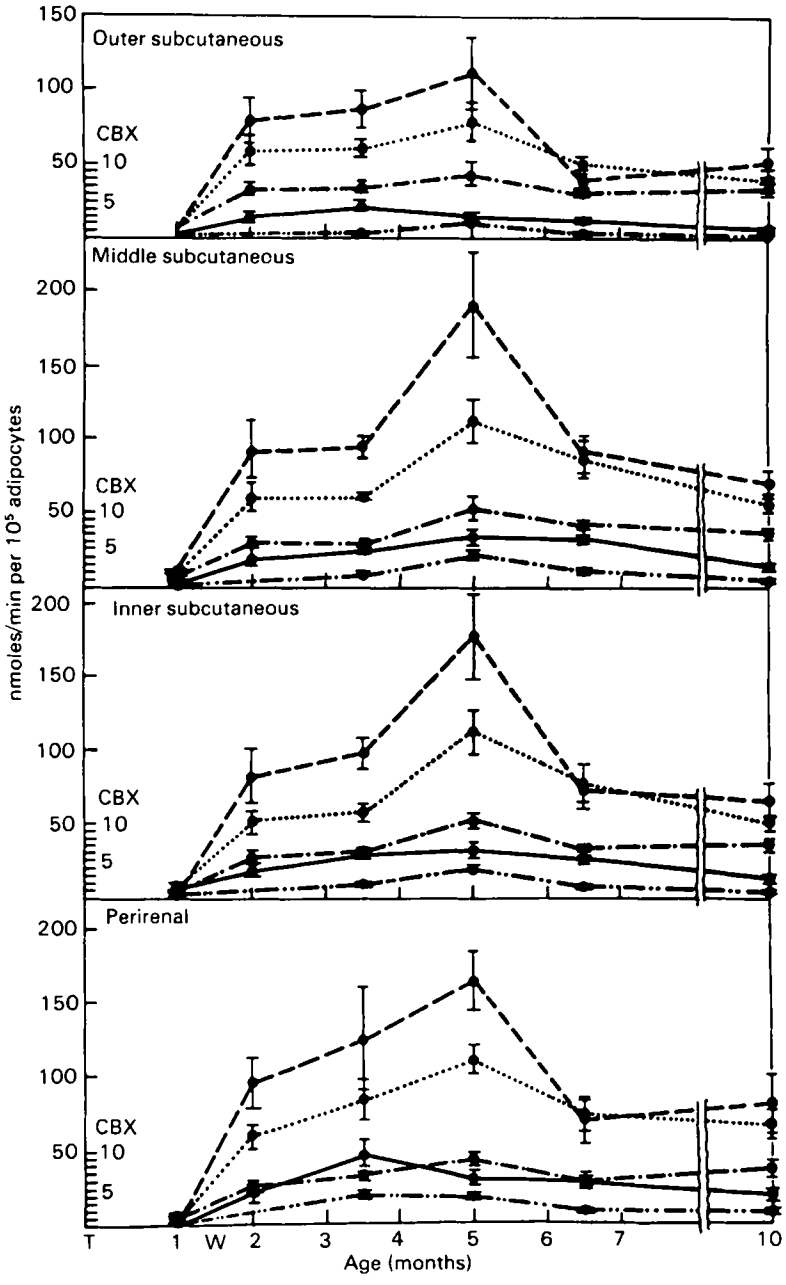

Fig. 2. Enzyme activities in adipose tissues of male castrate Chester White pigs expressed on a cellular basis. Mean values with their standard errors for outer, middle and inner subcutaneous backfat and perirenal sites. The enzymes studied were acetyl-CoA carboxylase (CBX, — · · —, *EC* 6.4.1.2), citrate cleavage enzyme (———, *EC* 4.1.3.8), 'malic enzyme' (— — —), glucose-6-phosphate dehydrogenase (· · · · · ·, *EC* 1.1.1.49) and 6-phosphogluconate dehydrogenase (— · —, *EC* 1.1.1.43). The 10-month values are for animals approaching maturity; T and W refer to term and weaning respectively (after Anderson & Kauffman, 1973).

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1971). Fatty acid synthesis (expressed per unit weight of tissue) was maximal at 67 d of age and declined linearly thereafter. When Anderson & Kauffman (1973) measured the activity of lipogenic enzymes in porcine adipose tissue from four anatomical locations, they observed maximal activities (expressed per 10^6 adipocytes) at 5 months of age (Fig. 2). Expression of these results on a tissue weight basis resulted in the conclusion that maximum rates of fatty acid synthesis occurred at 2 months of age. The increase in activity per adipocyte was particularly noticeable between the ages of 3·5 and 5 months. This corresponded to (a) a stage of rapid growth of the four adipose tissue depots and (b) an age when adipocyte volume increased at its most rapid rate. In a study of pigs with varying propensities to fatten, Hood & Allen (1973*b*) found that rates of lipogenesis also increased up to 5 months of age. In contrast, Lee & Kauffman (1974) observed that although enzyme activities in subcutaneous adipose tissue decreased after 16 weeks, activities of ATP-citrate lyase and malate dehydrogenase ($NADP^+$) continued to increase in intramuscular adipose tissue until the end of the experimental period of 24 weeks.

In young growing pigs on a typical maize–soya-bean meal diet, 76–84% of the total carcass fat was derived from *de novo* synthesis (Hood & Allen, 1973*a*). As an animal matures and enters the fattening phase of growth, *de novo* synthesis in adipose tissue declines yet lipid accumulation continues, suggesting that exogenous fatty acids contribute more to total lipid deposition as an animal ages. Lipoprotein lipase activity increases in pigs as body-weight increases (Lee & Kauffman, 1974). Etherton & Allen (1980) noted that in vitro esterification of radioactive palmitic acid also increased in pig adipocytes with increasing animal age: this coincided with a decline in fatty acid synthesis from glucose.

The activities of lipogenic enzymes and rates of lipogenesis in vitro are greater in adipose tissues of animals from breeds which show the greatest propensity to fatten (Hood & Allen, 1973*b*; Martin & Wilson, 1974; Hood & Allen, 1975; Allen *et al.* 1976). The capacity of adipose tissue to generate NADPH is a suitable indicator of potential for fatty acid synthesis although it responds to, rather than causes, changes in rates of fatty acid synthesis. The effects of growth, breed and food intake on the activity of lipogenic enzymes in meat animals have been documented by Allen *et al.* (1976).

Anatomical differences

Differences in metabolic activity of adipose tissue from several sites within an animal reflect the extent of fat deposition at these sites. Fat is preferentially deposited in the following tissue sites: perirenal and omental > subcutaneous > intermuscular > intramuscular. In animals grown for meat there are marked differences in the rates of fatty acid synthesis among anatomical sites; for example, in the activity of ATP-citrate lyase, a key enzyme for the generation of acetyl CoA, in pigs (Fig. 3). Contrasting findings between studies can often be accounted for by differences in the extent of animal maturity or differences in adipocyte sizes between tissues.

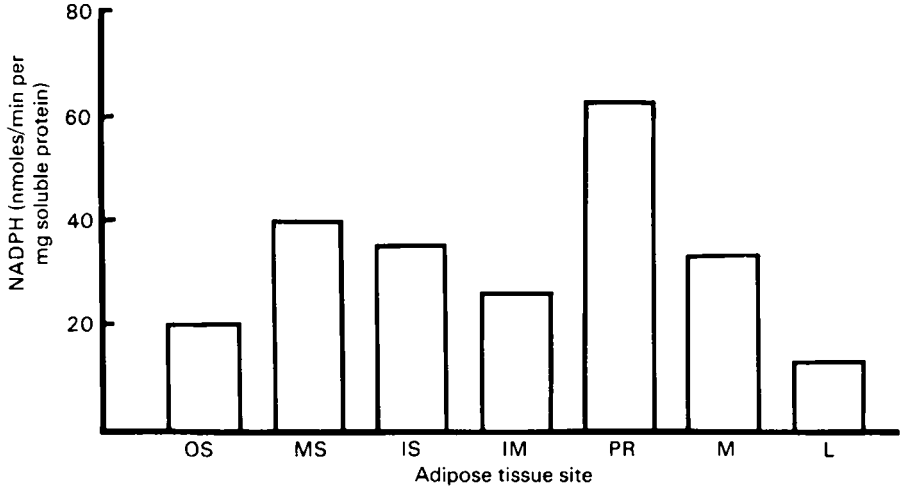

Fig. 3. Comparison of activities of citrate cleavage enzyme (*EC* 4.1.3.8) in adipose tissue from Yorkshire × Poland China crossbred male castrate pigs, each weighing 170 kg. Tissue taken from seven anatomical locations: outer (OS), middle (MS) and inner (IS) subcutaneous backfat; intermuscular (IM), perirenal (PR), mesenteric (M) and leg subcutaneous (L) adipose tissue (after Anderson *et al.* 1972).

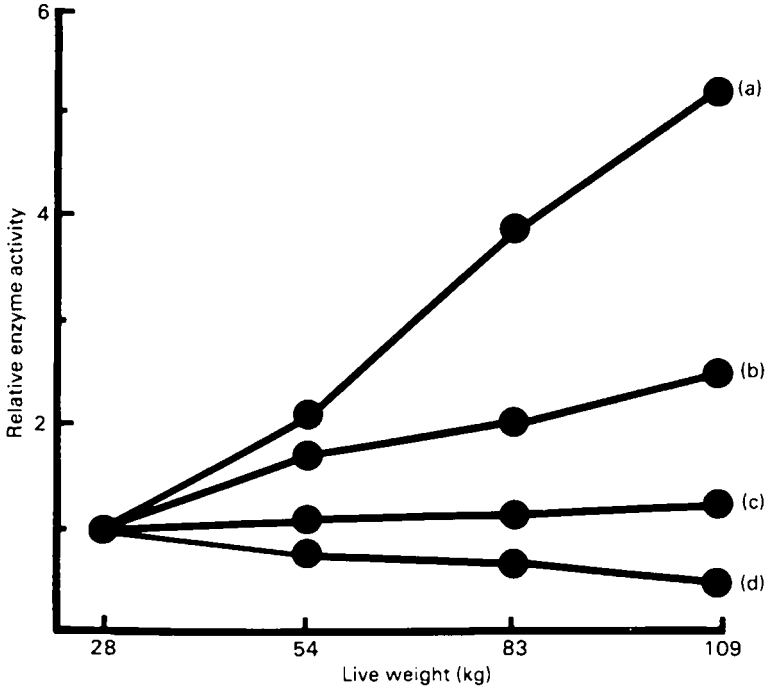

Fig. 4. Effect of live weight and method of enzyme activity expression (i.e. (a) per total weight of perirenal fat, (b) per adipocyte, (c) per mg protein solution, (d) per g of tissue) on the activity of malate dehydrogenase ($NADP^+$) (*EC* 1.1.1.82) in perirenal adipose tissue of pigs of the Minnesota 3×1 breed. Each activity is expressed relative to that found in the 28-kg pigs (after Hood & Allen, 1973*c*).

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Adipocyte size considerations

Adipose tissue can vary in size and cellularity; therefore, caution is required in the interpretation of metabolic studies of adipose tissue. Early studies (McMeekan, 1940; Gelhorn & Benjamin, 1965) noted an apparent paradox of a decreasing rate of fatty acid synthesis, on a wet-weight basis, with age and an increasing rate of fat deposition. Care is required in the interpretation of enzyme activities or rates of fatty acid synthesis, since the patterns that can be deduced are dependent on the reference unit selected for the expression of results.

Enzyme activity measurements are meaningful when they give some indication of lipogenic rate in an adipose tissue site. Hood & Allen (1973*c*) compared the activities of malate dehydrogenase ($NADP^+$) in perirenal adipose tissue of pigs of 28–109 kg live weight (Fig. 4) and found that the expression of activity per unit wet tissue or soluble protein considerably underestimated changes in total adipose tissue activity. Expression of activity on a cellular basis gave the best estimate though still underestimating the increase in total activity with increased fat deposition. Adipocytes are heterogeneous not only in size but also in lipogenic activity (Smith, 1971; Hood & Thornton, 1980*c*; Jamdar *et al.* 1980; Etherton *et al.* 1981). When comparisons are made using metabolic results obtained from a heterogeneous population of adipocytes, no account of size-dependent metabolic differences can be taken.

Adipocytes of different volume have different rates of fatty acid synthesis although the effect may be confounded with those of age, extent of fatness, diet and inherited metabolic disorders. Decreased utilization of glucose by large adipocytes has been reported in non-ruminant tissues. Often this conclusion has been made despite using heterogenous populations of adipocytes of known average volumes. The populations of large adipocytes are often obtained from older (or more obese) animals (DiGirolamo *et al.* 1974; Olefsky, 1977), different anatomical locations (Salans & Dougherty, 1971) or animals which have undergone nutritional treatments (Olefsky & Saekow, 1978). Techniques are available for measuring the

Table 1. *Effect of size of adipocyte, and age on the conversion of glucose to total lipid (nmol glucose* $\times 10^{-6}$*/adipocyte per 2 h) by perirenal adipose tissue of male castrate Hampshire* $\times$ *Yorkshire pigs*•

(Mean values with their standard errors for six pigs within an age group)

Age (days)		Weight (kg)		Adipocyte diameter (μm) 20–63		63–102		102–153		>153	
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
107	1	45	0·9	574	46	1229	148	1700	200		
171	4	91	0·5	366	67	976	146	818	133		
233	5	138	2·5	37	13	381	62	281	52	305	73

•From Etherton *et al.* 1981.

uptake of substrate into adipocytes which have been isolated from a single slice of adipose tissue, separated and grouped according to their diameters (Etherton *et al.* 1977; Hood & Thornton, 1980*c*; Jamdar *et al.* 1980). In one study on pigs of different ages (weights and extent of obesity), Etherton *et al.* (1981) concluded that (a) within each weight (age) group, more glucose is converted to glyceride fatty acids (per adipocyte) by large adipocytes than by small adipocytes, and (b) within a cell-size group, adipocytes from older, more obese pigs are less able to incorporate glucose carbon into fatty acids (Table 1).

When ovine adipocytes were separated into thirteen groups based on diameter, large adipocytes synthesized more fatty acids from acetate than did small adipocytes from the same tissue (Fig. 5) (Hood & Thornton, 1980*c*). Perirenal adipose tissue from fast-growing sheep incorporated more acetate into lipid than did that from slower-growing sheep (Hood & Thornton, 1980*a*), even though mean adipocyte volume was smaller in the sheep with the fast growth rate than in the obese sheep with a slow growth rate. Studies with pigs, sheep and rats (Holm *et al.* 1975) indicate that adipose tissue metabolism is related to nutritional status

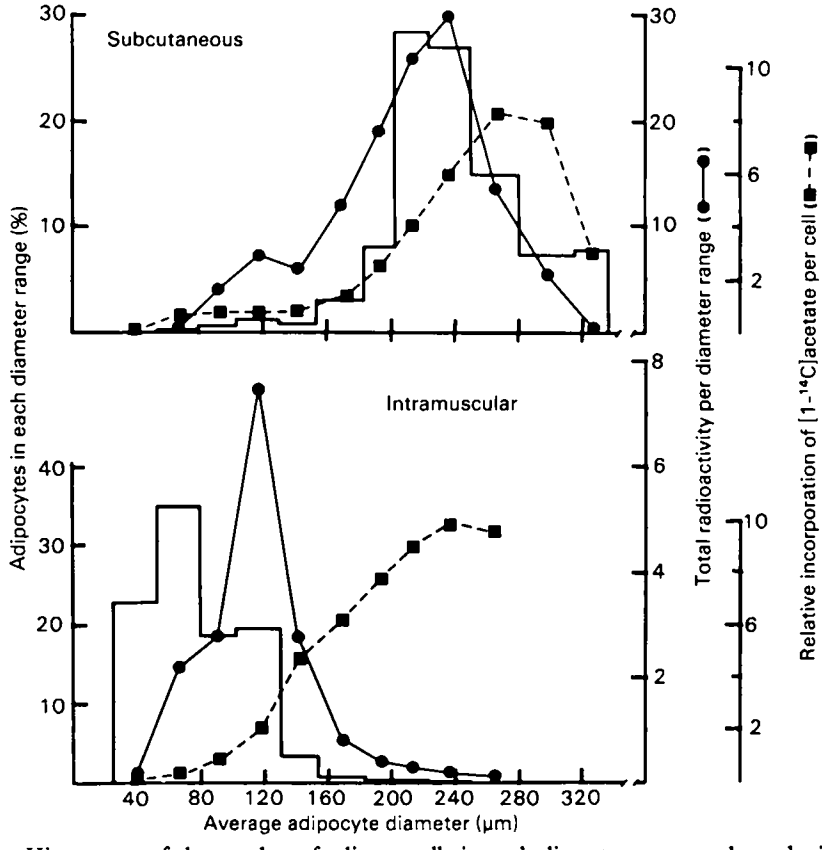

Fig. 5. Histograms of the number of adipose cells in each diameter range and graphs indicating the incorporation of [1-^{14}C]acetate into lipid in ovine intramuscular and rump subcutaneous adipose tissue. Each data point is the mean of three Dorset Horn × Merino wethers (after Hood & Thornton, 1980*c*).

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(e.g. fasting, carbohydrate:fat value) or animal age or both, and is not directly associated with adipocyte volume. These studies emphasize the importance of separating the effects of age and adipocyte size.

In general, fatty acid synthesis is positively correlated to adipocyte size. When activities are adjusted for surface area, however, incorporation rates are similar for cells of all sizes within a population (Hood & Thornton, 1980*a*; R. L. Hood, M. L. Trankina, D. C. Beitz and D. J. Best, unpublished results). In a study with very fat sheep (58% of the carcass as fat), very large adipocytes with diameters greater than 280 μm were found (Hood & Thornton, 1980*b*) which incorporated less acetate into lipid than did smaller cells (Fig. 5). In very large adipocytes, water concentration may be insufficient to maintain the action of the cytoplasmic enzymes which utilize water-soluble substrates (Hood, 1982).

Insulin effects

Insulin added to an incubation medium in vitro enhanced the rates of glucose metabolism in adipose tissue from young (12 kg) pigs (O'Hea & Leveille, 1970). In subcutaneous adipose tissue from older pigs (30–60 kg), however, glucose oxidation and fatty acid synthesis were not stimulated by insulin (O'Hea & Leveille, 1970; Steele *et al.* 1977; Etherton *et al.* 1981).

Nutritional effects

Fasting and consequent weight loss cause a marked decline in rates of lipogenesis and activities of related enzymes, but the capacity for lipid synthesis is restored on realimentation (O'Hea & Leveille, 1969; Ingle *et al.* 1973; Martin *et al.* 1973; Pothoven & Beitz, 1975). Rates of lipogenesis, measured by the incorporation in vitro of [^{14}C]acetate into fatty acids, were measured in wethers which were either fed *ad lib.*, or subjected to early or late periods of starvation (Fig. 6) (Hood & Thornton, 1980*b*). Refeeding resulted in faster growth and immature and mature sheep reached a fat content similar to that in the continuously fed sheep after 100 and 56 d respectively. In continuously fed sheep the rate of lipogenesis in subcutaneous adipose tissue increased with increasing live weight. Lipogenesis was almost eliminated after a period of weight loss in both starved groups and was slower to respond to refeeding in mature sheep; their biochemical pathways of lipid metabolism appear to take several days to respond fully to changes in physiological or nutritional state. Ingle *et al.* (1973) also found that in rump adipose tissue from fasted, refed fattening lambs, in vitro lipogenesis and the activity of acetyl-CoA carboxylase increased to only 13 and 26% respectively of the comparable values observed in the non-fasted control lambs.

A close association exists between the activity of acetyl-CoA carboxylase and in vitro lipogenic capacity in sheep adipose tissue (Ingle *et al.* 1973). Nakanishi & Numa (1970) have shown that during fasting and refeeding of rats, changes in acetyl-CoA carboxylase activity results from dramatic alterations in both the rates of synthesis and degradation of this enzyme; presumably, similar changes occur in other animals. Acetyl-CoA carboxylase, which catalyses the initial reaction in fatty

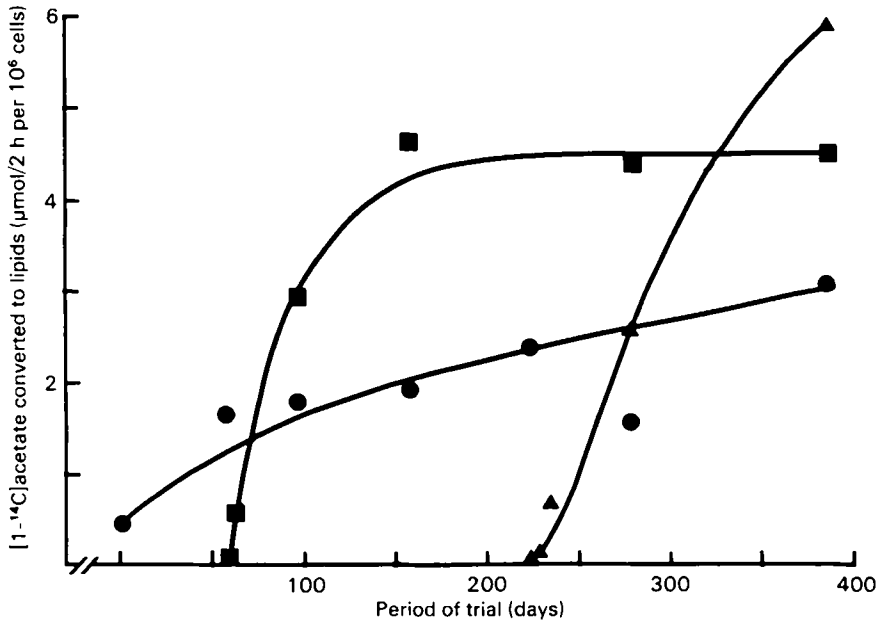

Fig. 6. Effect of early (■) and late (▲) periods of starvation and rehabilitation on lipogenesis in subcutaneous adipose tissue of Dorset Horn × Merino wethers. ●, Continuously fed sheep (after Hood & Thornton, 1980*b*).

acid biosynthesis, has been proposed as the rate-limiting enzyme in fatty acid synthesis (Nakanishi & Numa, 1970). If this is so, resynthesis of acetyl-CoA carboxylase in response to refeeding may occur very slowly in mature sheep, since approximately 1 month was required for full recovery of in vitro lipogenesis (Hood & Thornton, 1980*b*). The regulation of acetyl-CoA carboxylase activity is achieved by changes both in its cellular content and in its catalytic efficiency.

In both mature and immature groups of rehabilitated sheep, in vitro lipogenic capacity increased above that observed for the continuously fed sheep (Fig. 6), which indicates a possible sensitization of the metabolic pathways related to lipogenesis in response to fasting. A similar increase in fatty acid synthesis has been observed in rats when their pattern of food intake changed from nibbling to meal-eating (Leveille, 1966). The increase in the lipogenic capacity of ovine adipose tissue after refeeding contributes to the rapid deposition of fat in sheep which are undergoing compensatory growth; a phenomenon which is important in areas of the world where animals undergo large seasonal changes in growth depending on the availability of feed supply. However, during the initial phase of compensatory growth, particularly in mature sheep, fat of dietary origin rather than fat produced by *de novo* synthesis is the major source of fat deposited in adipose tissue.

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Summary

Rates of fatty acid synthesis alter during growth, and are responsive to physiological conditions which may be imposed on the animal. Typically, *de novo* fatty acid synthesis increases until an animal approaches physiological maturity and then declines, even though fat deposition may be increasing. Nutritional status and the availability of substrate are important factors in regulating fatty acid synthesis in adipose tissue. Enzymic capacity of adipose tissue is also important but appears to respond readily to changing substrate supply.

Adipocyte size also influences lipogenic enzyme activity and rates of synthesis of fatty acids in adipose tissue, and interpretation of results is assisted by using an equal number of adipocytes as the reference unit. Within a population, adipocytes are heterogeneous; large cells have a greater capacity to synthesize fatty acids than small cells. In studies relating metabolic activity to size of adipocytes, the age of the animal and the heterogeneity of adipocytes should be considered.

Many conflicting reports have been published on the influence of anatomical location on the rates of incorporation of substrate into fatty acids. Often, invalid comparisons are made among animals of different breeds at different stages of development. More systematic studies in this area are required to identify changing patterns of *de novo* fatty acid synthesis in adipose tissues from different sites, since rate of growth of these depots changes with maturity and differs among breeds.

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