Influence of Age on *in Vitro* Lipid Biosynthesis and Enzymatic Activity in Pig Adipose Tissue[1] (35597)

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Although the effect of age on carbohydrate utilization in the pig has been extensively studied (1, 2), no study has been conducted to determine the effect of age on lipid biosynthesis in the pig. In rat liver and adipose tissue, lipogenesis is depressed immediately after birth, increases after weaning, and steadily declines from about 35 days to maturity (3–6).

In the present study, we have examined the effect of age on the incorporation of glucose-U-^{14}C into fatty acids by pig adipose tissue slices. In addition, the activities of malic enzyme and citrate cleavage enzyme were studied.

Materials and Methods. A litter of Yorkshire pigs (6 intact females and 2 castrated males) were employed in the experiment. The pigs did not have access to any other food except sow milk until weaning at 35 days. After weaning the pigs were fed an 18% protein diet (corn-soybean meal), supplemented with adequate levels of vitamins and minerals. After 72 days of age, they were fed a 16% protein diet (corn–soybean meal) until the experiment was terminated, when the pigs reached an average weight of 90 kg. The two diets employed contained approximately 3% fat and 60% carbohydrate. Pigs were housed in an enclosed building and food and water were available *ad libitum* after weaning.

Biopsy adipose tissue samples (approx 1 to 2 g) were obtained (7) periodically at the ages indicated in the Results section. The first biopsy sample was obtained just prior to weaning. Duplicate adipose tissue slices were prepared using a Stadie-Riggs hand microtome. The incubation medium contained (per ml): 5 μmoles of glucose, 0.3 μCi of glucose-U-^{14}C, and 0.1 unit of insulin. Incubation conditions, extraction and CO_2 collection procedures and preparation of homogenates for enzyme assays have been described previously (7). Malic enzyme (EC 1.1.1.40) was assayed by the method of Ochoa (8) and citrate-cleavage enzyme (EC 4.1.3.8) according to the method of Cottam and Srere (9). The protein content of the tissue homogenates used for enzyme assays was determined by the procedure of Lowry *et al.* (10).

Results. The effect of age on glucose-U-^{14}C incorporation into fatty acids and oxidation to $^{14}CO_2$ is presented in Fig. 1. Fatty acid synthesis was extremely low in the biopsy samples obtained when the pigs were still nursing their dam on day 35. Lipogenesis increased rapidly after weaning when pigs began consuming the dry diet and reached a peak at 67 days of age. Thereafter, it declined linearly with advancing age. Production of $^{14}CO_2$ paralleled the response seen for fatty acid synthesis.

The influence of age on the activity of malic enzyme and citrate cleavage enzyme is shown in Fig. 2. Malic enzyme activity was extremely low at 35 days of age, increased rapidly after pigs began consuming the dry diets, and reached a maximum level at 109 days of age. After 109 days, malic enzyme activity decreased with advancing age. The activity of citrate-cleavage enzyme was also extremely low at 35 days and tended to reach a plateau from 50 to 109 days, and then

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decreased with advancing age. However, the magnitude of change observed for citrate-cleavage enzyme was less marked than that observed for malic enzyme.

The growth curve of the pigs employed in this study is presented in Fig. 3. After removal from the dam at 35 days, there was a slight loss of weight for the first 10 days,

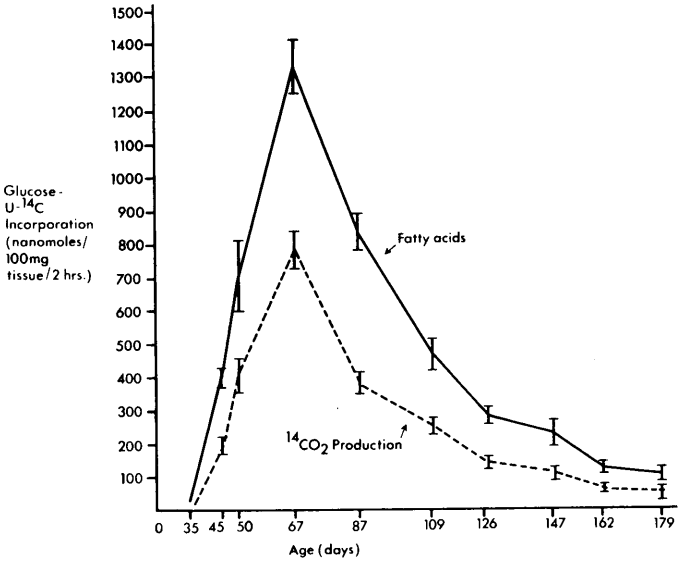

FIG. 1. Incorporation of glucose-U-^{14}C into fatty acids and oxidation to $^{14}CO_2$ by adipose tissue slices during development. Each point represents the mean ± SEM for 8 pigs.

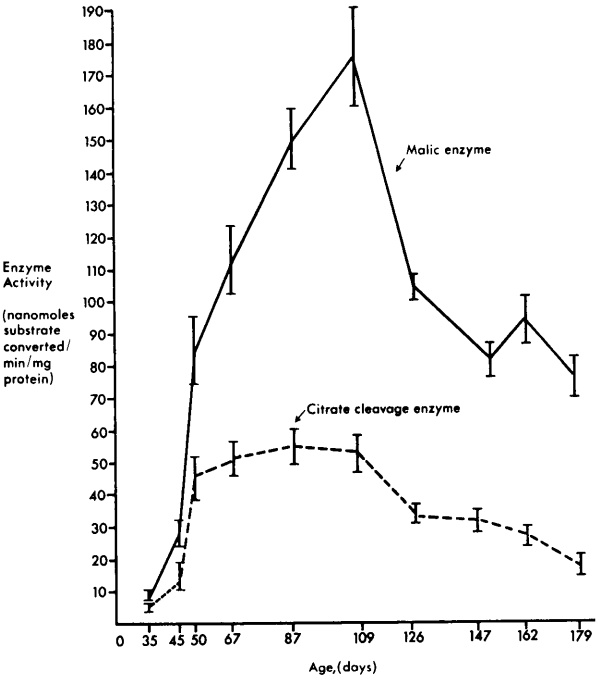

FIG. 2. Activities of malic enzyme and citrate-cleavage enzyme in adipose tissue homogenates during development. Each point represents the mean ± SEM for 8 pigs.

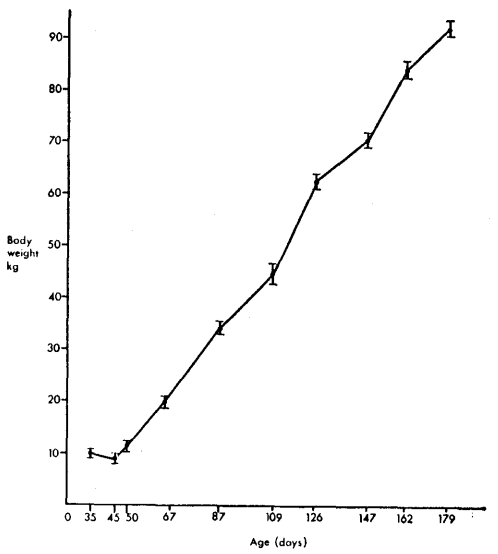

FIG. 3. Cumulative weight gain of developing pigs. Each point represents the mean ± SEM for 8 pigs.

during which time the pigs were adjusting to the dry, high-carbohydrate diet. From 45 days to 179 days there was a linear increase in body weight with advancing age.

Discussion. From the data presented, it appears that the changes in the capacity for fatty acid biosynthesis during development in the domestic pig can be divided into 3 periods: a very low rate of lipogenesis at the end of (and presumably during) the suckling period, a very rapid rate of fat synthesis from weaning to approximately 67 days of age and a gradual decrease in the capacity for fatty acid biosynthesis from 67 days to maturity.

We have reported previously (7) that the addition of fat to the diet of pigs triggers metabolic responses in adipose tissue resulting in a suppression of lipogenesis. It seems very probable that the extremely low rate of lipogenesis during the suckling period is due to the high-fat diet the pigs are ingesting. The dry matter of sow's milk contains approximately 40% fat, 30% protein, and 24% carbohydrate (11). At weaning the pigs were switched to a high-carbohydrate, low-fat (approx 3%) diet. Therefore, the rapid increase in lipogenesis at weaning may be due to the change from a high-fat, low-carbohydrate diet to a low-fat, high-carbohydrate diet. The third period of change in lipogenesis during development in the pig cannot be attributed to nutritional factors, since the diet from weaning to 179 days was essentially the same. The decreased rate of fatty acid synthesis expressed per unit weight of tissue seen with advancing age has been attributed to an increased size of the fat cells (12). According to this concept the weight of the individual fat cell increases due to fat accumulation while the fatty acid synthesizing capacity of each cell remains constant. Thus, there is an apparent decrease in fat synthesizing capacity per unit weight of tissue as the animal becomes older and fatter. However, this theory does not explain the enzymatic data since it is expressed on the basis of cytoplasmic protein which does not change as the fat cells increase in size due to the incorporation of more lipid material.

Lipogenesis requires an extramitochondrial supply of acetyl-CoA and NADPH. Citrate-cleavage enzyme is important in supplying cytoplasmic acetyl-CoA, since the mitochondrial membrane is essentially impermeable to acetyl-CoA (13). Young *et al.* (14), Wise and Ball (15) and Pande *et al.* (16) suggested that the oxalacetate derived from citrate cleavage could be converted to malate, then to pyruvate via malic dehydrogenase and malic enzyme, thereby transferring a hydrogen from NADH to $NADP^+$. After discovering that pyruvate carboxylase was present in the cytoplasm, Ballard and Hanson (17) proposed a "transhydrogenation cycle." Thus a mechanism exists for overcoming the mitochondrial impermeability to acetyl-CoA while at the same time generating NADPH, the essential source of reducing equivalents for fatty acid biosynthesis.

We have reported previously (7) a high correlation between malic enzyme activity and lipogenesis in pig adipose tissue. In the present study, the activity of both malic enzyme and citrate-cleavage enzyme in pig adipose tissue follows a pattern similar to lipogenesis. These results suggest that the "NADH–$NADP^+$ transhydrogenation cycle" is operating to supply reducing equivalents in pig adipose tissue as well as the pentose

pathway (18).

Evidence has been reported with rat liver (19), rat adipose tissue (20), pig adipose tissue (21), and chicken liver (22), which demonstrates that changes in lipogenesis precede the change in malic enzyme and citrate-cleavage enzyme activity. These data support the concept that changes in enzymatic activity respond to changes in fatty acid synthesis rather than cause the changes. The data reported in this study lend support to this concept. At weaning, the pigs were switched from a high-fat diet to a high-carbohydrate diet and an increase in lipogenesis was seen before any marked increase in the activity of malic enzyme occurred. The maximum rate of lipogenesis occurred several days before the maximum activity of malic enzyme was observed. The relationship of citrate-cleavage enzyme activity to lipogenic rate is not as obvious since no clear peak was observed for citrate-cleavage enzyme activity nor were the changes in activity as marked as those for malic enzyme.

Ballard and Hanson (5) and Taylor *et al.* (6) have reported effects of age on hepatic lipogenesis in the rat. Their results and the present results for the pig are in good agreement. In both species, there is a low rate of lipogenesis during the suckling period, a marked increase after weaning, and then a gradual decrease as the animal approaches maturity.

Summary. Lipogenesis, as measured by the incorporation of glucose-U-^{14}C into fatty acids by adipose tissue slices was determined periodically in 8 pigs from the age of 35 days (9.0 kg) to 180 days (90.0 kg). In the suckling pig, the capacity for fatty acid synthesis was very low, presumably due to the high-fat content of sow milk. Upon ingestion of the high-carbohydrate diet, fatty acid synthesis increased rapidly and reached a peak at 67 days of age. From 67 days of age there was a gradual decrease in the capacity for fatty acid synthesis with advancing age. Changes in the activities of citrate-cleavage enzyme and malic enzyme occurred in parallel with the changes in fatty acid synthesis, supporting the participation of these enzymes in lipogenesis in pig adipose tissue.

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