MUSCLE AND ADIPOSE TISSUE LIPOPROTEIN LIPASE IN FETAL AND NEONATAL SWINE AS AFFECTED BY GENETIC SELECTION FOR HIGH OR LOW BACKFAT

John P. McNamara and Roy J. Martin

University of Georgia, Athens 30602

Summary

The effect of genetic selection for backfat accumulation on adipose tissue and muscle lipoprotein lipase (LPL) in fetal and neonatal pigs was investigated. Fetal pigs at 110 d of gestation were taken surgically from sows of either a high backfat (High) or low backfat (Low) line of Yorkshire pigs selected over 18 generations on the basis of backfat thickness at 80 kg live weight. Fetuses of Yorkshire (Control) and Ossabaw (Obese) sows were used for comparison. Activities of LPL/mg cytoplasm protein in subcutaneous adipose tissue were 2.23 ± .19, 3.98 ± 1.06, 6.37 ± .83 and 7.57 ± .66 nmol FFA released/min and LPL/g tissue was 34.80 ± 4.06, 58.36 ± 14.23, 99.55 ± 15.15 and 159.94 ± 9.7, for Low, Control, High and Obese 110-d fetuses, respectively. Muscle (semimembranosus) LPL/mg protein was 1.06 ± .17 and 1.39 ± .20 and LPL/g tissue was 50.11 ± 6.9 and 59.07 ± 9.12 for High and Low line fetuses, respectively. Fetal body composition was not different for High and Low lines. In 14-d-old suckling pigs, adipose tissue LPL/mg protein had increased to 18.09 ± 3.48 and 17.76 ± 3.98 and LPL/g tissue was 291.12 ± 56.60 and 308.45 ± 64.43 in High and Low line pigs, respectively. Muscle LPL/mg protein had decreased to .83 ± .08 in High and 1.25 ± .13 in Low line pigs, while LPL/g muscle was similar between these lines. These effects of genetic selection on muscle and adipose tissue suggest that early in development, the partitioning of nutrients to lean or fat tissues may be altered, eventually leading to a marked difference in body composition.

(Key Words: Pig, Fetal, Genetic Selection, Lipoprotein Lipase, Muscle, Adipose.)

Introduction

At the luminal surface of capillaries, the enzyme lipoprotein lipase (LPL; EC 3.1.1.34) catalyzes the breakdown of lipoprotein triglycerides, allowing entry of fatty acids from triglycerides (TG) into cells. In the young pig on a milk diet, LPL allows utilization of chylomicron triglyceride, the major source of energy from this diet. Although circulating TG concentrations may be low in swine compared with other nonruminant species (Eisenberg and Levy, 1975; Kraeling et al., 1978) they could be a major source of lipid for tissues of fetal swine. Fetal swine have low rates of liver gluconeogenesis and lipogenesis (Mersmann, 1974). Fetal adipose tissue is actively synthesizing and storing lipid in late gestation (Desnoyers et al., 1980; Hausman et al., 1981). Lipoprotein lipase has been measured in fetal swine adipose tissue (Hausman et al., 1981) and is correlated with fetal adipocyte lipid filling. Tissue LPL activity estimates the tissue's capacity to take up circulating TG fatty acids, which are either from the liver or from the diet in the chylomicrons. Total tissue LPL activity would reflect the tissue's ability to derive energy from TG (as in muscle) or to store energy as fatty acids (as in adipose tissue).

The relative rates of muscle and adipose tissue growth determine the eventual body composition and, therefore, the economic and nutritional worth of meat animals. These rates

1 Supported in part by the Univ. of Georgia Exp. Sta. Project H666 and by NIH Grants HD 15064 and k04 Am00716.
2 Send reprint requests to R. J. Martin, Dept. of Foods and Nutrition, College of Home Economics, Univ. of Georgia, Athens 30602.
3 We gratefully acknowledge the technical assistance of Alayne Makula, Pat Mesta and Kenneth Williams.
4 Dept. of Foods and Nutr.
are determined by the genetic potential of the breed and by the nutrition supplied to the animal, as well as by environmental factors and animal health. Genetic selection for backfat accumulation has been used on Duroc and Yorkshire pigs (Hetzer and Harvey, 1967). This selection, now through 18 generations, has produced two lines designated High Backfat (High) and Low Backfat (Low). The lines have grossly different rates of adipose tissue accumulation, carcass composition (Hetzer and Miller, 1972a,b) and de novo lipogenesis in subcutaneous adipose tissue (Scott et al., 1981).

Previous studies have shown a correlation between LPL activity and the metabolic activity of muscle and adipose tissue of young (Planche et al., 1980) and adult rats (Linder et al., 1976; Tan et al., 1977) and of humans (Lithell et al., 1979a,b). This relationship has not been investigated in the pig. An alteration of either muscle or adipose tissue LPL in the High and Low lines should be consistent with a difference in the relative metabolic rates of either tissue between the two lines. Lipoprotein lipase is more active in red-type oxidative muscle than in white-type, glycolytic muscle. This is consistent with the theory that LPL activity is related to relative rates of lipid utilization. Therefore, we measured the LPL activity of dorsal subcutaneous adipose tissue and semimembranous muscle of 110 d fetuses to determine if fetal development was affected by genetic selection for increased backfat at 80 kg live weight. We also measured LPL at 14 d of age to determine if exogenous substrate (chylomicron) availability had an effect on the activity of this enzyme. Yorkshire control pigs and genetically obese Ossabaw pigs were contrasted to the two selected lines. Ossabaw pigs were studied to provide a comparison between the selected lines and a known genetically obese line (Martin et al., 1973).

Materials and Methods

High and Low Backfat animals were lines selected over 18 generations from the Yorkshire breed (Hetzer and Harvey, 1967; Hetzer and Miller, 1972a,b). Animals were housed in individual stalls before surgery and fed ad libitum a diet of 67.7% ground shell corn (IFN 4-02-935), 10% oats (IFN 4-03-309), 5% wheat bran (IFN 4-05-190), 8% soybean meal (50% protein, IFN 5-04-612), 4% poultry by-product meal (IFN 5-03-799), 2.5% alfalfa meal (17% protein, IFN 1-00-023) and 2.83% vitamin and mineral mix.

At 110 d of gestation, animals were laparotomized under halothane anesthesia and the fetuses removed and exsanguinated. At 110 d, six High, three Low, five Yorkshire and four Ossabaw sows were used to provide the desired number of fetuses. For 14-d-old pigs, two Low and three High line sows were used. The dorsal subcutaneous adipose tissue, including inner and outer layers, was excised from the cervical to the lumbar region and stored at -20 C until assayed (within 2 mo). Samples of semimembranous muscle (about 2 g) were also taken, cleaned of external connective tissue and frozen. Body composition was determined on median weight fetuses from each litter as follows: the frozen carcass was autoclaved, coarsely minced and homogenized in one volume of water. An aliquot was extracted with four volumes of 2:1 methanol:chloroform for 1 h, an additional volume of chloroform and acidic KCl was added and the entire mixture was held on ice for at least 5 min. Phases were separated by centrifugation, the top layer removed and bottom organic layer dried to determine lipid content. Dry matter was determined on an aliquot of the homogenate after drying for 48 h at 90 C. The dried sample was ashed for at least 12 h at 600 C for ash content. Protein content was calculated by difference.

Tissue samples were homogenized and LPL activity was assayed as described by Hausman et al. (1981) with the technique of Hietanen and Greenwood (1978) with some modifications: tissues were homogenized at a 4:1 (adipose tissue) or 8:1 (muscle) buffer:tissue ratio in .15 M NaCl, .005 M Na barbital, 20% glycerol and pH 7 buffer twice on ice at setting 8 on the Brinkman Polytron®. Homogenates were centrifuged at 12,000 x g, the fat was removed and the LPL assay performed on the supernatant. Lipoprotein lipase activity is expressed as 1 unit = 1 nmol free fatty acid released/min.

Statistical Analyses. The effect of breed, age, tissue and the interactions were determined with analysis of variance for fixed effects, and Fisher's Least Significant Difference test was used to determine significant differences among means (Steel and Torrie, 1960).
Results and Discussion

Selection for different amounts of backfat in swine caused a threefold increase in dorsal subcutaneous adipose tissue LPL activity in 110 d fetal pigs of the High line compared with the Low line (table 1). This effect was seen both in specific activity (units/mg cytoplasm protein) and in activity/gram of tissue. In Yorkshire fetal adipose tissue assayed at the same time, LPL activity was intermediate compared with High and Low lines (table 2). Ossabaw swine, a genetically obese strain with a decreased muscle growth rate (Martin et al., 1973), had the highest activity of adipose tissue LPL, although not significantly higher than that of the High line.

Muscle LPL of 110 d fetuses was not significantly different between Low and High lines (table 1). In the High line, LPL/g muscle was considerably lower than LPL/g adipose tissue. However, LPL/g tissue was higher in the low line muscle than in the Low line adipose tissue. These data suggest that at 110 d of gestation, High line fetuses had a greater capacity for TG fatty acid uptake in adipose tissue than in muscle; while the low line was opposite. At 110 d of gestation, the body compositions of High and Low line fetuses were not different (table 3). Selection, therefore, caused a change in activity of LPL before any measurable change in tissue accumulation. This early development of fetal adipose tissue LPL is possibly a key initial event in the excessive body fat accumulation in later growth phases in these genetically obese pigs. This is the first report of a change in adipose tissue LPL activity in lines selected for adiposity.

Previous studies in this laboratory have shown that the expression of swine adipose tissue LPL can be altered in utero by removal of hypothalamic control (Hausman et al., 1981). The energy status of the dam was not altered, therefore, the alteration may have been caused by a developmental difference in the tissue itself or by a change in fetal endocrine status. Similarly, the difference in adipose tissue LPL between High and Low line fetuses (present study) may be a genetic change in the tissue or due to an altered endocrine status. Growth hormone (GH) may be decreased in High line fetuses (J. A. Sheahan and R. J. Martin, unpublished data) and this may result in a greater LPL activity and eventual lipid accumulation.

<table>
<thead>
<tr>
<th>TABLE 1. LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE AND MUSCLE TISSUE OF 110-D FETAL PIGS GENETICALLY SELECTED FOR HIGH OR LOW BACKFAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Line</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Low</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± SE for 8 to 12 animals. Values are expressed as units/mg cytoplasm protein or gram wet tissue. One unit equals 1 nmol free fatty acid released/min.

<sup>b</sup>c'dDifferent superscripts indicate that High and Low lines differ (P<.05).

<sup>d</sup>eDifferent superscripts indicate that muscle and adipose tissue differ (P<.05).

<table>
<thead>
<tr>
<th>TABLE 2. LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE TISSUE OF 110 D FETAL PIGS OF FOUR GENETICALLY DIFFERENT STRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High backfat</strong></td>
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<td></td>
</tr>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± SE for 8 to 12 animals. Values are expressed as in table 1. High and Low backfat strains were developed from Duroc and Yorkshire breeds over 18 generations by selection for backfat thickness. Yorkshire pigs were control animals in the herd. Ossabaw pigs were an obese strain as described in the text.

<sup>b</sup>c'dMeans with different superscripts differ (P<.05).
TABLE 3. BODY COMPOSITION OF 110-D FETAL PIGS GENETICALLY SELECTED FOR HIGH OR LOW BACKFAT

<table>
<thead>
<tr>
<th>Item</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>% ash</td>
<td>2.55 ± .14a</td>
<td>2.95 ± .22</td>
</tr>
<tr>
<td>% protein</td>
<td>15.63 ± .78</td>
<td>14.74 ± .62</td>
</tr>
<tr>
<td>% fat</td>
<td>1.81 ± .08</td>
<td>1.68 ± .06</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>813.0 ± 57.4</td>
<td>966.9 ± 96.5</td>
</tr>
</tbody>
</table>

*aAll values are means ± SE for 8 to 12 animals. Values are expressed as percentages of carcass weight. There were no significant differences.

Greater LPL activity in the High line fetuses may be caused by differing energy status. A difference in maternal energy status can alter fetal adipose tissue LPL (T. R. Kasser, J. P. McNamara and R. J. Martin, unpublished observations). Adipose tissue and possibly muscle LPL are regulated by the relative energy balance of the animal, possibly via insulin (Nilsson-Ehle et al., 1980). Further research is needed to determine if the alteration in fetal development of LPL observed in this study was caused by direct genetic involvement at the tissue or by an indirect effect through maternal energy status.

To further characterize the change in tissue LPL in High and Low line pigs, we measured muscle and adipose tissue LPL in 14-d-old animals. These animals were suckling their own dams, with the same number of pigs/litter. Muscle LPL/mg protein was lower in both lines at 14 d of age than at 110 d of gestation, and the specific activity of High line swine muscle was lower than that of Low line swine muscle (table 4). Therefore, when the pigs were free from in utero maternal influence, muscle LPL activity still indicated that Low line muscle had a greater specificity for lipid uptake than High line pig muscle. Adipose tissue LPL activity in suckling pigs was increased over that of 110 d fetuses (three-to eightfold, table 1). This value was within the range of subcutaneous adipose tissue LPL activity reported for other pigs of the same age (Enser, 1973; Lee and Kauffman, 1974). Suckling pigs in this study were consuming a high fat (milk) diet that would increase plasma levels of chylomicrons. Lipoprotein lipase would be important in uptake of these chylomicrons. Both High and Low line pigs probably were storing lipid in adipose tissue at high rates during this period of high fat energy intake. Therefore, the genetic difference seen in adipose tissue in utero has been temporarily masked by mass action of substrate entry. The persistence of differences in muscle tissue and in older pigs of these lines (Hetzer and Miller, 1972b; Scott et al., 1981) indicates a maintenance of the genetic effect. Differences in yield and composition of milk also may affect neonatal LPL activity. Milk yield and composition from lean and obese sows were not measured in this study.

It has been reported that two breeds of swine (Duroc and Hampshire) had different levels of LPL in the same adipose tissue site (Lee and Kauffman, 1974). Results of the present study agree with those reported earlier in that a genetic effect on the expression of LPL activity was demonstrated. Muscle LPL was lower in neonatal genetically obese Zucker rats than in lean Zucker rats (Boulange et al., 1981). In adult Zucker rats, muscle LPL and lipid uptake were similar in muscle from lean and obese animals (McNamara et al., 1982). These findings support the concept that muscle LPL can be altered both by genetics (as seen in young animals) and by energy balance (as seen in adults). The finding of Althen and Gerrits (1976) of decreased serum and pituitary GH in High line pigs of the 16th generation provide a hypothesis for the change in muscle and adipose LPL in utero and in muscle tissue neonatally in

TABLE 4. LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE AND MUSCLE TISSUES OF 14-D-OLD SUCKLING PIGS FROM LINES SELECTED FOR HIGH OR LOW BACKFAT

<table>
<thead>
<tr>
<th>Line</th>
<th>Adipose tissue</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/mg proteina</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>18.09 ± 3.48bce</td>
<td>.83 ± .08cf</td>
</tr>
<tr>
<td>Low</td>
<td>17.76 ± 3.89bce</td>
<td>1.25 ± .13df</td>
</tr>
<tr>
<td></td>
<td>U/g tissue</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>291.12 ± 56.60bce</td>
<td>67.53 ± 9.33cf</td>
</tr>
<tr>
<td>Low</td>
<td>308.45 ± 64.43bce</td>
<td>71.54 ± 6.85cf</td>
</tr>
</tbody>
</table>

*aValues are means ± SE for 9 animals. Values are expressed as in table 1.
b,c,dDifferent superscripts indicate that High and Low lines differ (P<.05).
e,fDifferent superscripts indicate that tissues differ (P<.05).
this line. Pigs selected for high backfat may have less GH, causing a slower rate of muscle lipid uptake than normal, consistent with low muscle LPL. The lower GH would not directly affect adipose tissue LPL, however, the energy not used in muscle growth may then be used by the adipose tissue, leading to a greater LPL activity and eventually to a greater lipid accumulation.

In this study, the activity of LPL, a key enzyme in the control of tissue lipid uptake, was altered in utero by genetic selection for changes in the rate of lipid accumulation. A line selected for low backfat had increased muscle LPL specific activity compared with the High backfat line at 14 d of age. In addition, muscle LPL/g tissue in the Low backfat line was greater compared with Low line adipose tissue in utero. This may suggest that early in development, genetic selection alters the partitioning of nutrients to lean or adipose tissues that may eventually lead to a marked difference in body composition. Further studies of genetic control of lipid metabolism could lead to greater improvement in meat animal production.

**Literature Cited**


