THE EFFECT OF MATERNAL DIABETES AND FASTING ON FETAL ADIPOSE TISSUE HISTOCHEMISTRY IN THE PIG

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Summary

The effects of maternal diabetes (induced by 78 of gestation) and food deprivation (last 20 d of gestation) on the histochemical disposition of fetal adipose tissue at 112 d of gestation were determined. In both cases, there was an increased number of fat cell clusters by comparison with the control. In the fetuses of diabetic pigs, there were striking effects on adipocyte size and the extent of lipid filling of presumptive adipose space. In these fetuses, the adipocytes were large and many were unilocular, whereas, those in the fetuses of control and fasted pigs were smaller. The adipose tissue space of the control was “empty” compared with that of fetuses of diabetic pigs. Adipocytes from fetuses of diabetic pigs contained intracellular glycogen deposits, which were not present in adipocytes of control and fasted progeny. Maternal fasting and diabetes increased the number of lipid-containing adipocytes in fetal adipose tissue. An additional effect of maternal diabetes was to increase fetal adipocyte size over that of fetuses of control and fasted pigs.

(Key Words: Fetal, Histochemistry, Pig, Adipocyte, Diabetes, Fasting.)

Introduction

The term pig fetus and mature pigs contain similar numbers of extramuscular adipocytes (Vodovar and Desnoyers, 1978). Therefore, the fetal stage of porcine adipose tissue development is achieved primarily by an increase in adipocyte number (Vodovar and Desnoyers, 1978). The subcutaneous depot develops early in fetal life (Hausman, 1978) and by 2 to 3 wk after birth is filled with a large number of small adipocytes (Moody et al., 1978). The mechanism of “adipocyte filling” in the fetus involves the formation of a finite number of fat cell clusters with minimal increase in fat cell size (Hausman, 1978). Theoretically, an alternative mechanism for “adipocyte filling” is through an increase in fat cell size with minimal increase in fat cell cluster number. Adipocyte size relative to fat cell cluster number will indicate the primary “adipocyte filling” mechanism. Knowing which mechanism was primary will indicate if a given imposed factor is more effective in altering adipocyte size or adipocyte number (fat cell cluster number).

Interrelationships between adipose cellularity and the endocrine and metabolic status in the fetal pig have not been investigated. Previous research (Ezekwe and Martin, 1980) indicated that fetuses of diabetic sows had a twofold increase in percentage body fat but unaltered fetal hepatic lipogenesis. The increase in body fat may have been caused by greater lipid synthesis from glucose as a result of the elevated maternal levels of glucose. Alternatively, the body fat increase may have been caused by a greater uptake of free fatty acids as a result of the elevated maternal levels of free fatty acids, which is also associated with the diabetic condition. To determine which factors were most important in increasing body fat in the fetus we have analyzed adipose tissue of near term pig fetuses from diabetic and fasted sows. A group of chronically fasted sows served as a critical control group for the diabetic sows so that the effects of elevated maternal levels of free fatty acids per se could be evaluated. Without the fasted control group, the effects of maternal diabetes would be confounded due to elevated levels of both glucose and free fatty acids. Other studies have shown that 40 d fasting during a period of rapid fetal growth in the pig had no affect on total litter weight at birth (Hard and Anderson, 1979). In the present study, sows were fasted for 20 d during a period of slower fetal growth.
Figure 1. Cryostat (24 μm) cross sections of skin, hypodermis and underlying muscle from the dorsal shoulder area of control fetuses (A), fetuses from fasted sows (B) and fetuses from diabetic sows (C), (×23). These sections were stained for neutral lipid (dark material) with oil red O for the cellular nuclei with Harris hematoxylin. Indicated on the micrographs are dermis (d), epidermis (e), muscle (m), inner (i) and outer (o) layers of subcutaneous adipose tissue and clusters of lipid-containing fat cells (arrowheads). Note the thicker inner subcutaneous layer and the larger number of fat cell clusters in the fetuses of the diabetic (C) and fasted (B) sows than in the control fetuses (A). The complete line in A indicates the area and amount of tissue measured in the adipose tissue thickness data presented in table 1.

Materials and Methods

Sows. Toward the end of the second trimester, 15 pregnant sows were allocated to three treatment groups and individually housed in concrete pens. Four sows served as controls and were fed ad libitum a standard gestational diet. Alloxan diabetes was induced in seven sows by iv injection (40 mg alloxan/kg body weight via ear vein) on d 78 of gestation. The methods used for alloxan treatment have been
described by Romso et al. (1971) and modified by Ezekwe and Martin (1980). Blood samples were drawn on d 81 for determination of blood glucose. Sows with serum glucose levels greater than 300 mg/dl were considered diabetic. Another group of four sows was fed ad libitum until d 92, when a total fast of 20 d was begun. Sows were rendered unconscious by carbon dioxide and hysterectomy was performed on d 112, 2 d before the normal farrowing date.

**Fetuses.** Fetuses were rapidly removed from the uterus, revived, dried and ranked according to body weight. Average body weight for the two fetuses selected from each litter approximated the mean body weight for the litter. The total thickness of dorsal, interscapular subcutaneous tisse was measured on rectangular samples removed from the animal. A measurement was made on both sides of the most dorsal location of subcutaneous tissue (figure 1). The two measurements were averaged and this number represented the adipose tissue thickness for that animal.

**Histology.** The blocks of dorsal, interscapular subcutaneous adipose tissue were obtained from the designated fetuses after sacrifice. These blocks included epidermis, dermis and all underlying connective tissue including some muscle. The tissue blocks were quickly frozen in isopentane cooled in an acetone-dry ice mixture and stored in an ultracold freezer (−60 C) until analyzed. Air-dried, fresh frozen cryostat (−20 C) sections (10 to 30 μm) were stained with oil red O (Barka and Anderson, 1963), picro ponceau (Humason, 1972), the periodic acid-Schiff (PAS)-diastase procedure (Humason, 1972), toluidine blue, pH 4.2 (Humason, 1972) and Harris hematoxylin (HH). Slide holders with a 25-slide capacity were used for the various staining procedures. By this procedure, eight sections from each treatment group were simultaneously stained for each of the histochemical techniques. Comparative analysis of the various treatment effects were restricted to those sections that were stained together.

**Fat Cell Diameter.** Fat cell diameters were determined on lipid stained cryostat sections according to the method of Sjostrom et al. (1971). One hundred fat cell diameters were measured for each of five pigs from the fasted, control and diabetic groups. It was impossible to determine the size of all the small adipocytes in sections from the fetuses of diabetic sows because of the compact nature of groups of adipocytes. Therefore, the fat cell size determined for progeny of diabetic sows may be biased towards larger cells.

**Statistical Analysis.** Differences between two means were analyzed by the Student's t-test as described by Steel and Torrie (1960).

**Results**

The adipose tissue of the fetuses of diabetic sows (FDS) and of the fetuses of fasted sows (FFS) was thicker than that of the fetuses of control sows (FCS; figure 1 and table 1). Adipose tissue from FDS had a much greater lipid content than that from either the FFS or the FCS (figure 1). The adipose tissue from the FDS was more lipid filled than was the adipose of FFS and FCS (figure 1). In the FCS, lipid deposition was evident in both layers of subcutaneous adipose tissue (figure 1). The FFS had slightly more lipid in the adipose tissue (figure 1).

| Table 1. Body Weights, Backfat Thickness and Fat Cell Diameters of Fetuses of Control (FCS), Diabetic (FDS) and Fasted Sows (FFS) |
|-------------------------------|-------------------------------|-------------------|
| **Animal** | **Body wt, g** | **Adipose thickness** | **Fat cell diameter (μm)** |
| | | **Inner layer** | **Outer layer** |
| FCS | 1,077 ± 69 | 3.7 ± 2c | 18.8 ± 1.0c |
| FFS | 1,085 ± 41 | 5.0 ± 2d | 18.7 ± 1.4d |
| FDS | 1,013 ± 54 | 5.1 ± 3d | 28.7 ± 2d |

aBody weights expressed as means ± SE for eight animals.

bSubcutaneous adipose tissue thickness expressed as means (mm) ± SE for eight animals.

Means ± SE for five animals.

Values in a column with different superscripts differ (P<.05).
Figure 2. Cryostat (24 μm) sections of adipose tissue from control (A) and diabetic (B) progeny, (×250). These sections were stained by the picro ponceau method and Harris hematoxylin. Indicated on the micrographs are small multilocular adipocytes (a) and unilocular adipocytes (u). Note the large number of unilocular adipocytes in the fetus of the diabetic sow as compared with the smaller, multilocular adipocytes that predominate in the control fetus. Note, too, that the reaction for connective tissue (arrowheads) around many adipocytes from the diabetic progeny is more obvious than that around adipocytes from control progeny.
Figure 3. Cryostat (24 μm) sections of dorsal subcutaneous adipose tissue from control (A) and diabetic (B) progeny, (X 250). These sections were reacted for metachromasia (due to glycosaminoglycan) with toluidine blue staining. Indicated on the micrographs are fat cell clusters (FC). In these black and white micrographs, the darkly stained material is actually a purple-red colored reaction product. Note the darkly stained metachromatic area (m) around fat cell clusters from control fetuses (A) and the absence of this phenomenon in diabetic progeny (B).
Figure 4. Cryostat (24 μm) sections of dorsal subcutaneous adipose tissue from control (A) and diabetic (B) progeny, (X250). These sections were reacted for glycogen with the PAS-diastase procedure of Humason (1972) and counter stained with Harris hematoxylin. Note the positive reaction within adipocytes (arrowheads) from the FDS (B) whereas there is only a positive reaction around adipocytes (arrowheads) from the FCS (A). The reaction within the adipocytes from the FDS was abolished by pretreatment with diastase.

1), which was associated with the increased thickness.

The greater lipid concentration in the adipose tissue from the FDS was attributable to an increase in fat cell size (figure 2 and table 1). Adipocytes from the FFS and FCS were similar in morphology and size (figure 2 and table 1). Concentrations of fat cell clusters were similar in adipose tissue of the FFS and FCS (figure 1). The increased thickness of adipose tissue from the FFS (table 1) was, therefore, associated with a larger number of fat cell clusters relative to FCS (figure 1).

When the sections were stained for connective tissue fibers (picro ponceau), there were no apparent differences between the three groups in amount or organization of fascicular connective tissue (strands of connective tissue separating
adipocyte clusters). Adipocyte membranes from FDS were obvious in sections stained for connective tissue fibers, whereas membranes from the FFS and FCS were not as obvious (figure 2).

Toluidine blue-stained sections of adipose tissue from FCS and FFS demonstrated a metachromatic substance around fat cell clusters (figure 3). The intensity of the metachromasia was positively associated with the concentration of small, unorganized connective tissue fibers (figure 3). Treating sections with lysozyme before staining abolished the metachromatic reaction, indicating the presence of sulfated glycosaminoglycans (Humason, 1972, p 329). There was no metachromatic reaction in sections from FDS (figure 3).

In sections stained with PAS there was a positive reaction within the adipocytes from the FDS only (figure 4). When treated with diastase before staining, the adipocytes from the FDS were negative, indicating the presence of glycogen (Humason, 1972, p 329). The positive PAS reaction around fat cell clusters from the FCS and FFS (figure 4) was resistant to diastase treatment.

Discussion

Maternal diabetes increased the size and altered the histochemical characteristics of fetal adipocytes. It is widely accepted that insulin can affect adipocyte cell size markedly (Kazdova and Vrana, 1970; Salans et al., 1972). Elevated insulin levels were found only in the FDS (Kasser et al., 1978) and they may have been responsible for the multi- to unilocular adipocyte conversion and subsequent adipocyte hypertrophy.

The multi- to unilocular conversion normally begins when the pig is 3 d old (Hausman, 1978). This is also a time when insulin levels are increased greatly from nonmeasurable levels in the near-term fetus (Herbein et al., 1977) and newborn animals (Swiatek et al., 1968). Therefore, the lack of insulin during the fetal life of the pig may prevent significant adipocyte hypertrophy. The presence of glycogen inside the adipocytes from FDS indicates that the chemically determined glycogen (Kasser et al., 1981) in the FDS may be involved in adipocyte metabolism. These intracellular glycogen stores probably facilitated the greatly increased lipogenic capacity observed in the FDS (Kasser et al., 1981). The higher glucose and insulin levels may have caused the intracellular glycogen deposition, which in turn facilitated lipogenesis and esterification.

A network of collagen fibers surrounds the basal lamina of mature adipocytes from many species (Slavin, 1972). The positive reaction for connective tissue fibers around adipocytes from the FDS may indicate more advanced structural maturity. Large amounts of metachromatic (toluidine blue) glycosaminoglycan-containing material accumulate in degeneration of mesenchymal cells in a variety of tissues and in adipose tissue atrophy (Cheville, 1976). The absence of a metachromatic reaction in adipose tissue from the FDS may indicate a trophic affect of the maternal diabetic condition on fetal adipose tissue accretion.

Relative to the FCS, there was an increased number of fat cell clusters and an increased tissue thickness in the FFS and FDS. The elevated levels of maternal lipids (Kasser et al., 1978) in fasted and diabetic pigs were associated with this "hyperplastic adipose growth" in the FDS and FFS. The feeding of a high fat diet to adult rats increased adipocyte proliferation as measured by incorporation of tritiated thymidine into DNA (Klyde and Hirsch, 1979). The exposure to high concentrations of "dietary lipids" may have accelerated fetal adipose tissue growth.

Several researchers have histologically analyzed subcutaneous adipose tissue of newborn pigs from lean and obese dams (Moody et al., 1978; G. J. Hausman and R. G. Kauffman, unpublished data). When the obesity was represented by only one breed (G. J. Hausman and R. G. Kauffman, unpublished data), there were no differences between lean and obese offspring. When the lean and obese animals represented different breeds (Moody et al., 1978), the adipose tissue of the obese (Large White breed) dams' offspring was more "lipid filled" than that of the lean dams' offspring (Pietrain breed). In neither of these studies were the animals compared at similar weights as they were in the present study. Differences in body weight alone may account for differences in adipose tissue development. A comparison at similar body weights must be conducted to provide meaningful results.

The results of this study demonstrate that abnormalities in maternal metabolic or endocrine profiles are reflected by alterations in fetal adipose tissue development. These alterations indicate possible reasons for the normal
pattern of fetal adipose tissue development. The extremely slow rate of adipocyte hypertrophy in fetal adipose may be due to low levels of insulin. The number of fat cell clusters and the tissue thickness may reflect the level of fetal exposure to the maternal free fatty acids.

Literature Cited


