MINIREVIEW

Regulation of Adipose Cell Development *In Utero* (44333)

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Abstract. **The condition of obesity is impacted by increases in fat cell number, fat cell size, or a combination of the two. It is generally believed that fat cell number is dependent on the age of onset and the degree of obesity. This review provides an update on intrauterine growth of fetal adipose tissue, the earliest period of proliferation onset, and the factors that interact to enhance or suppress development. Fetal adipose tissue development is regulated by the complex interaction of maternal, endocrine, and paracrine influences that initiate specific changes in angiogenesis, adipogenesis, and metabolism. Developmental stages and metabolic processes influenced by specific hormones and paracrine factors have been identified through examination of the offspring of obese and diabetic pregnancies, hormonal manipulation during late pregnancy in animal models, and the use of cell culture. Collectively, the results of the studies cited herein delineate the basis for imprinting or conditioning of fetal preadipocytes at the paracrine/autocrine level and a role of thyroxine, glucocorticoids, and other hormones in fetal adipose tissue development and metabolism.**

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Obesity is one of the most pervasive public health problems. An epidemic of obesity among both adults and children is being reported in the United States (1). The prevalence has increased to approximately 35% in women, 31% in men and 25% in children. Many Americans will successfully lose weight only to regain it. Most weight control programs have a very high percentage (>90%) of weight recovery when examined 5 years later. The high rate of failure is likely related to endogenous mechanisms for body weight maintenance and adipose cell development.

Our understanding of differentiation of preadipocytes has been advanced by the use of cell lines and primary cell culture. Swiss 3T3 fibroblasts differentiate into cells with morphological and biochemical characteristics of adipocytes (2, 3). Biochemical differentiation is accelerated by additions of serum, hormones, and autocrine and paracrine factors (4–6). Adipose precursor cells derived from stromal-vascular cells of adipose tissue have also been investigated (7, 8). Cells from both sources are able, after injection into animals, to differentiate into mature fat cells (9). The use of cell culture has enabled investigators to characterize direct endocrine regulation of preadipocyte differentiation. To appreciate the physiological significance of these many cell culture studies, it is important to understand what factors act *in vivo* to initiate early adipogenesis (Fig. 1). Therefore, this review is focused on *in vivo* events (maternal, endocrine, and paracrine) that in concert initiate unique changes in the pattern of fetal adipogenesis and metabolism.

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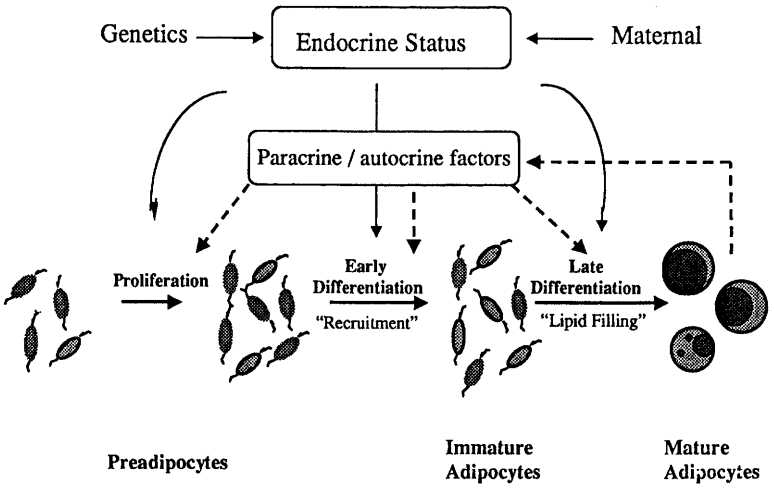

Figure 1. An overview of the developmental stages and regulatory influences associated with adipogenesis.

Maternal Factors Influencing Adipose Cell Development

To determine the effect of maternal obesity on fetal weight gain in normal pregnancies, birth weights in 326 singleton term gestations were studied in nondiabetic, nonsmoking women (10). Fetal weight gain was only 0.6 g per day greater for obese pregnant women than for women of normal weight for height. It was concluded that the effect of maternal obesity on fetal weight gain at term is small and not clinically significant. In animal studies, similar results were obtained; however, some metabolic differences were observed (11, 12). Fetuses from lean and obese pigs and reciprocal crossmatings were examined at 110 days of gestation for maternal and heterotic effects. There was no significant maternal effect on fetal weight or fat composition. Significant maternal effects were observed for both adipose tissue lipoprotein lipase activity and serum triglycerides since both parameters were elevated by maternal obesity.

Minor changes in diet composition or maternal energy status do not usually alter fetal metabolism and growth (13, 14). The effect of excessive intake of dietary fat during late gestation on maternal, placental, and fetal metabolism was studied in pigs. Palmitate oxidation and incorporation into fatty acids by the placenta, and fetal liver and adipose tissue were determined. Fetal development and metabolism were, for the most part, unaffected by maternal diet; thus, the fetus appears to be protected from dietary excess through adaptations of both maternal and placental metabolism (13). Mobilizing maternal energy stores by fasting pregnant pigs from Day 100 to Day 110 of gestation caused maternal nonesterified fatty acid levels to increase 7.5-fold, β-hydroxybutyric acid levels to increase 4.8-fold, and triglyceride levels to decrease 1.8-fold (14). Fasted fetuses had a 1.3-fold increase in nonesterified fatty acids, a 1.9-fold decrease in triglycerides, a 1.5-fold decrease in glucose, and no change in β-hydroxybutyrate levels as compared with control fetuses. Esterification of [14C]-palmitate by maternal placenta and fetal adipose tissue was reduced by fasting, but other parameters of fatty acid metabolism were unaffected. Fasting decreased lipoprotein lipase activity per milligram of protein by 33% in maternal placenta and by 44% in fetal adipose tissue. There was no detectable effect on percentage of body fat of the fetus. However, in a similar study that included a longer fasting period (15), an increased number of lipid-containing adipocytes in fetal adipose tissue was found. These studies suggest that fasting mobilizes maternal fuel stores that are not effectively utilized by the fetus for adipose tissue lipid deposition but may stimulate adipose cell hyperplasia. Fatty acids have been shown to stimulate adipogenesis in cell culture studies (16). The role of fatty acids in adipose cell growth is discussed below.

Severe metabolic and nutritional stresses influence fetal growth and development. Both maternal semistarvation and severe diabetes in rats are accompanied by perinatal growth retardation (17). Both stresses are associated with fetal hypoinsulinemia. As discussed below, fetal insulin levels are important in the development of adipose cells. The gestational period of nutritional stress may alter the postnatal outcome. For example, during the winter of 1944–1945, the Netherlands experienced an acute famine known as the Dutch Hungerwinter. Male offspring of mothers who were subjected to this famine during the first two trimesters of pregnancy had a higher than expected incidence of obesity (18). Furthermore, *in utero* stunting or runting in pigs results in fatter animals that have alterations in adipocyte cellularity (19).

Gestational Diabetes

Infants of diabetic mothers tend to have more adipose tissue than normal infants at birth and are predisposed to develop obesity during childhood generally between the ages of 5–9 years (20). This is thought to be caused by maternal hyperglycemia and subsequent fetal hyperinsulinemia. Babies born to mothers who had a mean fasting blood-glucose above 90 mg/dl had more subcutaneous fat tissue than newborn infants of mothers with a mean fasting

blood-glucose under 90 mg/dl. Maternal fasting blood-glucose and mean blood-glucose in the third trimester correlated significantly with neonatal weight, skin-fold thickness, and body mass index (21).

The type of diabetes may influence the magnitude of the fetal response. Both infants of mothers with pregestational insulin-dependent diabetes mellitus (IDM) and infants of mothers with gestational diabetes mellitus (IGDM) were found to have increased mean relative weights (20). IDM infants had higher insulin levels and a higher frequency of obesity than IGDM at birth. Throughout childhood, frequencies of overweight were elevated in both IDM as well as IGDM. Infants who were large for their gestational age displayed a significantly higher percentage of overweight in childhood than those whose weight was appropriate. Infants of mothers with diabetes during pregnancy are predisposed to develop overweight and obesity during childhood (20).

Women who were not treated for glucose intolerance had higher rates of newborns with a birth weight of more than 4000 g, large-for-gestational-age newborns and newborns with hypoglycemia than women without carbohydrate intolerance or than women with carbohydrate intolerance who received treatment (22).

Experimental diabetes in the pregnant pig has been used to study in more detail the mechanisms of increased fetal growth and adipose tissue development (23). Diabetes during pregnancy was produced in pigs with alloxan at 70 days of gestation. At 112 days of gestation, the fetuses were delivered alive by hysterectomy, and utilized for analyses. Progeny of diabetic mothers had a significant increase in the percentage of body fat. Liver lipogenic enzyme profiles did not indicate a significant change in activity to account for the increased body fat. In follow-up studies, maternal diabetes increased the number and size of lipid-containing adipocytes in fetal adipose tissue (15) and induced a 40-fold increase in fetal adipose tissue lipogenesis (24). Enzymes normally associated with lipogenesis were also affected in a similar fashion. These observations support the concept that *de novo* fatty acid synthesis in fetal adipose tissue is stimulated by diabetic pregnancies and is a primary mechanism by which increased lipid accumulates in the fetus.

Hyperglycemia and hyperinsulinemia may play a key role in enhanced fetal adipose tissue lipogenesis (Fig. 2). Glucose-infused sheep fetuses had higher serum insulin and were significantly heavier and fatter than saline-infused fetuses, indicating that growth and lipid deposition in the sheep fetus are responsive to increased glucose supply, an effect that may be mediated through the actions of insulin (25).

In addition to insulin, other factors may be involved in the increased adipose cell development of offspring of diabetic pregnancies. Cells obtained from adipose tissue of fetuses of diabetic pigs had a greater proliferative response and higher rates of differentiation as determined by sn-glycerol-3-phosphate dehydrogenase activity (26). In addition, an increase in adipogenic activity in the sera from fetuses of diabetic dams was observed when tested on normal fetal adipose cells in culture (26). Fetal serum IGF-1 concentrations were increased by maternal diabetes but IGF-1 mRNA levels were depressed in fetal adipose tissue (27). Maternal diabetes causes changes in the preadipocyte fraction of developing adipose tissue. These changes result in formation of more adipocytes and thus a greater capacity for lipid accumulation in the growing fetus of the diabetic. In addition, increased fetal serum IGF-1 may play a role in increased adipose tissue growth (Fig. 2).

Leptin in Fetal Development

Leptin is a 167-aa protein that is secreted from adipose tissue and is important in the regulation of energy balance, hematopoiesis, and reproduction (28, 29). The study of serum leptin levels during pregnancy has revealed some interesting patterns and relationships (30–33). Maternal leptin concentrations were elevated throughout gestation, especially during the second trimester, but postpartum, circulating leptin levels fell sharply to below pregnant values. There was no correlation between maternal serum leptin levels and birth weight (32). In contrast, fetal leptin levels (cord blood) correlated positively with birth weight (31–34) and body weight/height (31). Leptin concentration in cord blood also correlated closely with cord blood insulin concentration and with placental weight (32, 34). Within the first 6 hr of life,

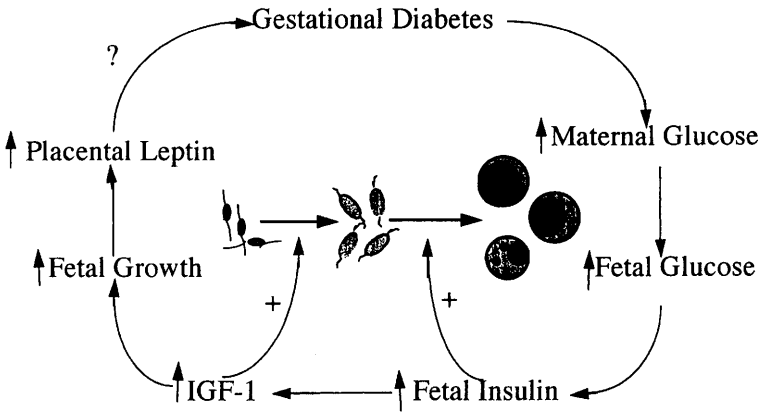

Figure 2. Proposed cascade of events that promote excessive fetal adipose tissue growth in gestational diabetes.

the concentration of serum leptin in large-for-gestational-age infants was three times the level found in average-for-gestational-age infants (35). These observations suggest that leptin is synthesized *in utero,* and that the circulating leptin concentration relates to the intrauterine growth pattern.

Further evidence for a role of leptin in fetal development can be found. The distribution of mRNAs encoding leptin and the leptin receptor was examined in the 14.5-day postcoitus mouse fetus and in the placenta (36). High levels of gene expression of leptin, the leptin receptor, and the long splice variant of the leptin receptor with an intracellular signaling domain were observed in the placenta, fetal cartilage/bone, and hair follicles. Western blotting and immunocytochemistry, using specific antibodies, demonstrated the presence of leptin and leptin receptor protein in these tissues. These results suggest that leptin may play a role in the growth and development of the fetus, both through placental and fetal expression of the leptin and leptin receptor genes.

Leptin significantly stimulates the proliferation and differentiation of yolk sac cells and fetal liver cells and directly stimulates hematopoietic precursors. Leptin alone can increase the number of macrophage and granulocyte colonies (29). The receptor for leptin is an isoform of B219, a novel hemopoietin receptor with a nearly identical ligand binding domain. The leptin receptor (B219/obr) is expressed in the yolk sac, early fetal liver, enriched hematopoietic stem cells, and a variety of lymphohematopoietic cell lines. These observations strongly support the hypothesis that leptin has a role in hematopoietic and immune system development (29).

The role of placental leptin in fetal development may be linked to its effects on thyroxine secretion (Fig. 3). Leptin stimulated thyroxine levels in postnatal animals (37). Our studies have demonstrated the importance of thyroxine in the development of fetal adipose cells, mediated in part by an increased production of IGF-1 (38). Taken together, it is possible that leptin may enhance fetal adipogenesis by increasing the thyroxine levels which, in turn, cause an increase in adipogenesis. Fetal serum leptin may be of placental and/or adipocyte origin.

Thyroxine may also act directly on fully differentiated adipocytes to increase leptin production. Triiodothyronine significantly increased the expression of leptin mRNA (39). Other hormones, including insulin, also increase leptin expression in adipose cells (40, 41).

Characteristics of Early Adipose Cell Development and Enzymology

De novo lipid synthesis in human fetal subcutaneous adipose tissue, measured as the ability to incorporate acetate into neutral lipid, was shown to increase with gestational age. An increased activity of acetyl coenzyme A carboxylase paralleled this development of adipose cell lipid accretion (42). In addition, fetal plasma triglyceride concentration decreased exponentially with gestation. This is likely the result of either increased triglyceride utilization by the fetus for energy or deposition into adipose tissue or a greater dependence on *de novo* lipogenesis (43).

Enzyme cytochemical changes, as they relate to the morphological differentiation of the subcutaneous depot, show that there are distinct temporal lags between the appearance of specific enzymes in adipocytes (44). For example, NADH-tetrazolium reductase activity appeared earliest, whereas esterase activity appeared before lipoprotein lipase (LPL) activity. Lipid and enzyme cytochemistry demonstrated physical continuity between primordial cells and differentiated fat cell clusters. Blood vessel architecture and vascular alkaline phosphatase activity clearly demarcated perirenal and subcutaneous depots in the fetus. At each of three stages of gestation studied in the pig, 70, 90, and 110 days, the largest fat cell clusters were consistently located near points where large blood vessels entered the loose connective tissue (45). Enzyme cytochemistry disclosed that reactions for glucose-6-phosphate dehydrogenase (G6PDH), lipoprotein lipase (LPL), and NADH-TR enzymes were reduced in distal (relative to entry points of large arterioles) adipocytes compared with proximal adipocytes.

Mitotic activity in fetal porcine adipose tissue showed that in the youngest fetuses there was a period of intense stromal cell mitotic activity before any adipocyte lipid accumulation (46). During subsequent fetal development, clusters of tightly arranged stromal cells were formed. Lipid accumulation occurred only in these cell clusters. Primary cultures of stromal-vascular cells of adipose tissue from fetuses at 70 and 110 days of gestation were evaluated as potential model systems for studies of fetal adipocyte differentiation and proliferation (8). In the cultures, fat cells developed as very discrete clusters with histochemical and anatomical features similar to those observed *in vivo.*

The study of early development of adipose tissue and

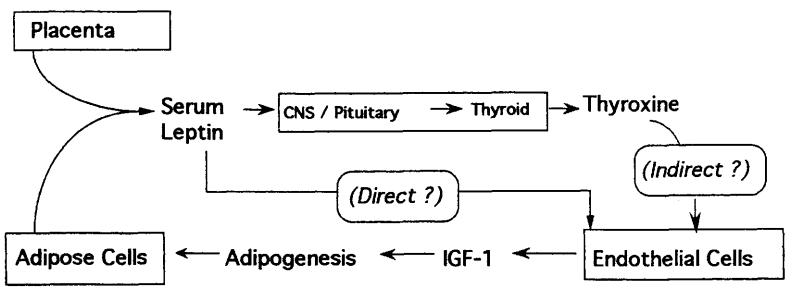

Figure 3. Proposed direct and indirect role of leptin on fetal adipose cell development.

supporting structures was enhanced by the development of specific antibodies that allowed early identification of fat cells. Monoclonal antibodies prepared by fusion of mouse myeloma cells and lymph node cells of mice immunized with porcine adipocyte plasma membranes were used to demonstrate a temporal relationship between adipogenesis and angiogenesis (47). Immunofluorescent staining of cryostat sections with each antibody revealed antigen-positive cells in fetal subcutaneous mesenchyme prior to lipid deposition. From 70 to 110 days of development, the monoclonal antibody detected all adipocytes examined, as well as capillaries associated with fat cell clusters in subcutaneous tissues. These results established the presence of cells expressing surface determinants found on mature adipocytes and associated capillaries prior to adipogenesis and supported a lineage relationship between adipocytes and capillary endothelial cells.

It is commonly assumed that once formed, fat cells do not go away and there is no turnover of fat cells (48). However, there is recent evidence that fat cells undergo apoptosis (i.e., programmed cell death (49, 50)). In addition, there is evidence that adipose tissue from humans (51) and pigs (52) contain adipocyte precursor cells that represent a dormant reserve, but may be recruited to become new fat cells. So the process of adipose cell development is dynamic, involving cellular death as well as cellular expansion.

Transcription Factors Involved in Adipose Cell Differentiation. Adipose cell differentiation is accompanied by transcriptional activation of lipid related genes that eventually lead to fatty acid synthesis and storage as triglycerides in large lipid droplets. From studies of adipose cell lines, it is proposed that adipocyte differentiation is achieved through the action of primarily two transcription factor families: the peroxisome proliferator activated receptors (PPARs) and the CCAAT/enhancer binding proteins (C/EBPs) (53, 54). PPAR γ-2 is selectively and abundantly expressed in fat tissues (55) and induced early in adipocyte differentiation (56). Overexpression of PPAR γ leads to conversion of fibroblasts to adipocytes (57). C/EBP α is expressed during terminal differentiation of adipocytes and, when overexpressed, promotes an adipogenic program in fibroblastic cell lines (58). Ectopic expression of C/EBP β induces PPAR γ and stimulates lipogenesis of fibroblasts (59). C/EBP α knockout mice fail to develop adipose tissue (60) indicating the physiological impact of this transcription factor.

Very little data are available on the level of various transcription factors during the *in vivo* development of adipose cells. Furthermore, there is evidence that observations of the pattern of expression of transcription factors in cell lines may not parallel the pattern found in fetal adipose tissue. For example, C/EBP β and δ expression precedes expression of C/EBP α in cell lines and suggests that C/EBP β and δ may, in fact, activate C/EBP α expression (61). However, C/EBP α was expressed by porcine adipocytes throughout fetal development; but there was no evidence that C/EBP δ and β expression preceded C/EBP α expression (62). Clearly, the characteristics of C/EBP isoform expression during fetal development are not always predicted from studies of preadipocyte cell lines. More information on the *in vivo* pattern of expression of various transcription factors is needed before the physiological significance of observations in cell lines can be determined.

Genetic Obesity

Studies involving large numbers of identical twins have demonstrated that a significant portion of human obesity is genetically based (63, 64). Furthermore, the use of genetic animal models of obesity has proven very useful in identifying key regulatory events involved in the development of obesity. The obese pig has a size advantage over other animal models of obesity and is particularly useful for the study of fetal adipose tissue development. Pigs that have been genetically selected for the amount of subcutaneous fat, have over two times as much body fat and have an adipose cell volume nearly 3-fold larger than that of the lean pigs (65–67). When an extreme difference like this exists between experimental and control subjects, it permits easier identification of the regulatory events that are important for *in vivo* development of adipose cells. Pig fetuses from genetically obese dams are not yet "obese" and still have only about 1%–2% body fat. However, they have several characteristics that distinguish them from fetuses of lean dams. For example, differences have been found in early fetal adipose cell lipid metabolism in genetically lean and preobese fetal pigs (68, 69). In subcutaneous adipose tissue, *de novo* lipogenesis and lipoprotein lipase activities increased with fetal age and were higher in preobese than in control fetuses by 110 days of gestation. At 75 days of gestation, rates of glucose oxidation and incorporation into fatty acids were similar in adipose tissue from both strains. However, by 110 days of gestation, both basal and insulin-stimulated rates of glucose metabolism were greater in preobese compared to control fetuses. Palmitate esterification increased with fetal age but was similar in pre-obese and control fetuses. Basal lipolysis was not affected by obesity or fetal age. However isoproterenol, which had no effect on lipolysis in the 75-day-old fetuses, stimulated glycerol release to a comparable degree in 110-day-old fetuses of both strains. These observations demonstrated that metabolic differences between genetically obese and control pigs are already apparent in the pre-obese state prior to birth when fetuses have only 1%–2% body fat.

Comparison of stromal-vascular cultures derived from lean and pre-obese fetuses indicates inherent differences at this early stage of development. Primary cultures of stromal-vascular (S-V) cells from lean and preobese fetuses were studied at several fetal ages (50, 75, and 110 days) (52). The rate of preadipocyte development in lean and preobese fetuses diverged between 75 and 110 days, resulting in many more preadipocytes in preobese fetuses at 110

days. Therefore, S-V cells from preobese fetuses (late term) may be inherently more sensitive to adipogenic agents than S-V cells from lean fetuses.

Hormonal Factors that Influence Fetal Adipose Tissue Metabolism

Studies of fetal pig hypophysectomy or decapitation revealed the importance of the brain and pituitary in fetal development of adipose tissue. When compared to control fetuses, hypophysectomized fetuses have three times the fatty acid synthesis rate in adipose tissue and deposit more body lipid (70–72). A study of histochemical and cellular aspects of adipose tissue development indicated that fetal hypophysectomy exerts a positive influence on enzymes involved in lipid synthesis (72, 73). In addition, hypophysectomy also exerts a negative influence on fat cell hyperplasia. Fewer fat cell clusters in hypophysectomized fetuses were associated with fewer blood vessels in the subcutaneous layers. In addition to adipose tissue lipogenesis, hormone sensitive lipase was altered in adipose tissue from hypophysectomized pig fetuses. Adipose tissue lipolysis was not stimulated by norepinephrine in the absence of the pituitary, but was stimulated by dibutyryl-cyclicAMP (74). These findings demonstrated the necessity of a functioning pituitary for the normal regulation of fatty acid synthesis and receptor-mediated lipolytic response in developing fetal adipose tissue.

To restore the metabolic abnormalities found in adipose tissue of hypophysectomized fetal pigs, hormones were replaced in a systematic manner. Hypophysectomy of fetal pigs causes a loss of serum thyroxine and growth hormone, a decrease in glucocorticoids, and an increase in serum insulin and glucagon (73, 75). Thyroid hormone has a profound influence on fetal adipose cell development and metabolism. Preobese pig fetuses have elevated thyroid hormone levels relative to lean fetuses. However, thyroxine implants did not influence adipose tissue development in intact fetuses, but markedly enhanced development in hypophysectomized fetuses (76–78). For instance, thyroxine supplementation restored the lipolytic response to isoproterenol and enhanced the response to dibutyryl cyclic AMP in hypophysectomized pig fetuses (76, 77) but had no effect on basal or stimulated lipolysis in intact fetuses (76). In addition, fat cell size and lipogenic enzyme activities in hypophysectomized fetuses were increased with thyroxine treatment, with a marked increase in apparent fat cell number (78). Thyroxine also induced a dramatic increase in lipogenesis (^{14}C-glucose incorporation into ^{14}C-triglyceride fatty acids) in hypophysectomized fetuses. This dramatic increase in lipogenesis was normalized with the combination of thyroxine and growth hormone treatment in the hypophysectomized fetuses (78a). As thyroxine had an effect on fat cell parameters and lipid metabolism only in the hypophysectomized fetal pigs in these studies, this would suggest that thyroxine may have a primary influence on fetal adipose tissue metabolism only in the absence of inhibition from counter-regulatory hormones of pituitary origin. These results further indicated that growth hormone may be, at least in part, responsible for modulating the response of adipose cells to thyroxine.

Pituitary growth hormone may play a role in early development of adipose tissue metabolism. High growth hormone levels in normal fetal pigs may be responsible for inhibiting lipogenesis, whereas fetal decapitation would remove this inhibition and be associated with greater lipid deposition (70). However, pancreatic insulin release was greater in decapitated fetuses than in intact fetuses, an indication that elevated lipid deposition may also be due to greater fetal insulin secretion.

Unique Properties of Fetal Serum. Fetal pig serum contains adipogenic factors that promote the proliferation and differentiation of adipocytes in culture. Fetal pig serum stimulated more adipocyte proliferation in culture than adult pig sera, whereas serum from adult pigs promoted greater differentiation and lipid filling of adipocytes (73). Fetal pig sera stimulated histochemical expression of enzymes, but did not induce lipid filling.

The adipose conversion of 3T3-L1 cells depends on a serum factor present in high amounts in fetal calf serum, which is heat stable, and can be extracted from serum by ethanol precipitation (79). Further study showed that fetal serum contains two large molecular weight proteins bearing adipogenic activity that could play an important role in the control of the adipose differentiation process (6).

Serum from hypophysectomized fetuses has a lower level of adipogenic factors. In primary cultures of fat cells treated with serum from hypophysectomized pig fetuses, there was a lower number of fat cells and a lower level of preadipocytes when compared to control fetal serum (80). Serum from decapitated fetal pigs specifically reduced the number of proliferating preadipocytes in culture (81). In addition, serum from decapitated fetal pigs induced less differentiation as measured by sn-glycerol-3-phosphate dehydrogenase activity than serum from intact pigs. Histochemical staining for enzymes of differentiating preadipocytes was also reduced in cultures incubated with serum from decapitated fetal pigs in comparison to serum from intact pigs. The *in vivo* effect of decapitation on fetal adipose tissue development is a consequence of alterations in systemic factors in response to removal of central regulation by the hypothalamic-pituitary axis.

An altered *in vivo* fetal endocrine environment affected subsequent development and differentiation of preadipocytes *in vitro* (82). Stromal-vascular cultures from hypophysectomized fetuses contained fewer fat cell clusters and concomitantly lower glycerol-3-phosphate dehydrogenase enzyme activity than did cultures from litter mate controls. Fluorescence-activated flow cytometry using a monoclonal antiadipocyte/preadipocyte antibody designated AD-1 revealed that hypophysectomy also reduced the proportion of cells expressing the AD-1 cell surface antigen. These results demonstrated that effects of fetal hypophysectomy on cells

from adipose tissue are maintained and measurable in culture. In addition, one consequence of fetal hypophysectomy may be a diminution in the relative proportion of adipocyte precursors.

What Are the Likely Hormonal Candidates for a Role in Adipogenesis? *Glucocorticoids.* Hydrocortisone is a major fetal adipogenic hormone. The *in vivo* effects of hydrocortisone have been investigated in fetal pigs. In one study the glucocorticoid analog dexamethasone markedly increased the percentage of lipid-free cells expressing a "marker" AD-1 reactive cell surface antigen *in vivo* (83). In another study, hypophysectomized fetuses were treated with hydrocortisone, and S-V cultures were prepared from intact, treated and untreated hypophysectomized fetuses 15 days later[1]. The proportion of preadipocytes was doubled in cultures derived from hydrocortisone-treated hypophysectomized fetuses compared to cultures from intact and untreated hypophysectomized fetuses. The responsiveness to dexamethasone was also examined in S-V cultures derived from 50-, 75-, and 105-day-old pig fetuses (52). Dexamethasone induced 10- to 20-fold more cytodifferentiated cells in cultures from 75- and 105-day-old fetuses than in cultures from 50-day-old fetuses (52), thus showing that responsiveness of porcine preadipocytes to glucocorticoids increases progressively with fetal age. These data are consistent with the observation that the number of glucocorticoid receptors was very low in 50-day-old fetal porcine preadipocytes and increased progressively to a peak at Day 105, a 6-fold increase (84). Insulin markedly increased the number and binding affinity of glucocorticoid receptors in preadipocytes, indicating its potential involvement in regulation of glucocorticoid receptors. The effect of glucocorticoids on differentiation in preadipocytes is, at least in part, mediated by the number of glucocorticoid receptors.

It is well established *in vitro* that glucocorticoids, or the glucocorticoid analog dexamethasone, enhances differentiation in the presence of insulin or serum in nearly every preadipocyte line studied and in all stromal-vascular (S-V) cells studied (5, 81, 85–88). Therefore, glucocorticoids may be the major modulator of insulin (or serum) action on adipogenesis *in vitro,* and they may do so in a direct manner. Modulation of prostaglandin (PG) secretion is a likely means of glucocorticoid action on adipogenesis (5, 89, 90).

Thyroxine. Fat cell number and lipid deposition were markedly increased by thyroxine treatment in hypophysectomized fetuses (78). The response to thyroxine in adipose tissue may also be mediated by autocrine/paracrine factors or through modulation of major hormone receptors. Serum insulin-like growth factor 1 (IGF-1) was significantly elevated by thyroxine, indicating that thyroxine indirectly influences the development of adipose tissue by increasing production of a locally produced adipogenic growth factor (see below). In addition, thyroxine has an *in vivo* effect on the secretion of IGF binding proteins (IGFBPs) in stromal-vascular cells (91). Thyroxine treatment completely normalized IGF-1 secretion in hypophysectomized pig fetuses. Four IGFBPs (BP-1, BP-2, BP-3, and BP-4) detected in S-V cultures derived from 95-day-old fetuses were decreased in concentration by hypophysectomy and were increased by thyroxine treatment of hypophysectomized fetuses. Thyroxine has a major influence on fetal preadipocyte development and production of IGF-1 and IGFBP.

Growth hormone. The influence of human growth hormone (hGH) on the differentiation of preadipocytes was examined in primary cultures of stromal-vascular (S-V) cells from porcine adipose tissue (87). Growth hormone reduced the number and size of fat cell clusters and decreased differentiation of preadipocytes. These studies indicated that hGH significantly impedes porcine preadipocyte development *in vitro.* Therefore, the decreased rate of adipose tissue growth observed in pigs chronically treated with GH could be due in part to impaired preadipocyte growth.

Fetal serum IGF-1 levels are reduced by hypophysectomy but, in contrast to thyroxine treatment (38), are not influenced by growth hormone treatment (78a). In fact, fetal growth hormone treatment abolishes hypophysectomy induced adipose tissue lipogenesis and markedly antagonizes thyroxine stimulated lipogenesis (78a). Apparently, the role of growth hormone in the fetus is limited to modulating adipose tissue lipogenesis with little influence on adipocyte hyperplasia or growth in general.

Autocrine/Paracrine Factors May Control Adipocyte Development

An involvement of paracrine/autocrine factors in preadipocyte development has been established (72, 92–95). Criteria that must be met to establish paracrine regulation of adipogenesis are as follows: (1) the factor influences either adipocyte proliferation or differentiation; (2) the factor is located and produced in adipose tissue cells or neighboring cells; (3) the level and/or secretion rate of the factor is dependent on physiological states of energy balance; (4) receptors or binding proteins for the factor are found on or within preadipocytes or adipoblasts; and (5) the elimination of the factor causes a disruption of adipose tissue development.

Primary evidence for paracrine influence on adipogenesis comes from studying the effect of medium "conditioned" by *in vitro* exposure to mature fat cells on the bioactivity of preadipocytes cultures (i.e., differentiation or proliferation of preadipocytes (7, 92–95). Mature fat cells from obese subjects release compounds exhibiting increased adipogenic activity when compared to cells from lean subjects (93, 94). Conditioned medium prepared from adipose tissue from obese Zucker rats stimulated the proliferation of

[1] G. J. Hausman and Z. K. Yu, unpublished data.

preadipocytes in a cell culture bioassay system (95). Changes in adipose cell morphology during the development of obesity in humans (94) and in genetically obese rats (95) are consistent with the concept that there is an association between the enlargement of fat cells and the appearance of a locally produced factor(s) capable of stimulating adipose cell proliferation or differentiation in a paracrine fashion.

Several studies provide evidence that fatty acids and several prostaglandins (PGs) may act in a paracrine/autocrine fashion (72, 96, 97). Fatty acids have been proposed to play a role as signal transducing molecules that are involved in adipose cell differentiation (98). There is evidence for PGI_2 as a positive growth factor in Ob1771 preadipocyte cell line cultures, however, PGI_2 had no influence on rat (96) and pig stromal-vascular cultures (99). In fact, studies with rat S-V cells provided substantial evidence that $PGF_{2\alpha}$ acts locally as a negative growth factor (72). The inconsistencies in these studies may be due to the presence or absence of vascular cells. Vascular endothelial cells and isolated adipocytes have been shown to cooperate in PG production (100). It has been reported that the ability of angiotensin II to stimulate differentiation of preadipocytes was dependent on the presence of mature adipocytes (97). These coculture experiments showed that angiotensin II stimulated prostacyclin from mature adipocytes and that prostacyclin stimulated differentiation of preadipocytes.

Insulin-like growth factor-1 (IGF-1) may act as a locally produced positive factor in the development of adipose tissue. Expression of IGF-1 and IGF binding proteins (101) during adipogenesis has been shown, as well as IGF-1 binding in undifferentiated cells but not in mature fat cells (102). In addition, IGF-1 enhanced the development of fat cell clusters in S-V cultures by increasing the number of cells per cluster (102).

Several locally produced factors including tumor necrosis factor-α (TNFα) and transforming growth factor β (TGF-β) may act in a negative manner on adipogenesis. TNFα production from adipose tissue is elevated in human obesity (103, 104) and has a major negative influence on *in vitro* adipogenesis (105, 106). The mechanism by which TNFα influences adipogenesis may be through its action to decrease peroxisome proliferator activated receptors (PPARs) (107). PPARγ has been shown to be important in the regulation of adipose cell differentiation (53–57).

Various types of studies indicate that TGF-β may be a locally produced mitogen that markedly antagonizes differentiation *in vitro* (72). TGF-β blocks differentiation by inhibiting the production/secretion of positive factors like IGF-1 (108). Possibly, the interaction of positive and negative acting factors would dictate the net autocrine/paracrine regulation. Clear delineation of autocrine/paracrine control of adipogenesis awaits further research of potential preadipocyte/adipocyte and vascular interactions.

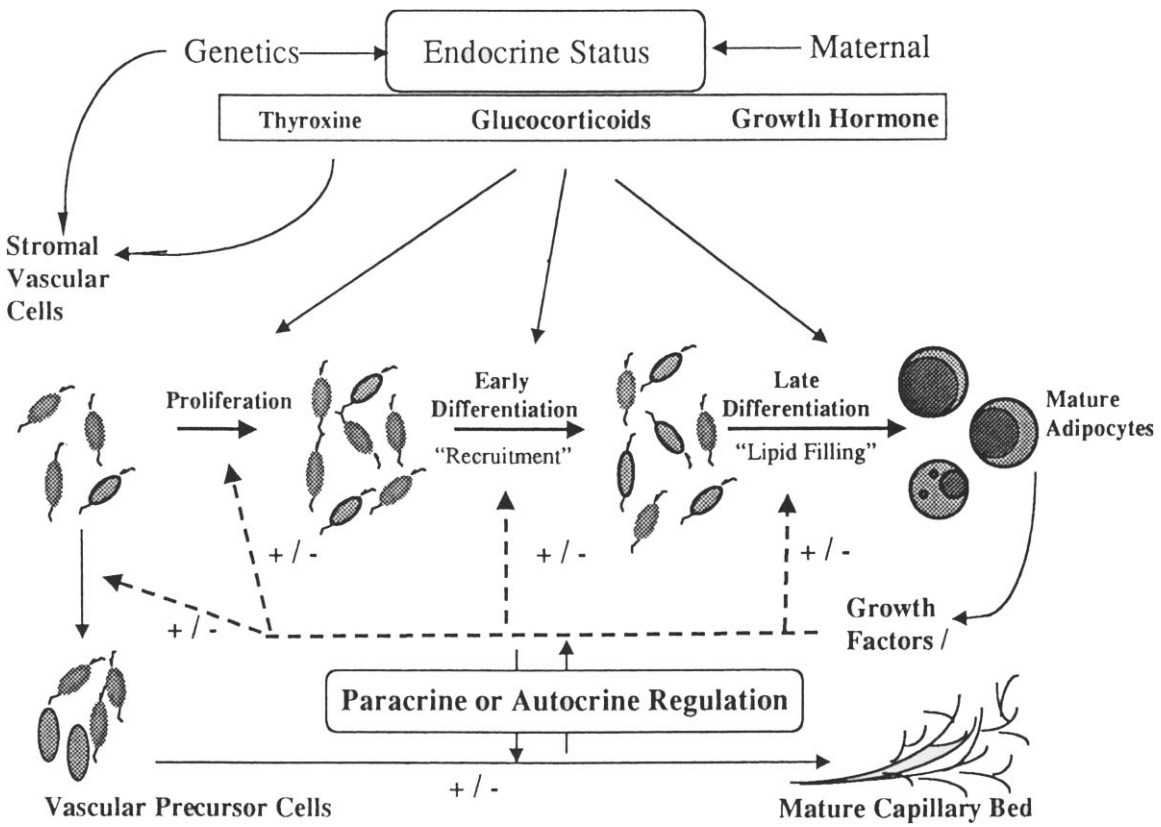

Figure 4. *In utero* regulation of adipose tissue development: Summary of factors involved.

Summary

This review summarizes recent studies reported specifically on fetal adipose tissue growth and development. Fetal development occurs *in vivo* under the influence of maternal, genetic, and endocrine factors (Fig. 4). The genetic influence is evident in adipose cell histology and metabolism of the genetically obese pig, before birth and before major changes in total body fat. The early development and endocrine requirements of adipose tissue development involve the pituitary, thyroid, and adrenal glands. For example, marked changes in cellular growth and metabolism occur in the absence of thyroxine and are completely reversed when thyroxine status is restored. Growth hormone appears to be a major inhibitor of adipogenesis in the fetal pig. The maternal diabetic status has been shown to influence adipose cell growth as well. Experimentally produced diabetes in the sow results in an increase in fat storage through an increase in fetal glucose and insulin levels that trigger greater fetal adipogenesis and lipogenesis. Two evolving stories are the role of paracrine factors in altering the microenvironment of the developing preadipocyte and the intracellular transacting factors required for adipose cell differentiation. Although there is limited information concerning the *in vivo* ontogeny of transacting factors during fetal adipose cell development, some studies have been done on the *in vivo* role of preadipocyte paracrine regulation. It is evident that an interaction between stems cells and the surrounding microenvironment is important for both adipogenesis and angiogenesis. This microenvironment consists of stromal cells such as fibroblasts, macrophages, endothelial cells, and adipocytes and their secreted products. These cells regulate adipogenesis by either stimulating growth factor interaction with stem cells or inhibiting the interaction through negative regulatory factors. Additional knowledge of the ontogeny of these interactions during intrauterine growth is essential for the complete understanding of this, the earliest period of proliferation/development onset of fetal adipose tissue.

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