Fetal Growth and Long-Term Consequences in Animal Models of Growth Retardation

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Fetal growth and development are primarily determined by genetic information in the fetus, but the genetic regulation of fetal growth is influenced by various factors that can exert a stimulatory or inhibitory effect. On the one hand, fetal growth is determined by the capacity of the mother to supply nutrients and by the capacity of the placenta to transport these nutrients to the fetus. On the other hand, the fetus has its own factors that influence its growth and differentiation: the fetal growth factors.

Normal growth demands an equilibrium in the interaction between these different compartments and between the stimulatory and inhibitory factors affecting each of these steps. Disturbance of this equilibrium at any stage can result in intrauterine growth retardation (IUGR) and microsomia or in fetal overgrowth and macrosomia.

The mother’s metabolic condition is the first important determinant of fetal growth. Adequate maternal and fetal blood flow is important for placental function, and therefore also for an efficient nutrient supply to the fetus. Malnutrition, as well as diabetes, results in a decreased uteroplacental blood flow during gestation (1,2) and a decreased total milk volume during lactation (3), which hamper normal fetal and neonatal growth. These two conditions can thus provide insight into the adaptations of fetal development in an abnormal intrauterine milieu and thereby into the mechanisms that regulate such development in normal pregnancies as well.

PLACENTAL NUTRIENT TRANSPORT

Normal Pregnancy

Normal fetal growth and development depend on nutrients derived from maternal fuels.

Glucose is the major substrate for the fetus, because—at least in the rat fetus—it cannot be synthesized from placentally transferred substrates. Maternal glucose is therefore retained for transplacental transport and traverses the placenta freely by
facilitated diffusion, mediated by glucose transporters. In the rat, GLUT-1 and GLUT-3 have been demonstrated in the placenta within different placental layers (4), but only GLUT-3 is exclusively expressed within the labyrinth, which is the zone of physiologic exchange between the maternal and fetal circulations. It has been suggested, therefore, that GLUT-1 is responsible for supplying glucose as a placental fuel and that GLUT-3 is important for glucose transfer to the fetus. In the human, GLUT-1 is abundant in both syncytiotrophoblast and cytotrophoblast and in fetal endothelial cells in placental villi at term. There is controversy as to whether GLUT-3 is also expressed in human placentae (5). So far as possible regulatory factors are concerned, it has been found that insulin has no effect on glucose uptake and metabolism in a perfused in vitro system; only maternal glucose concentration has been shown to direct its uptake.

Amino acids are concentrated in the fetus against a transplacental gradient; the levels of amino acids are higher in the fetus than in the pregnant mother (ratio = 1.5 and 2.0). A similar relation has also been reported in the rhesus monkey and the rat. The transport of amino acids through the placenta is active, with a variety of transport systems for individual amino acids (6). Essential branched-chain amino acids are transported rapidly through the placenta, whereas the transfer of straight-chain amino acids is rather slow.

The transport of lipids across the placenta depends on the maternal plasma lipid concentration and the species; placentae of different species have very different permeabilities and transport capacities for fatty acids. As a result, the fat content of the fetuses varies markedly among species in direct relation to placental lipid transport (e.g., the human infant at term has about 18% body fat, whereas the rat fetus has only 1% to 2%). The net flux of lipids across the placenta can occur by at least three mechanisms: (a) direct transfer of fatty acids by specific transport proteins, (b) synthesis of complex lipids from fatty acids within the placenta and subsequent release into the umbilical circulation, and (c) hydrolysis of triglycerides, lipoproteins, and phospholipids and subsequent release into the fetal circulation (7,8).

Diabetic Pregnancy

In diabetic pregnancy, the fuel supply to the fetus is abundant, owing to the accentuated catabolism in the mother. Fetal glucose levels are increased relative to the mother’s glycemia. GLUT-3 messenger RNA and protein in diabetic rat placentae increase up to fivefold, whereas the expression of GLUT-1 remains unaltered. The same effect could be obtained in pregnant rats during hyperglycemia, but not during euglycemic hyperinsulinemia (4). The transport of amino acids depends on an adequate uteroplacental blood flow. The transplacental transport of \( \alpha \)-aminoisobutyric acid correlates with the placental blood flow and fetal weight in guinea pigs (9). In diabetic rat pregnancy, the influx of amino acids from the maternal side is largely increased in macrosomic fetuses, whereas amino acid transport to their underweight littermates is significantly reduced (10). Amino acid levels in growth-retarded fetuses
of rats with streptozotocin-induced diabetes are decreased (11,12). In human pregnancies, amino acid concentrations are also decreased in small-for-gestational-age (SGA) fetuses (13) and newborns (14). The passage of lipids to the fetus in a diabetic pregnancy is promoted by high levels of very-low-density lipoprotein (VLDL) triglycerides and nonesterified fatty acids in maternal plasma (15).

**FETAL ENDOCRINE PANCREAS**

In the fetal rat pancreas, ‘‘islet-like’’ formations, composed mainly of insulin-containing cells, are clearly present from day 18 of gestation; these cells already co-express GLUT-2, the glucose transporter of the β cell (16). By day 20, the endocrine cells accumulate in clusters, appearing organized into real ‘‘mantle-islets’’ with a core of insulin-producing β cells (17). At the ultrastructural level, the β cells appear as mature synthesizing and secreting cells. Granulation of β cells increases with fetal age, parallel to the increase in pancreatic insulin content (18). With the appearance of small islets of Langerhans (day 20 of gestation), glucose-stimulated insulin release can be triggered in vivo. The transition from a fetal to an adult type of insulin release in response to glucose occurs during the last days of gestation and parallels quantitative rather than qualitative changes within the β cells (19). In fetuses of streptozotocin-induced diabetic rats, an evolution similar to that in control fetuses is observed, but the development of the endocrine pancreas is enhanced by the raised blood glucose concentrations, which results in hypertrophy and hyperplasia of the islets from day 20 of gestation until birth (17). When maternal hyperglycemia is severe, fetal pancreatic islets are overstimulated by the excessive glucose concentration. The fetal β cells become degranulated, disorganized, and incapable of reacting to any stimulus (19). Indeed, glucose-stimulated insulin release was absent from pancreases of severely hyperglycemic (15.09 mM) fetuses (19). Incubation of fetal islets with other secretagogues also results in the absence of an insulin response in the fetuses of highly hyperglycemic rats. Only arginine induced a sustained monophasic insulin release, suggesting that the defect may concern stimulus-secretion coupling (20,21). Severe diabetes is associated with fetal growth retardation as a result of fetal malnutrition. Similar findings have also been reported in the fetuses of very poorly controlled diabetic women (22).

Poor nutrition of the mother during pregnancy obviously leads to IUGR. The decreased availability of nutrients for transplacental transport and a decreased placental blood flow result in a decreased nutrient supply to the fetus (1). An inadequate stimulation of the fetal pancreas leads to hypoplasia of the pancreatic endocrine tissue, owing to a smaller size of the islets of Langerhans; pancreatic insulin content is decreased and insulin response to stimuli is altered (23,24), resulting in fetal hypoinsulinemia. The effect was similar whether the maternal rats were restricted in their total food intake or only the protein component of the food was diminished, with or without complementing the diet up to isocaloric levels (23–25).
INSULIN ACTION

In the rat, insulin receptors are present from day 17 of gestation in fetal liver, lung, gastrointestinal tract, and heart. Internalization of the hormone in the hepatocytes occurs as early as insulin receptors are detected, and the rate at which this mechanism proceeds increases with the degree of liver maturation (26).

Fetal hypoinsulinemia and a reduced number of insulin receptors on target cells (27) in fetuses of severely diabetic rats may lead to a reduction in fetal glucose uptake; a reduced fetal glucose uptake has been shown in hypoinsulinemic streptozotocin-injected fetal lambs (28). The growth of the fetal protein mass is suppressed; fetal protein synthesis is consistently lower than control rates, whereas protein degradation increases sharply toward the end of gestation (29).

With maternal malnutrition, the combination of fetal hypoinsulinemia and low substrate availability decreases fetal whole-body glucose utilization rates in sheep (30) and rat (31), mainly through a decrease in glucose uptake by the fetal skeletal muscles and heart; glucose uptake by liver and brain is unaffected (31). A decrease in glucose transporter activity, protein, and messenger RNA has been reported in the lung of SGA fetuses in the rat (32). Also, lipid deposition and protein breakdown are decreased, retarding the growth of muscle and adipose tissue (33).

Malnutrition of the maternal rat prematurely induces gluconeogenesis through a decreased insulin-to-glucagon ratio in the fetus. An increased activity of phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme of gluconeogenesis, was shown in fetal liver after maternal malnutrition (34) and after administration of streptozotocin (35) or anti-insulin serum (34) to fetuses. Moreover, PEPCK activity is increased in neonatal and adult rat liver after perinatal protein deprivation. Glucose-6-phosphatase plays a crucial role in the regulation of hepatic glucose production through either glycogenolysis or gluconeogenesis. Glucose-6-phosphatase activity in fetal liver can be induced by administration of glucagon, cyclic AMP, or epinephrine to the fetus, whereas administration of glucose to the maternal rat prevents an increase in glucose-6-phosphatase activity (36). Insulin injection in the newborn rat also prevents an increase in glucose-6-phosphatase activity (36). Messenger RNA expression of glucokinase, an enzyme of glycolysis, is stimulated by insulin and inhibited by glucagon/cyclic AMP (37). A premature induction of glucokinase messenger RNA expression can also be induced by glucose (38). Glucokinase enzyme activity is decreased in neonatal and adult rats after perinatal protein deprivation. The messenger RNA expression of GLUT-2 seems also to be regulated by glucose (39).

With respect to the peripheral tissues, the perinatal period is also very important for the regulation of the glucose transporters (GLUT-1 and GLUT-4). During fetal life, the glucose transporter GLUT-1 dominates in peripheral tissues. GLUT-1 seems to be negatively regulated by glucose.

After birth, the glucose diet of the fetus changes into the high-fat diet of the suckling rat. The amount of endocrine tissue does not further increase, whereas the pancreatic insulin content exceeds adult values. Plasma insulin concentration
ANIMAL MODELS OF GROWTH RETARDATION

decreases and remains low until weaning (11). At weaning, the high-fat diet of the suckling rat is changed into a high-carbohydrate diet. The mass of endocrine tissue and the plasma insulin concentrations increase while pancreatic insulin content decreases (11). The suckling-weaning transition in rats is associated with an increase in insulin sensitivity of the peripheral tissues (40), which may be conferred by an enhanced expression of the GLUT-4 glucose transporter (41). During the neonatal period, GLUT-1 expression decreases while GLUT-4 expression increases. The regulation of GLUT-4 expression depends on the composition of the diet; the suckling-weaning transition in rats is associated with a shift from a high-fat (milk) to a high-carbohydrate diet (rat chow). An increase in GLUT-4 messenger RNA expression can be partly prevented by weaning on a high-fat diet. The expression and translocation of GLUT-4 are also regulated by circulating insulin concentrations (41).

ADULT OFFSPRING

Female offspring of severely diabetic rats have a lower body weight from fetal life onward (Table 1). At adult age (3 months), these offspring appear to have recovered from the influences of a perinatal diabetic environment. They have a morphologically normal endocrine pancreas and normal plasma glucose concentrations (11). Plasma insulin concentrations are normal (Table 1) or increased. During

<table>
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<th>TABLE 1. Body weight, plasma glucose and insulin concentrations in the offspring of control, diabetic, and food-restricted rats at various ages</th>
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a 3-hour glucose infusion, however, these offspring have a raised insulin-to-glucose ratio, suggesting insulin resistance (42). Euglycemic hyperinsulinemic clamp studies combined with isotopic measurement of glucose turnover using [3-3H]-glucose have clearly demonstrated the existence of an insulin resistance in liver and peripheral tissues of adult offspring of severely diabetic rats (43).

The peripheral tissues of offspring of severely diabetic rats are less sensitive to insulin (half-maximal effect), but they display a normal responsiveness to insulin (maximal effect), confirming previous results of the 123I-insulin captation experiments (44). Because in the clamp studies all rats were in the postabsorptive state, the glucose production rate in these studies equals the actual glucose production rate. Endogenous glucose production in offspring of severely diabetic rats is both less sensitive and less responsive to insulin (43).

With the exception of the liver, the hyperinsulinemic clamp does not allow identification of the tissues contributing to the peripheral insulin resistance. To determine the peripheral tissues contributing to the decreased glucose disposal, we used the 2-deoxy-[1-3H]-D-glucose technique in basal conditions and during a clamp at physiologic hyperinsulinemia. We thus determined the glucose metabolic index in five skeletal muscles, the diaphragm muscle, white adipose tissue, and two control tissues (brain and duodenum) (Fig. 1). As could be expected, skeletal muscles are primarily responsible for the peripheral insulin resistance that characterizes the adult offspring of severely streptozotocin-induced diabetic rats (45). Indeed, the glucose metabolic index in the skeletal muscles of adult offspring of severely streptozotocin-induced diabetic rats was 9% to 29% lower under basal conditions and 25% to 70% lower at physiologic hyperinsulinemia than that of control rats. Muscles are the main reservoir of insulin-sensitive tissues within the mammalian body, representing 36% to 40% of the body weight. Their contribution to the whole glucose turnover is about 36% in postabsorptive control rats and 50% during euglycemic hyperinsulinemia (46).

Female offspring of rats that are food-restricted (50% of normal food intake) during pregnancy and lactation have a lower body weight from fetal life onward; by contrast, the offspring of rats food-restricted only during pregnancy increased their body weight above control values (Table 1). Nonfasting plasma glucose levels were increased in offspring with malnutrition during both the fetal and neonatal periods (Table 1), indicating that glucose tolerance had deteriorated in this group (25).

With the euglycemic-hyperinsulinemic clamp technique, we have shown that adult female rats subjected during the perinatal period to malnutrition, caused by impaired uteroplacental and mammary transfer of nutrients, are resistant to the action of insulin, as evidenced by the decreased infusion rate of glucose to maintain euglycemia (Fig. 2).

This resistance to insulin was found to be the result of a decreased responsiveness of the liver—that is, a dampened suppression of glucose production during hyperinsulinemia (47). Insulin action at the peripheral tissues, however, remained normal (25).
Apart from the slight difference in plasma glucose concentrations and a clear difference in body weight (growth retardation at the time of the clamp only in rats undernourished during fetal life), there were no significant differences in the plasma insulin levels or tissue insulin sensitivity of rats subjected to food restriction during the fetal period alone or the fetal and neonatal periods combined. This suggests that fetal malnutrition is the main determinant of hepatic insulin resistance in the rat. By
FIG. 2. Insulin dose-response curves for steady-state glucose infusion rate in adult offspring of rats food-restricted during pregnancy (group A; squares) or during both pregnancy and lactation (group B; triangles) and in rats fed ad libitum (group C; circles). Data are means ± SEM of five to 10 experiments. *p < .05; **p < .01; ***p < .001 vs. control values. From Holemans et al. (25).

contrast, although fetal muscle glucose utilization was found to be suppressed by maternal fasting, this did not affect peripheral glucose utilization at adult age. Thus, there is no long-term "imprinting" of perinatal malnutrition on muscle glucose utilization.

The growth retardation in adult rats induced by malnutrition during gestation and lactation has different implications for glucose homeostasis than the growth retardation induced by severe diabetes in the maternal rat. We found that the adult offspring of severely diabetic rats had normal nonfasting glucose concentrations and normal or increased nonfasting insulin concentrations. The insulin resistance in these rats was characterized by a decreased sensitivity of the peripheral tissues—that is, the skeletal muscles—and by a decreased sensitivity and responsiveness of the liver (43,45). The insulin secretion in response to glucose in vitro was greater in the offspring of diabetic rats than in control rats (44), whereas the inverse seems to be the case in the offspring of food-restricted or protein-restricted rats (25). Thus, the long-term imprinting caused by maternal diabetes on insulin secretion and tissue insulin sensitivity of the adult offspring is very different from that caused by malnutrition.

When the second-generation offspring of severely streptozotocin-induced diabetic rats become pregnant, they exhibit signs of glucose intolerance; they have higher
TABLE 2. Body weight, plasma glucose and plasma insulin concentrations of pregnant offspring of diabetic rats and their third-generation fetuses

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight, g*</th>
<th>Glucose, mmol/l*</th>
<th>Insulin, nmol/l*</th>
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<tbody>
<tr>
<td>Pregnant offspring</td>
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<tr>
<td>Control</td>
<td>293 ± 3 (35)</td>
<td>4.3 ± 0.1 (49)</td>
<td>0.40 ± 0.02 (34)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>270 ± 3*** (37)</td>
<td>4.7 ± 0.1** (45)</td>
<td>0.34 ± 0.01* (40)</td>
</tr>
<tr>
<td>Fetuses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.10 ± 0.03 (65)</td>
<td>2.3 ± 0.1 (21)</td>
<td>0.72 ± 0.05 (10)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.12 ± 0.02 (112)</td>
<td>2.7 ± 0.1** (48)</td>
<td>0.96 ± 0.05** (44)</td>
</tr>
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* The measurements were made on day 20 of gestation in pregnant animals and in their fetuses. Values are means ± SEM for the number of rats in parentheses.

* p < .05 vs. control rats; ** p < .01 vs. control rats; *** p < .001 vs. control rats.

glucose levels than normal pregnant rats (Table 2), and the number of granulated β cells in the endocrine pancreas does not increase as in normal rat gestation (11). These data would suggest that a defect is present in offspring of diabetic rats in the pregnancy-induced response of the β cells to glucose. As normal pregnancy is a state of severe physiologic insulin resistance, we wanted to investigate whether the insulin resistance present in the offspring of diabetic rats is further aggravated during gestation. For this purpose, again we used the euglycemic-hyperinsulinemic clamp technique combined with isotopic measurement of glucose turnover (48). The insulin dose-response curve for the increase of glucose metabolic clearance rate over basal values and for inhibition of endogenous glucose production obviously shows that the pregnancy-induced insulin resistance is not found in the offspring of diabetic rats. There is no further decrease in the peripheral tissue sensitivity to insulin, and there is only a small decrease in the hepatic insulin sensitivity. Overall, there are no differences in insulin sensitivity between pregnant control rats and pregnant offspring of diabetic rats. This is also apparent from the glucose metabolic indices determined in various peripheral tissues of both pregnant control rats and pregnant offspring of diabetic rats (Fig. 1). Although the insulin resistance was not markedly aggravated during pregnancy in offspring of diabetic rats, a syndrome of "gestational diabetes" evolved in these rats. The pregnancy-associated increase in circulating insulin concentrations was blunted in offspring of diabetic rats; as a consequence, pregnant offspring of diabetic rats had lower insulin concentrations than pregnant control rats. Their nonfasting glucose levels were also increased, and levels of nonessential fatty acids were markedly elevated (48).

Their fetuses, the third generation offspring, also develop in an abnormal intrauterine milieu. They display islet hyperplasia, β-cell degranulation (11), hyperinsulinemia, and hyperglycemia (48) (Table 2). At adult age, the third generation offspring of streptozotocin-induced diabetic pregnant rats have impaired glucose tolerance with high glucose levels (42). These data clearly show that a diabetogenic tendency
is transmitted from the pregnant streptozotocin-induced diabetic rat to her fetuses, with consequences persisting into adulthood and into the next generation. The transmission of diabetes through the maternal line has been confirmed in offspring of rats made hyperglycemic by a continuous glucose infusion during the last week of pregnancy (49).

The inducing factor of the insulin resistance that characterizes the adult offspring of severely streptozotocin-induced diabetic rats must be the abnormal perinatal milieu of the diabetic maternal rat, to which the developing fetus has to adapt. Indeed, normalization of the maternal glycemia from day 15 of gestation by islet transplantation prevents the occurrence of a disturbed glucose tolerance in the offspring (50). The studies of Grill et al. (51) and Ktorza et al. (49) strongly suggest that the diabetic or hyperglycemic intrauterine milieu must be responsible for the metabolic alterations observed in the offspring, as in both studies the offspring were reared by nondiabetic or normoglycemic foster mothers.

SUMMARY

Perturbations of the maternal environment create an abnormal intrauterine milieu for the developing fetus. The altered fuel supply (depending on substrate availability, placental transport of nutrients, and uteroplacental blood flow) from mother to fetus induces alterations in the development of the fetal endocrine pancreas and adaptations of the fetal metabolism to the altered intrauterine environment, resulting in IUGR.

The alterations induced by maternal diabetes or maternal malnutrition (protein energy or protein deprivation) have consequences for the offspring that persist into adulthood and into the next generation.

REFERENCES


DISCUSSION

Dr. Stuart Campbell: I would like Professor Battaglia to follow on from the statement he made that glucose restriction does not cause IUGR. I find that slightly difficult to accept for the following reason, which is that Peter Soothill showed very elegantly that the insulin-to-glucose ratio was low in IUGR in prenatal samples of cord blood (in other words, a relatively low insulin in relation to the glucose levels that were already low), and it has also been shown that the \( \beta \) islet cells are reduced in volume and in percentage, and the volume of the endocrine tissue is reduced. So in other words, there is hypoinsulinemia and there are reduced numbers of \( \beta \) cells; surely low glucose would cause hypotrophy because of low insulin levels.

Dr. Battaglia: In animal research, in the sheep models of severe growth retardation you certainly develop a lower glucose and a lower insulin, just as you would if I starved you. Now if I starve you and you lower your insulin and glucose, we don't say the low insulin and glucose cause you to grow poorly; we say that's a perfectly reasonable adaptation of the body to the fact that your food is restricted. We looked at that in our animal model of growth retardation, in which there is a very small placenta. The only way a small fetus with a small
placenta will get enough glucose is by resetting a low glucose concentration and increasing
the glucose gradient across the placenta. I would not interpret that as a cause, but rather as
an adaptation. So we have to make clear here what we are talking about as cause and effect.
As far as Barker's work is concerned, he had no gestational age data. Growth rate implies a
change with time. If all you have is the weight of a baby but no age, you can't talk about
growth retardation. So I think this is still a very confusing field. I think we need to know a
lot more about it. I have no doubt that in fetal life any organ, including the pancreas, will
change the way it grows and develops if you impose on it a different environment. So if we
restrict the supply of nutrients to a fetus because of a small placenta for a long time, I think
we could find changes in many fetal organs, including the pancreas.

Dr. Godfrey: In relation to the epidemiologic data, in six of seven populations in which
there are gestational age data in relation to later diabetes, the effects we see are independent
of gestational age; adjusting for gestational age if anything strengthens some of the associa-
tions. In the initial population that we studied, we didn't have gestational age data, but in
populations in the United States, in other populations in the UK, Sweden, and India, and in
Australian aborigines, associations between size at birth and later diabetes have being shown
independently of gestational age at birth.

Dr. Talamantes: I am trying to put all this together. Several years ago, my lab showed
that if you fasted mice you got an increase in placental lactogen. More recently, we showed
that the increase that you see in pancreatic islets in the $\beta$ cells is a consequence of mouse
placental lactogen or rat placental lactogen in the case of the rat. If in your fasted rats you
get an increase in placental lactogen and if you keep them fasted for long enough, you are
going to get a hypertrophy of those islets. I am trying to see how that in turn will have an
effect in terms of diabetes.

Dr. Van Assche: We didn't look at human placental lactogen, but I can follow your sugges-
tion. We are not claiming to know the origin of this hypertrophy or this hyperplasia. I am
not saying it is glucose, because in severe diabetes there is high glucose and low insulin. I
think the main point, which could be the critical event leading to fetal problems, is a reduced
uteroplacental circulation in all these situations. And that is the thing that we would like to
figure out, but we have no data. If there is reduced uteroplacental circulation, we can explain
a lot of things.

Dr. Talamantes: In the human, if you increase glucose you are going to reduce placental
lactogen. The growth of the islets in humans, shown by the work of Sorenson, depends on
placental lactogen, so if you raise the glucose you are going to lower placental lactogen and
you are going to have a reduction in $\beta$ cell multiplication and release of insulin.

Dr. Van Assche: I follow your comments. The only thing is that you can have hyperplasia
of the islets with high glucose levels when there is a normal uteroplacental circulation. So
there are a lot of mechanisms working against each other. I completely agree with your
comment about human placental lactogen, but there are a lot of factors acting together and
I don't know which is the final determinant.

Dr. Soothill: In the human fetus, you get low levels of glucose in IUGR, and even after
adjusting for the level of glucose the insulin is still lower. One of the ideas we had about
why that might be was that the redistribution process might lead to the pancreas, with other
organs in the abdomen, being relatively ischemic. Have you any comments about that in
relation to your models?

Dr. Van Assche: We have no data in the rat concerning the vascular system. I have shown
that in the human, when there is macrosomia there is an increased amount of vasculature in
the endocrine pancreas. So your postulation could be right. Your suggestion that there could be a redistribution of the vascular system is also possible.

*Dr. Ogata:* Are your adult former IUGR rats smaller than normal?

*Dr. Van Assche:* Their weight is relatively reduced until about 20 to 30 days of weaning, and you could propose that maybe the mammary glands are not developed enough, but then they increase in weight and they are overweight in adulthood.

*Dr. Ogata:* This complicates the mechanism of insulin resistance. We have used the maternal uterine artery ligation model and have found postnatally—and we have grown our rat pups under reasonably well-controlled nutritional conditions—that up to 4 weeks they remain smaller than expected compared with controls. We have also found evidence of insulin resistance in the neonatal period; for example, PEPCK is not induced as quickly as in the normal, and if you look at group 1 modulation and insulin-sensitive glucose transporter in various tissues, they are reduced and not appropriate to the insulin. But we also have seen that beyond a certain point the rats get fat. To my mind, that changes the possible mechanisms of the insulin resistance, because now you have a big rat, not a small one. I think that complicates the mechanism considerably.

*Dr. Van Assche:* I agree with you.

*Dr. Pardi:* The weight of the pancreas of growth-retarded fetuses is reduced compared with controls. If you look at the ratio with total body weight, the difference is much less. Have you any data on the weight of other organs in the same fetuses—brain and liver, for example—to give an idea about the degree of weight loss of the fetal pancreas in growth retardation?

*Dr. Van Assche:* Let me first give you some explanation about the morphometric data. When we say that the volume density of the endocrine tissue is reduced, it means that there is an absolute reduction. So in comparison with the total body weight and in comparison with the pancreatic weight, we have a particular reduction of the endocrine tissue. Second, of course the body weight is reduced and the placental weight is increased in severe diabetes, and all the other organs are reduced. The liver is reduced in weight.

*Dr. Stuart Campbell:* As a humble clinician, I had always believed that maternal diabetes caused macrosomia, and that if you had an IUGR fetus associated with maternal diabetes, it was caused by vascular disease and impaired perfusion. Now you found in your severely diabetic rat mothers that you had IUGR with a low percentage of $\beta$ cells. Did they have vascular disease, or are you saying that severe maternal diabetes can cause IUGR irrespective of maternal vascular disease?

*Dr. Van Assche:* In the human situation, in the majority of cases of maternal diabetes you have macrosomia, except when there is an association with pre-eclampsia or with vascular disease. In the rat, when there is severe diabetes (and severe diabetes means glycemia levels up to 400 mg%), we have shown that in this situation there are endothelial lesions to the kidney. We have also shown that in the mesometric triangle in the placenta of the rat, which may be equivalent to the region of the spiral artery in the human, endothelial lesions are also found. Therefore, we think that in severe diabetes we have a reduction of uteroplacental blood flow.

*Dr. Girard:* Perhaps I could suggest a possible explanation for the liver insulin resistance. It could be postulated that these mothers are making more glucose by gluconeogenesis than by glycolysis to explain why insulin is less active in this group.

*Dr. Godfrey:* As mentioned before, a maternal low-protein diet during pregnancy alone is associated with a persistent tendency toward increased hepatic gluconeogenesis. In our Southampton studies, we found that studying liver metabolism in free-living adults is very
difficult, but we have done studies of skeletal muscle metabolism and have shown by magnetic resonance spectroscopy and other techniques that thin babies with insulin resistance as adults do have persisting abnormalities of muscle metabolic fuel selection, so marrying up the species differences and the model differences may be important.

Dr. Battaglia: When you say that uteroplacental perfusion may be abnormal, remember that if there is one thing that is well established it is that the hormones that control angiogenesis are linked to cell division in an organ. So if you have cancer tissue with rapid cell growth, you get a lot of angiogenesis, and if you slow down cell division, then the vascular bed also doesn’t develop as fully. The change is in the cell division rate of the tissue you are studying, and angiogenesis follows that—they are nicely coordinated. When we talk about reduced uteroplacental perfusion, my question is whether we are talking about a reduction per kilogram of uterine tissue supplied, because at least in the model we work with, in which we measure flows, we don’t see a reduction per kilogram of tissue. Certainly if you have a small fetus, you are going to have a low flow in absolute terms, but for me that isn’t underperfusion. How do you visualize this uteroplacental problem?

Dr. Stuart Campbell: Normally, uterine blood flow from before pregnancy to late pregnancy increases from 60 ml/min to about 700 to 800 ml/min, so there is a huge increase in the amount of blood flow that the intervillous space requires, and there has to be this adaptation of maternal blood vessels, principally in the spiral arteries. If that adaptation doesn’t occur, then there is, I suppose, a relative obstruction to this flow getting into the intercotyledonal space. I think it helps us understand why IUGR occurs if you realize that blood is going right into the middle of the cotyledon and filtering through the villi, and it’s obstruction at that level that is the source of a lot of problems relating to IUGR. In diabetes, there might not be a vascular adaptation problem, but there are abnormal blood vessels in the placental bed that prevent the increase in blood flow, just as in sickle cell disease you get pre-eclampsia because of obstruction by clumps of sickling cells in the placental bed. Anything that obstructs the normal perfusion of the intercotyledonal space, causing slowing of the blood, platelet aggregation, and fibrin deposition within these cotyledons, will I think cause this problem.

Dr. Battaglia: Suppose you have a 10-kg mother and a 100-kg mother, and the 100-kg mother is the one in whom you define normal flow, and it goes up to 700 ml/min. But if 700 ml/min occurs in a 10-kg mother she is dead, because there is no perfusion of the brain and other organs. So her adaptation might be 300 ml, perfectly normal, but 300 because she is smaller. I’m trying to get at the problem of when a disease should be called a uteroplacental perfusion problem. I accept that a placenta that is half the size of another will have a reduced blood flow, but for me that is not a uteroplacental perfusion problem.

Dr. Stuart Campbell: In the human fetus, you often see early IUGR with a fetus adapting to a situation of relatively low perfusion with a small placenta; the fetus adapts, almost hibernating in the uterus, and can often get to viability because it has constrained its growth and has grown along a particular path, even if suboptimally. However, in late pregnancy, with a fully grown fetus or a fairly normally growing fetus, when the effects of impaired perfusion to the placenta and secondary obliteration to the villous circulation occur, then you see the fetus becoming acidemic and dying very quickly because the blood flow is inappropriate to its size. These are the problems we see very often in clinical practice.

Dr. Meschia: You keep implying that the blood flow is decreased, but you don’t know that. I could say it’s because the mass of the trophoblast is less. How do you respond to that?

Dr. Stuart Campbell: I suppose it is because we use Doppler to identify abnormal waveforms in the placental bed, which are typical of failure of trophoblast invasion and therefore
identify a group that we believe has impaired perfusion. If you do volume flow studies, which I know are not particularly accurate, we definitively see a reduced volume flow into the placenta.

*Dr. Meschia:* But there is also a reduced trophoblast mass, so the disease could primarily be a reduction in the growth of the trophoblast.

*Dr. Van Assche:* I completely agree with the explanation of Professor Campbell, but I also understand your critical approach, because you have another view with your experimental models, and what is flow? The only thing I can say is that when we look at the morphology of the spiral arteries, we find that they are blocked, and you see infarctions of the placenta on the side where there is blocking of the uteroplacental circulation. We are speaking about the reduction of the flow to the placenta.

*Dr. Stuart Campbell:* What we have shown is that in many normal small IUGR fetuses at term in which there are no clinical abnormalities, the placenta is actually smaller than average quite early on, at about 20 weeks. But in most of those cases in which we find abnormal uteroplacental waveforms, the placenta is within the normal range for volume, so there are abnormal waveforms in a placenta of normal volume. That is why I believe the blood flow is inappropriate for the placenta.