

Rodent models of intrauterine growth restriction

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Scientific and epidemiological background. Intrauterine growth restriction (IUGR) is a major cause of perinatal death, neonatal morbidity and mortality. There are numerous causes of IUGR such as genetic/chromosomal abnormalities (e.g. family history, trisomy and Turner's syndrome), toxic embryopathies (e.g. rubella syndrome and toxoplasmosis), toxic/negative environmental factors (e.g. chemicals, alcohol and other drugs) and substrate limitation through placental insufficiency (e.g. oxygen and nutrients) (Peleg et al., 1998, Pollack et al., 1992). Substrate limitation in early pregnancy retards embryonic growth and results in symmetrically small low birth weight babies (Barker et al., 1993b). In mid gestation the placenta grows faster than the fetus, and nutrient deficiency during this period may therefore affect fetal growth by changing the complex interaction between fetus, placenta, and mother. Severe maternal undernutrition during this time restricts growth of both fetus and placenta. In contrast, mild substrate limitation leads to increased placental weight but does not change fetal size. This placental overgrowth may be an adaptation to sustain nutrient supply from the mother to the fetus (Barker et al., 1993a). In late gestation maternal substrate limitation immediately slows fetal growth and alters the metabolic interaction between fetus and placenta. Fetal growth slows down to maintain placental function, and oxygen, glucose, and amino acids are re-distributed. The placenta reduces its consumption of oxygen and glucose while maintaining a large output of lactate to the fetus. The lactate is partly derived from amino acids of fetal origin (Barker et al., 1993a). The effect of substrate limitation in late gestation on the

fetus depends on its duration. The fetus is capable of regaining lost weight after a short substrate limitation period. Longer-term substrate limitation slows fetal growth rate and leads to irreversible stunting of fetal growth (Barker et al., 1993a).

Over the past decade a number of epidemiological studies have revealed strong and reproducible links between indices of poor fetal and early postnatal growth and susceptibility to the development of glucose intolerance and insulin resistance syndrome in adult life. The "thrifty phenotype" hypothesis has been proposed to explain some of these associations. Key features of the hypothesis are: (I) Intrauterine growth restriction has a nutritional basis and the resulting altered fetal environment permanently alters the development and metabolic functions of organs; (II) These alterations are beneficial to survival in a poor nutritional environment, but may lead to diseases such as non-insulin dependent diabetes mellitus if nutrition is abundant and obesity occurs in adult life (Hales, 1997).

High blood pressure in adult life is associated with disproportional growth indicated by thinness at birth and short body length in relation to head size (Barker, 1994). Furthermore, Barker (1995, 1993b) has shown that placental enlargement is associated with Syndrome X (elevated blood pressure, impaired glucose tolerance, disordered blood coagulation, altered lipid metabolism, and death from coronary heart disease) in adult life.

The scientific and epidemiological data explain why there is continued interest in developing animal models to investigate this important clinical problem. Experimental models of intrauterine growth restriction have generally used

either interference with placental function, uterine blood flow, maternal hypoxia, drugs or limitation of maternal energy and protein intake to restrict fetal growth. This review describes four approaches to induce IUGR: "Nutritional models", "Surgical models", "Hypoxia models" and "Drug induced models".

Nutritional models.

Prolonged fasting and pan-undernutrition. Girard et al. (1977) have studied rat fetuses' responses to altered maternal nutritional supply. The effects of prolonged maternal fasting, begun 24-96 h before term (term = 22 days), were examined and compared with values from normally fed term animals. To see a significant effect on fetal weight at term the mothers needed to be fasted for more than 48h. Prolonged maternal fasting was associated with low fetal blood glucose and plasma insulin, and increased glucagon. The authors hypothesised that maternal fasting induces fetal substrate limitation that leads to impaired fetal growth.

Ledermann and Rosso (1981) have looked at the changes in fetal and maternal weight and in maternal body composition during a 2-day fast, between day 17 and 19 of gestation in the rat. They examined ad libitum fed and undernourished (50% of ad libitum) pregnant and non-pregnant rats to see whether maternal nutrient stores are mobilized to aid fetal growth during fasting and whether pre-existing undernutrition alters the effect of fasting on maternal weight and fetal growth. Fasting between day 17 and 19 of gestation resulted in a greater loss of total maternal body weight and body fat in ad libitum-fed pregnant than in food-restricted pregnant rats, that in fact maintained their net body weight and body fat during the fast, as did nonpregnant rats. Fetal weight was not significantly reduced by fasting in the ad libitum-fed rats, but was reduced by 25% in the previously food-restricted rats. The results demonstrate that prior maternal nutritional status strongly influences the effects of fasting on the fetuses.

Straus et al. (1991) examined the effect of maternal fasting on days 17-21 of gestation on the

gene regulation of IGF-I, IGF-II, IGFBP-1 and IGFBP-2 in fetal rat liver. Fetal weight was significantly reduced in the offspring from the fasted dams compared to offspring from non fasted dams. Maternal fasting caused a decrease in fetal IGF-I gene expression, in fetal serum IGF-I, and a slight decrease in fetal serum IGF-II, while IGFBP-1 gene expression was increased. The authors hypothesise that this combination of changes in the fetal IGF-axis may well contribute to the fetal growth restriction observed in this model.

The importance of the fetal IGF-axis in the regulation of fetal growth is further emphasized by the results from gene targeting experiments in mice. Single gene deletions of either the IGF-I or IGF-II gene reduced fetal weight 40% compared to wild type controls, while a deletion of the IGF-I receptor (IGFR) resulted in a 55% reduction of fetal weight. Double null mutations of the IGF-I and IGF-II genes or the IGF-II and the IGFR showed the most severe phenotype with a 70% fetal weight reduction at term (term = 20 days) (Liu et al., 1993; Baker et al., 1993). Mice carrying a double null mutation for the IGFR and insulin receptor (InsR) also show a 70% weight reduction, indicating that the InsR is a vital part of the IGF signalling cascade in the absence of IGFR (Louvi et al., 1997). Interestingly enough, only IGF-II null mutants showed impaired placental growth, which became apparent after embryonic day 13 (Baker et al., 1993).

Woodall et al. (1996a) have developed an IUGR model in the rat with nutritional restriction of the mother throughout gestation. They examined the effects of fetal growth restriction on endocrine and metabolic status during the perinatal period. Food was available ad libitum throughout pregnancy to a control group and a restricted group was fed 30% of the ad libitum intake, determined by the amount of food consumed by the control group on the previous day. After birth, all pups were cross-fostered to an ad libitum mother. Litter size was not affected by maternal undernutrition. The mean body weights of fetuses in late gestation from the restricted fed dams were significantly lower in comparison with fetuses from control dams. Placental weights were also significantly reduced

in the restricted fed dams compared with control dams. Postnatal body weights were significantly lower in the offspring of restricted fed dams compared to controls from birth until 18 weeks of age (Woodall *et al.*, 1999). At 50 weeks of age, body weights were similar between offspring of ad libitum fed and restricted fed mothers. Plasma insulin levels were significantly reduced in the pups of restricted fed dams at birth but not at later time points (Woodall *et al.*, 1996a). Circulating IGF-I was significantly reduced in the restricted fed group from day 22 of gestation until postnatal day 9, but not at later time points. Plasma IGFBP-1 and -2 levels were significantly increased from day 22 of gestation and were still lower at postnatal day 9 in the restricted fed group. The ¹²⁵I-bGH specific binding of liver membranes was significantly reduced by 20% in offspring from restricted fed dams at 21 days of age; this difference was equalized at 90 days of age (Woodall *et al.*, 1996a). Liver IGF-I mRNA was

reduced during the first 21 days in the restricted fed group (Woodall *et al.*, 1998). Systolic blood pressure was significantly elevated in the restricted fed group from 30 weeks of age and onward (Woodall *et al.*, 1996b). These data demonstrate that nutritional deprivation in the pregnant rat leads to IUGR and postnatal allometric growth patterns, to delayed catch-up growth and to elevated blood pressure in adulthood.

Holemanns *et al.* (1996) have investigated insulin sensitivity in adult rats after perinatal malnutrition. Wistar rats were food-restricted (about 50% of normal food intake) from day 11 of pregnancy until delivery (group A) or from day 11 of pregnancy and during lactation (group B) and compared with rats fed ad libitum during pregnancy and lactation (group C). The offspring from the restricted dams were growth retarded and relatively hypoglycaemic and hypoinsulinemic at term (day 22) (see Table 1).

Table 1: Body weight and non-fasting plasma glucose and insulin changes in response to perinatal malnutrition in rats.

Age	Group	Δ% Body weight	Δ% Glucose	Δ% Insulin
Day 22 of fetal life	A/B	-20*	-25†	-52‡
Day 20 after birth	A	14*	-	-
	B	-58*	-	-
Day 100 after birth	A	8*	3	-42*
	B	-8*	10†	-42*

Group A: food-restriction (50%) from day 11 of pregnancy until delivery. Group B: food-restriction (50%) from day 11 of pregnancy and during lactation. Group C: fed ad libitum during pregnancy and lactation. *P<0.001 vs group C, † P<0.01 vs group C, ‡ P<0.02 vs group C (Holemanns *et al.*, 1996).

IUGR pups from group B became more growth retarded at weaning and this difference was maintained at least until day 100 after birth, in contrast to offspring from group A, which gained more weight than the control group. Non-fasting plasma glucose concentrations were normal in group A 100 days after birth but were increased by 10% in group B offspring, whereas insulin concentrations were markedly lower in groups A and B. During an euglycemic hyperinsulinemic clamp experiment glucose infusion rates were significantly lower in both groups A and B compared to the control groups with an insulin infusion rate of 10 and 50 mU/kg/minute, which indicates insulin resistance in groups A and B. Garofano et al. (1998) have designed a rat model of perinatal malnutrition to study the role of nutrition in postnatal somatic growth and insulin stores until adulthood. Maternal food restriction (50%) from day 15 of pregnancy resulted in IUGR in the offspring. The outcome of moderate or severe IUGR was investigated. Neonates with moderate IUGR and normal postnatal nutrition showed normal body and organ weights and normal pancreatic insulin contents in adulthood. Offspring with severe IUGR and normal postnatal nutrition also rapidly recovered normal body and pancreatic weights, but liver and kidney weights were significantly reduced at adult age. Malnutrition until weaning in offspring with severe IUGR induced marked growth restriction (50%) in body and organ weights at weaning. Although pancreatic weight recovered at adult age, body, liver and kidney weights were irreversibly affected, despite several months of normal nutrition. Furthermore, severe IUGR at birth resulted in decreased pancreatic insulin content at adult age, irrespective of postnatal nutrition. This animal model demonstrates that normalization of adult size and weight can be dissociated from organ growth. Furthermore altered insulin stores in adulthood are more dependent on the severity of IUGR at birth than on postnatal catch-up in organ growth.

A guinea pig model of IUGR induced by maternal pan-undernutrition throughout pregnancy has also been described (Kind et al., 1999; Sohlstrom et al., 1998). Pregnant guinea pigs were fed 85% of

ad libitum food intake, which resulted in a 13% reduction of fetal body weight at birth (term = 65 days). Multiple regression analyses showed that maternal weight gain was negatively associated with plasma IGF-II and IGFBP-2. Fetal weight was positively associated with maternal IGF-II, but negatively associated with maternal plasma IGFBP-1 and IGFBP-2. Most interestingly, the impact of maternal IGF-II and IGFBP-1 on fetal growth was dependent on the nutritional status of the mother (Sohlström et al., 1998).

The model was used to test the impact of restricted fetal growth on cholesterol handling in postnatal life. The offspring were divided into two groups: "High" birth weight above median birth weight and, "Low" birth weight below median birth weight. Total plasma cholesterol did not differ between groups before the onset of a 6 week cholesterol supplemented (0.25%) diet. After 6 weeks total and LDL cholesterol were 30% higher in males from the low birth weight group compared to the high birth weight group. Female offspring did not respond to the cholesterol enriched diet (Kind et al., 1999).

Protein restriction.

Resnick et al. (1982) have examined the physiological weight changes seen in rat dams and their offspring as sequela of either an overt or a hidden form of chronic protein malnutrition. In the overt model, which was produced by feeding dams a very low protein diet (6% casein) starting 5 weeks prior to conception and continued through lactation, the females showed significant weight losses at all ages compared to dams maintained on a normal diet (25% casein). This caused the malnourished 6% dams to have offspring that were categorized as small-for-date at birth in terms of their weight indices and peripheral metabolic profiles. Also, the inadequate milk production of these dams resulted in their pups displaying an almost total failure of growth (greater than 60% decreases in body weights) and peripheral imbalances characteristic of infantile marasmus by day 8 of lactation. Consequently at all times examined, the 6% casein dams and pups showed most of the typical responses seen in the more severe forms of in utero and lactational

malnutrition in man. In contrast, the hidden form of malnutrition produced by feeding dams a somewhat higher protein diet (8% casein) throughout the same time periods caused no marked weight loss by these females during their pregnancy compared to the normal dams. Although the 8% casein pups had the same birth weight as the normal offspring, the 8% casein dams displayed lactational insufficiencies as noted by retarded postnatal growth of their pups. Nursing of these offspring by 25% dams allowed them to maintain a normal lactational growth curve. However, not only was this cross-fostering unable to rehabilitate most of the prenatally determined biochemical alterations affecting the 8% casein pups but, additionally, this form of malnutrition would remain undetected if weight indices alone were used as assessors of normalcy. Thus, it appears that the 8% casein rats may serve as a useful model for the hidden forms of malnutrition in man. The most prevalent form of malnutrition in humans is characterized by its chronic and generational nature. Resnick and Morgane (1984), therefore, have carried out a study in rats on the generational effects of protein malnutrition. Their results indicate that a relatively mild protein restriction (8% casein diet) in the first generation produces a phenotype typical for a more severe protein restriction in the second generation (F2). This is based on weight gain of the dams during pregnancy, the mean number of pups (F2) per litter, and the mean pup (F2) body weight from birth to weaning. They consequently propose this as an animal model for some types of chronic undernutrition in socioeconomically underprivileged human populations. Langley-Evans and Jackson (1994) have looked at the possible associations between maternal nutrition in pregnancy and non-communicable diseases of adulthood using a rat model. Rats were habituated to diets containing a range of protein levels (18, 12, 9 and 6% casein) over a 14-day period before mating. The low protein diets were maintained throughout pregnancy. Lactating mothers and their offspring were transferred to a standard rat chow

diet (20% casein). Pregnant rats demonstrated a graded response to these diets, with those fed 9 and 6% protein tending to consume less energy and gain less weight than 18% protein fed controls. Litter size and newborn death rates were not significantly altered by the low protein diets. Offspring of 12 and 9% protein fed dams gained weight at a similar rate to those born to 18% protein fed control rats. Offspring of the 6% protein fed dams were smaller than pups from all other groups, over a 21-week period (see Table 2). At 9 weeks of age, systolic blood pressure was determined in the offspring. All offspring from the three low protein groups were found to have significantly elevated blood pressure (15-22 mmHg) relative to the control group. An inverse relationship between maternal protein intake and the systolic blood pressure of the offspring was observed; $r = -0.52$, $p < 0.005$. There was also a relationship between maternal energy intake and systolic blood pressure, although to a lesser extent; $r = -0.33$, $p < 0.05$. Blood pressure remained elevated in the offspring of the 9 and 6% protein fed dams until 21 weeks of age. In the same model, Langley-Evans et al. (1994) performed a glucose tolerance test at 9 weeks of age by infusing glucose (2 g/kg-BW i.v.). The glucose load was cleared in 60 min by control rats, 18% and 12% protein exposed rats. Peak blood glucose concentrations in these animals were 27.2 and 27.1 mmol/L, respectively. Rats exposed to 9% protein in utero also cleared the glucose load in 60 min, but peak blood glucose concentrations were lower (24.4 mmol/L), and the area under the glucose curve was 28% lower than in controls (see Table 2). Rats in the 6% protein exposed group cleared the glucose load even faster (40 min) and peak blood glucose levels were only 22.1 mmol/L. The area under the glucose curve was reduced by 40% relative to controls. When the glucose tolerance test was repeated in animals aged 44 weeks, no differences in peak glucose concentrations, area under the curve or rates of glucose clearance were observed. These observations differ from another

Table 2: Effect of protein malnutrition during pregnancy on postnatal growth, systolic blood pressure and glucose tolerance in rats.

Dietary Protein %	Body weight			BP	Glucose tol. AUC	
	1 week	9 weeks	21 weeks	9 weeks	9 weeks	44 weeks
18	12.5	204.0	290.0	137	452	355
12	12.9	210.0	276.0	152‡	435	346
9	12.1†	197.0	272.0	153‡	328‡	365
6	9.0*	173.0*	238.0*	159‡	275‡†	348

Effect of fetal exposure to maternal low protein diets on postnatal growth body weight [g], systolic blood pressure "BP" [mmHg] in female adult rats (*Langley-Evans & Jackson, 1994*) and the response to intravenous glucose load over 60 min., expressed as area under curve "AUC" [mmol/L/h] (*Langley et al., 1994*). * p<0.01 compared with other groups, † p<0.05 compared to 12% protein, ‡ p<0.05 compared to 18% protein.

report (*Dahri et al., 1991*) in which an impaired glucose tolerance was noted. Langley and co-workers speculate that they might have found the same impaired glucose tolerance in the 9 and 6% casein animals later in life. They hypothesise that their rats were already hyperinsulinemic, and that these animals would have become insulin resistant over time. This is however highly speculative since they did not measure insulin. These experiments show that in utero exposure of rats to low maternal protein diets alters glucose tolerance in young adulthood through an as yet undefined mechanism. To try to explain these observations, Latorraca et al. (*1998*) have looked at *in vivo* and *in vitro* insulin secretion from low protein (LP ~ 6 %) and normal protein (NP ~ 17 %) fed groups. They were able to reproduce the results of Langley et al. (*1994*). Additionally, they found that the *in vitro* insulin secretion of isolated pancreatic islet cells was reduced in rats that had been exposed to a low protein diet. This observation, taken together with the improved glucose tolerance, implies that insulin target tissues in low protein exposed rats are most probably more insulin sensitive than those in control rats.

A large variety of nutritionally induced IUGR models exist, based on total starvation during short periods of time, or more long-term pan-undernutrition. The rat has also proven to be very sensitive to iso-caloric protein malnutrition. The nutritionally induced IUGR models have a number of advantages: 1) they are very easy to establish; 2) the need for technical equipment is small; 3) they are highly reproducible. Most importantly however, the combination of duration, interval and severity of the nutritional insult can produce a number of different phenotypes, which more or less reflect the human IUGR phenotypes.

Surgical models

Uterine artery ligation.

Wigglesworth's (*1964*) approach of completely ligating the uterine artery of one horn has been the most popular small animal model of intrauterine growth restriction. The problem with this model is the high incidence of fetal death and resorption among the fetuses in the manipulated horn. The model was originally designed to demonstrate the importance of the uterine blood supply as a controlling factor in fetal growth.

Price et al. (1992a) have looked at the changes in IGF-I and -II, IGF binding protein and IGF receptor transcripts in liver, carcass and placenta of fetal rats with IUGR resulting from uterine artery ligation. They formed the ligation on day 17 of gestation followed by caesarean section on day 20. There they saw a significant reduction in body weight, liver weight and placental weight in the fetuses from the ligated uterine horn (UA-lig) compared with those from the opposite, nonligated horn (UA-nonlig) and those from dams with no surgery or anaesthesia (nonop). Liver IGFBP-1, IGFBP-2 and IGF-II m-RNA transcripts were increased in the UA-lig fetuses compared to UA-nonlig. In the placenta, however, expression of IGF-II was decreased by 44% in the AU-lig compared with nonop pups. The serum IGFBP-1/-2 doublet band level, analysed by ligand blot, increased 2.4-fold in the UA-lig fetuses compared to UA-nonlig and nonop. When they looked at immunoreactive IGFBP-2 alone there was no difference between the three groups, which indicates that IGFBP-1 accounted for the increase in doublet intensity. The authors speculate that the increased serum IGFBP-1 concentration may decrease IGF activity in serum and thus inhibit IGF-stimulated cell proliferation or, by crossing the endothelial border, inhibit the activity of locally produced IGF. Furthermore, the decreased IGF-II expression in the placenta may also contribute to the decreased placental growth and, in turn, contribute to the observed IUGR. It may therefore be possible that IUGR in fetal rats with a restricted arterial blood supply could be caused by decreased IGF bioavailability and/or activity (Price et al. 1992a). Similar results were reported by Unterman et al. (1993) after 24 hours uterine artery ligation, from day 19 to 20 of gestation. Tanaka et al. (1994) mimicked a phenomenon called "postischemic hypoperfusion" of uteroplacental circulation to create a new IUGR rat model with a low mortality rate and favourable reproducibility. Instead of ligating the uterine artery, they used 2 small artery clamps to occlude the uterine vessels near the lower and upper ends of the right horn for a period of 5 to 60 minutes. After a 60 min occlusion, they saw a significant decrease in body weight for the fetuses on the

ischemic side compared to those on the non-ischemic side. Death rate on the ischemic side was 14% compared to none on the non-ischemic side. Lafeber et al. (1984) have looked at the effect of reduced placental blood flow on organ growth and development of the fetal guinea pig following ligation of the uterine artery. The ligation was performed on day 30 of pregnancy (term = 65 days) and the fetuses were investigated 20 or 30 days later. Thirty percent of the fetuses in the ligated horns died. The surviving fetuses were growth-restricted by 50% and they did not show any postnatal "catch up" growth. Brain, liver and kidney weight were reduced, also when expressed relative to body weight. Using the same model, Jansson et al. (1986) measured placental blood flow (microsphere distribution) along with fetal and placental weight at day 45, 55 and 65 day of gestation. Placental blood flow and placental weight were reduced in the ligated horn at all times. At day 45 of gestation there was no change in fetal weight, but at 55 and 65 day of gestation fetal weight was significantly reduced. These results show that ligation of the uterine artery indeed reduces placental blood flow which, in turn, restricts placental growth without a measurable effect on fetal weight before day 45 of gestation. A time lag is seen between the onset of the placental blood flow restriction and the manifestation of fetal weight reduction. This is probably due to the relative hyperperfusion of the placenta at this stage of gestation, as illustrated by an increasing fetal weight/placental blood flow ratio in the nonligated horn between day 45 and 55, and the lack of correlation between fetal weight and placental blood flow at day 45. This also suggests that before day 45 fetal growth is not limited by placental blood flow, but when ligation impedes the normally seen pronounced increase in blood flow, fetal growth rate declines. Jones et al. (1987) studied plasma sulphation-promoting activity (a measure for IGF bioactivity) and concentration of IGF-I and -II from day 49 to 65 of gestation in a similar experimental set-up. The ligation caused a reduction in fetal growth of more than 45% at all time points investigated. Uterine artery ligation was associated with substantial delays in the development of a number

of fetal tissues and in particular that of the skeleton, which remained cartilaginous for longer than normal. At day 50 of pregnancy clear evidence of epiphyseal ossification in the long bones of the fore- and hind limbs was seen in the fetuses from the non-ligated horn, while in growth restricted fetuses ossification was reduced by more than 50%. Delayed skeletal development and the slowing in fetal growth rate correlated well with the marked depression of plasma sulphation-promoting activity seen in the growth-restricted fetuses. The plasma levels of immunoreactive insulin and IGF-I were reduced while levels of IGF-II were increased in the growth-restricted fetuses. The authors conclude that in uterine growth restriction caused by uterine artery ligation IGF-I falls, potentially slowing cell growth. IGF-II rises sharply to preserve essential metabolic events such as glycogen deposition necessary for postnatal survival.

In general, results obtained in the guinea pig match very well the observations made in the rat, and they again point towards the fetal IGF-axis as one of the central elements in the regulation of fetal growth.

Electrically induced thermal placental injury.

Intrauterine growth restriction involves haemodynamic modifications in the uterus and placenta. In order to study intrauterine growth restriction, Rosati et al. (1995) electrically induced a thermal placental injury in the rabbit. Fetal growth restriction was recorded in 71% of fetuses exposed to 15 mA direct current for 40 seconds. In this model, fetal growth restriction was particularly evident in the liver with a relative sparing of the brain, while the kidney and heart did not seem to be affected in growth. This growth restriction model is simple and readily reproducible. It has the advantage of a direct effect on the maternal-fetal exchange area, modelling exchange.

functional alterations of the fetal-maternal. The surgically induced IUGR models are relatively simple and most of them are highly reproducible. Many of them have the advantage that unaffected littermates in the non-manipulated horn can serve as controls, thereby reducing between litter variations. A clear disadvantage is the high mortality rate of some of the models and the prolonged exposure of mothers and fetuses to anaesthetic agents, which themselves could have detrimental effect on fetal growth. Furthermore, the insult is irreversible, which makes it impossible to study the effects of intermittent insults on fetal development.

Hypoxia models.

A number of IUGR models have been developed by subjecting the pregnant rat to hypoxia at different levels of oxygen in the last half of the gestation. Some of the results have been summarized in Table 3.

All the studies used pair-fed groups to control for the reduced food intake due to hypoxia. It was, however, found that the reduced food intake was not severe enough to account for IUGR.

As can be seen from the data summarized in Table 3, the effect of hypoxia is very powerful.

Even if the animal is only exposed to brief intermittent episodes of hypoxia (Schwartz et al., 1998), fetal growth restriction will result. These findings support the hypothesis that intermittent episodes of hypoxia acutely decrease fetal oxygen delivery, which can have a deleterious effect on fetal growth in the rat. This observations is particularly important, because in humans, intermittent rather than prolonged episodes of hypoxia are most likely to occur in utero. These studies do involve a significant amount of technical equipment, which makes them more difficult to establish than for example nutritional or surgical models.

Table 3: Effect of hypoxia during pregnancy on fetal growth parameters in rats.

Reference	Strain	Induction of IUGR	$\Delta\%$ Litter Size	$\Delta\%$ Body Weight	$\Delta\%$ Brain Weight	$\Delta\%$ Liver Weight	$\Delta\%$ Placenta Weight
van Geijn et al., 1980	Sprague-Dawley	9.5 % oxygen chronic day 10-22 of gestation	-75*	-36*	-23*	-44*	-15
de Grauw et al., 1986	Wistar	11.6 % oxygen chronic day 11-21 of gestation	-7*	-10*	-4*	-20*	11*
		10.7 % oxygen chronic day 11-21 of gestation	-8*	-28*	-13*	-37*	5
		9.0 % oxygen chronic day 11-21 of gestation	-29*	-37*	-22*	-48*	-6
Tapanainen et al., 1994	Sprague-Dawley	13 % oxygen chronic day 14-21 of gestation	n.m.	-24*	n.m.	-20*	-10*
Schwartz et al., 1998	Sprague-Dawley	9.5 % oxygen 1 h/day day 15-19 of gestation	20	-4*	-2	-12*	-13*
		9.5 % oxygen 2 h/day day 15-19 of gestation	-12	-7*	-8*	-8*	2

* $p < 0.05$ compared to own controls. n.m. : not measured.

Drug induced models

Glucocorticoids

Glucocorticoid induced IUGR models are highly relevant, since glucocorticoid administration to women threatened by premature labour is widely used in clinical practice. It is well established that such treatment significantly reduces the incidence and severity of the respiratory distress syndrome in prematurely born infants by inducing fetal lung maturation (Liggins & Howie, 1972; Schellenberg & Liggins, 1987).

The fetus is normally protected from corticoid exposure by the placenta, which contains 11 β -hydroxysteroid dehydrogenase (11 β -HSD), which converts physiological glucocorticoids into inactive products. Treatment of pregnant rats during pregnancy with dexamethasone, which can not be metabolised by 11 β -HSD, lowers birth weights and leads to elevated blood pressure in postnatal life (Benediktsson et al., 1993). Price et al. (1992b) studied the gene expression of IGF's, the IGF-I receptor, and IGFBP's in a dexamethasone induced IUGR model in rats. They

dosed the animal with 100µg/day from day 15 to 19 of gestation. Fetal body weight was significantly reduced (32%), together with a reduction of liver and lung weights. The DNA content of the liver and lungs was also reduced by the dexamethasone treatment. Hepatic IGFBP-1, IGFBP-2, IGF-II and type 1 IGF receptor mRNA expressions were significantly increased, whereas IGFBP-3 expression was decreased in the glucocorticoid treated group. Lung IGFBP-1 mRNA was increased in the treated group, while IGFBP-2, type 1 IGF receptor and IGF-I expression was unchanged. Serum IGFBP-1 was increased in the treatment group. The authors conclude that decreased IGF bioactivity through the increased IGFBP levels may be an important factor in the etiology of IUGR in this model.

Inhibitors of 11 β-hydroxysteroid dehydrogenase
Benediktsson et al. (1993) found that rat placental 11 β-HSD is positively correlated with term fetal weight but negatively correlated with placental weight. Langley-Evans (1997) has studied birth weight and blood pressure in offspring from mothers treated with carbenoxolone, an inhibitor of 11 β-HSD, and compared them to offspring from mothers fed a protein-replete diet. Pregnant rats were fed control (18% casein) or low protein (6% casein) diet throughout gestation. Half of the animals fed with control diet were injected with carbenoxolone. Injections were administered either throughout pregnancy (day 0-22), or targeted to specific periods in early (days 0-7), mid- (days 8-14) or late (days 15-22) gestation. Fetuses from mothers fed a low protein diet had a reduced birth weight and at 4 weeks of age significantly elevated systolic blood pressure compared to the control fed group. These hypertensive animals had small kidneys in proportion to body weight. Fetuses from control fed mothers treated with carbenoxolone during gestation had lower body weight at birth regardless of time or length of treatment. These IUGR fetuses had a higher systolic blood pressure at 4 weeks of age than control animals. The greatest elevation of blood pressure was associated with carbenoxolone treatment in late (days 15-22) gestation. None of the animals with carbenoxolone

induced hypertension had any evidence of retarded renal growth. These results imply that increased fetal exposure to maternal glucocorticoids impairs fetal growth and programmes elevated blood pressure in later life.

Dihydroergotamine

Dihydroergotamine is a vasoactive drug, which is widely used to treat postural hypotension in both pregnant and nonpregnant women. Hohmann and Kunzel (1992) dosed pregnant guinea pigs with 14 µg/kg/day dihydroergotamine from day 30 – 60 of pregnancy. Fetal weights were significantly reduced (21%) in the group treated with dihydroergotamine compared to the control group. They hypothesised that dihydroergotamine might have a variable effect on the arterial constrictor activity in different vascular beds, including the placenta, which then effects fetal growth.

A very large number of drug induced IUGR models is conceivable. The models presented were chosen because of their clinical relevance. However, most other drug induced IUGR models represent a direct toxic effect of the drug, and are therefore very limited in their use as a model in a more general sense to investigate the effect and mechanism of IUGR.

Conclusion

A number of IUGR models in rodents have been reviewed in this paper. These models make it possible to investigate different phenotypes of IUGR in rodents, which more or less resemble the human phenotypes, such as differential growth restriction, alteration in liver, kidney and placenta growth, delayed catch-up growth, and different aspects of syndrome X, such as elevated blood pressure, hyperinsulinemia, insulin resistance, and dyslipidemia. These rodent models permit detailed studies of many aspects of IUGR, which are inaccessible through direct study of human pregnancy. However, necessary caution must be used when extrapolating from rodent models of IUGR to the growth restricted human infant. The rat for example has a relatively short gestation, and many important developmental events occur postnatally in rats that are part of fetal development in humans. This is one of the reasons

why some investigators use the guinea pig, which also gives birth to a more developed fetus. Nevertheless, the existing animal models have already given valuable insights into IUGR in man, and are currently the only workable instrument to increase our knowledge.

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