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Fetal Growth Restriction: a Workshop Report☆

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Intrauterine growth restriction (IUGR) is associated with significantly increased perinatal morbidity and mortality as well as cardiovascular disease and glucose intolerance in adult life. A number of disorders from genetic to metabolic, vascular, coagulative, autoimmune, as well as infectious, can influence fetal growth by damaging the placenta, leading to IUGR as a result of many possible fetal, placental and maternal disorders. Strict definitions of IUGR and of its severity are needed in order to eventually distinguish among different phenotypes, such as gestational age at onset, degree of growth restriction and presence of hypoxia.

This report explores and reviews some of the most recent developments in both clinical and basic research on intrauterine growth restriction, by seeking mechanisms that involve genetic factors, utero-placental nutrient availability and vascular growth factors.

New exciting findings on the genomic imprinting defects potentially associated with IUGR, and the placental anomalies associated with the decreased nutrient transport are summarized. Moreover, recent data on angiogenic growth factors as well as new information arising from application of gene chip technologies are discussed.
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INTRODUCTION

Intrauterine growth restriction (IUGR) presents a complex management problem for the clinician. The failure of a fetus to achieve its genetically determined growth potential is associated with significantly increased perinatal morbidity and mortality [1] as well as being a major determinant of cardiovascular disease and glucose intolerance in adult life [2].

IUGR is not a specific disease entity with a unique pathophysiology, but the result of suboptimal intrauterine growth conditions in conjunction with a variety of disorders from genetic to metabolic, vascular, coagulative, autoimmune, as well as infectious. New challenging fields, like genomic imprinting defects potentially involved in regulation of fetal and placental growth, or trombophilic diseases potentially damaging the placenta are now being explored and may represent additional causes of IUGR.

Understanding the etiology of IUGR in each specific pregnancy is the basis for the clinical management of that patient. Fetal growth is a complex process regulated by genetic factors, utero-placental nutrient availability and hormones. However, independently from the underlying cause, a fetus that fails to achieve its full growth potential has, by definition, undergone a process of reduced tissue deposition due to a reduced nutritional supply from the utero-placental circulation (Figure 1). While the knowledge of IUGR resulting from a shallow trophoblast invasion during the first trimester, together with Doppler studies showing changes in the utero-placental circulation, is widespread among both clinicians and scientists, the discoveries that the nutrient supply can also be decreased by changes in the placental transport properties is not so well known. Moreover, recent studies in a number of mouse knockout and transgenic models have allowed to link defects in

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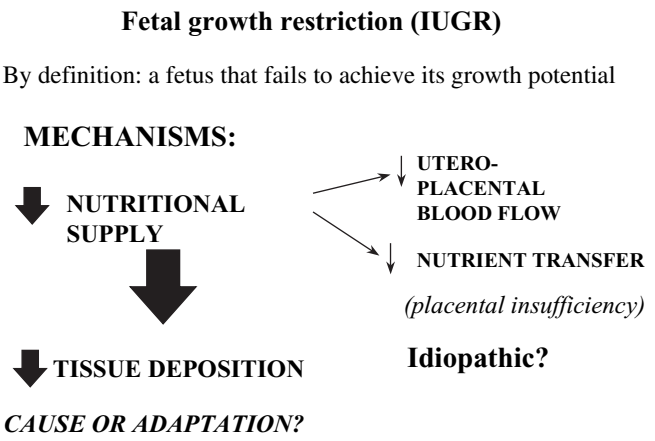

Figure 1. Mechanisms for reduced tissue deposition in fetal growth restriction.

imprinted genes to changes in specific transport systems [3]. However, the lessons of the murine experiments need to be compared to results obtained in human pregnancies. We will briefly review some of the most recent developments in both clinical and basic research on intrauterine growth restriction. In particular, we will describe advances in clinical understanding of the natural history of IUGR, the genomic imprinting defects potentially associated with IUGR, and the placental anomalies possibly responsible for the decreased nutrient transport. We will also summarize new results demonstrating involvement of angiogenic growth factors in the pathogenesis of preeclampsia and IUGR.

CRITERIA FOR DIAGNOSIS AND EVALUATION OF SEVERITY OF IUGR

There is still considerable debate as to how IUGR should be defined. The most commonly used definition is based on a birth weight below the 10th percentile. However, a significant number of small-for-gestational age babies (SGA) is not truly growth restricted and is instead part of the normal variation in the birth weight distribution. Therefore, we should include the evaluation of growth in utero, for example as a defined shift in centiles on a standard growth curve of the routine ultrasound measurements in the definition of a population of truly growth-restricted fetuses. However, in large population studies, in utero diagnosis might not be available, and a population of SGA is often utilized. This is acceptable as long as explained and recognized as a study limitation.

Assessment of intrauterine growth is particularly relevant for diagnosis of IUGR early in gestation. Diagnosis of IUGR during pregnancy before 32 weeks of gestation might result in babies having a weight over the 10th percentile at birth. There are two potential reasons for this discrepancy. 1. In conventional birth weight curves, the assumption that the weight-for-gestation profile of babies born prematurely represents that of the healthy babies who continue to grow in utero might be incorrect [4]. 2. Alterations in growth occurring early in gestation might lead to a very sick baby before the weight falls below the 10th centile.

This again underlines the need for endpoints that allow for definitions of severity. There are three main general determinants of severity: 1. gestational age, 2. degree of nutritional deprivation, and 3. degree of oxygenation.

All these parameters are associated with short- and long-term adverse outcome. However, the prognosis for neurological function is strictly related to oxygenation and adequate timing of delivery. Not all IUGR fetuses are subjected to a reduced oxygen supply [5]. Fetuses that exhibit normal umbilical arterial blood flows evaluated by Doppler blood velocimetry and normal fetal heart rate are indeed associated with normal oxygenation [5]. Recently, a temporal sequence of early and late Doppler velocimetry alterations which occur in a subset of early and severe IUGR fetuses has been described [6]. Abnormalities of the pulsatility index of the middle cerebral artery and of the umbilical artery (absent end diastolic flow) represent early changes that precede blood flow abnormalities of the ductus venosus, reverse end-diastolic flow of the umbilical artery, decreased pulmonary artery peak velocity, reverse flow in the ductus venosus and, lastly, decreased aortic peak velocity, which represent "late" changes, associated with an increased risk of perinatal death [6].

GENOMIC IMPRINTING DEFECTS AND IUGR

There is a strong association between abnormal fetal karyotype, Mendelian single gene disorders, uniparental disomies (UPDs) and IUGR. Fetal genetic changes include trisomies of chromosomes 13, 18 and 21, microdeletions, confined placental mosaicism and more than 50 inherited single gene disorders. The risks associated with UPDs include genetic dysfunction if the chromosomal segment involved harbours imprinted genes. IUGR is the most common feature, a phenotype shared among matUPD7, matUPD14, patUPD6q24 and matUPD20. The fetal growth disturbance in UPD carriers is consistent with the action of imprinted genes during fetal growth, as recently reviewed [7]. In murine models, most of the 60 recognized imprinted genes are expressed during fetal life and, in particular, in the placenta, in which they may regulate fetal demand and maternal supply of nutrients. This peculiar mechanism of growth regulation has been demonstrated for Igf2 in mice lacking specific Igf2 placental transcript [3].

There are many mouse models in which targeted candidate gene mutations result in compromised placental function and IUGR, although the pathological effects differ among the investigated genes. ESX1 mutants show changes of vascularization in the placental labyrinth [8], Igf2 null mice have an inefficient placental transport [3], and p57/Kip2-deficient mice present changes of labyrinth and spongiotrophoblast [9]. Data from murine models strongly indicate that IUGR cannot be considered a disease per se, rather a manifestation of many possible fetal gene defects that primarily affect placental development and function. The reverse approach (from phenotype

to gene defect) aimed to demonstrate that genetic lesions in human IUGR fetuses are complicated by the heterogeneity of predisposing causes. A useful approach could be to segregate the IUGR cases in different sub-phenotypes, as recently suggested by Cross [10] for human preeclampsia. In this view it could be helpful to evaluate the severity of IUGR [5], placental morphology and additional pregnancy complications such as preeclampsia. Finally, in order to focus on possible genetic causes of IUGR it is important to take into account and investigate a familial aggregation of IUGR by assessing a detailed pedigree analysis.

PLACENTAL TRANSPORT

In the human placenta, it is the two plasma membranes of the syncytiotrophoblast that represent the primary barrier to transport of glucose, amino acids and most ions. Consequently, studies of isolated microvillous (MVM) and basal plasma membranes (BM) may provide valuable information concerning transplacental transport processes. In MVM isolated from small-for gestational age babies the activity of the amino acid transporter system A was shown to be markedly reduced [11]. Intrauterine growth restriction is also associated with a reduced activity/expression of placental transport systems for essential amino acids, such as taurine, leucine and cationic amino acids. In contrast to the effect of IUGR on placental amino acid transport the expression and activity of glucose transporters is unaltered in IUGR [12].

Placental ion transporters also appear to be altered in IUGR. For example, the activity and protein expression of the Na^+/K^+ ATPase in the MVM, but not BM, is reduced in the IUGR placenta [13]. It is possible that this change may impair the ability of the syncytiotrophoblast to maintain a low intracellular Na^+ concentration, thereby affecting all Na^+-coupled transport processes. Furthermore, the activity and protein expression of the Na^+/H^+ exchanger in MVM, the primary mechanism for syncytiotrophoblast pH regulation, is diminished in preterm, but not in term IUGR [14]. It is speculated that these alterations may contribute to the development of acidosis in the IUGR fetus delivered preterm. Interestingly, the activity of the Ca^{2+} ATPase in BM has been shown to be markedly increased in association with IUGR [15].

Collectively, these in vitro data clearly suggest that IUGR is associated with a number of changes in the activity and/or expression of placental nutrient and ion transporters. These alterations may be present also in vivo as shown by the recent demonstration of decreased transplacental transport of leucine in human pregnancies complicated by IUGR [16].

The changes observed in IUGR are likely to be specific and not secondary to a general pathological process such as altered membrane composition. Some of the observed changes, for example, the reduction in amino acid transporter activity, may be "primary" events in the development of IUGR whereas other alterations, e.g., increased Ca^{2+} ATPase activity, may be secondary to restricted fetal growth. There are also new possibilities emerging as the molecular characterization of nutrient transporters progresses. For both glucose and amino acid transporters the concept of a single transporter is being displaced as multiple isoforms of a transporter are defined. If these isoforms have different properties, altered isoform ratios or distribution may have significant effects on activity in vivo. For example the system L amino acids transporter has two isoforms; the first, LAT-1, is an exchange transporter [17], while there are reports that the second, LAT-2, is a uniporter [18]. Changes in the isoform ratio might have significant effects on transport without apparent changes in overall transporter expression.

It is speculated that the placenta functions as a "nutritional sensor" and responds to changes in maternal nutrition or placental blood flow by altering the activity and/or expression of placental nutrient transporters, thereby regulating fetal growth. There is however an equally powerful argument to be made that the fetus, as the "end-user" of nutrients transported across the placenta, may also act as the nutritional sensor through output of hormones controlling fetal (and possibly placental) growth by circulating nutrient concentrations.

EXPRESSION OF VEGF, PlGF AND THEIR RECEPTORS IN PREGNANCIES COMPLICATED BY PREECLAMPSIA AND IUGR

Several growth factors such as vascular endothelial growth factor (VEGF-A) and placental growth factor (PlGF) are involved in placental vascular development. Therefore, it is of interest to understand whether dysregulation in the VEGF family may be associated with preeclampsia and IUGR. Recent studies show evidence for a significant deregulation of the expression of VEGF, PlGF and their receptors in the placenta of severe early onset preeclampsia or IUGR [19].

VEGF-A, PlGF and VEGFR-1 that are produced by villous and extravillous trophoblast cells are significantly upregulated in placentas from preeclamptic women and from cases with IUGR [19,20]. It seems that during pregnancy most of the circulating VEGF is bound to sVEGFR-1 produced in high amounts by the placenta [19–21]. In preeclampsia, placental production of VEGFR-1 and sVEGFR-1 is likely increased because of low oxygen levels in the placenta. The massive increase of sVEGFR-1 in preeclamptic women results in low levels of circulating free VEGF and free PlGF.

Results from the placental bed biopsies show that VEGFR-1 expression is significantly reduced in cases with preeclampsia. Low levels of VEGFR-1 in the placental bed may explain the defective uterine vascularization frequently associated with early onset preeclampsia. Maynard et al. [20] have shown in a rodent model that VEGF, PlGF and sVEGFR-1 are likely to play a role in the pathogenesis of preeclampsia. Excess placental production of sVEGFR-1 contributes to hypertension, proteinuria and glomerular endotheliosis.

In conclusion, these studies indicate the involvement of sVEGFR-1 as an antagonist to VEGF and PlGF in

preeclampsia. The downregulation of the membrane bound form of VEGFR-1 in the placental bed may also result in the decreased maternal vascular adaptation to pregnancy.

NEW DEVELOPMENTS OF THE GENE CHIP STUDIES APPROACH

New areas, like gene chip studies fishing for new genes, are exploring still unknown possibilities. These preliminary studies reveal that genes involved in lipid metabolism are being differentially upregulated in the growth-restricted group. Moreover, few other genes that have not been associated with IUGR before have been found to have expression levels that are markedly different in IUGR.

Aberrant gene expression in the placenta of IUGR pregnancies has been investigated on pooled RNA purified from tissue biopsies taken at term [22]. The expression profiles have been analysed by microarrays (Human Genome U133A, Affimetrix) and the expression levels of a selection of genes verified by real-time PCR. A number of genes implicated in lipid metabolism were differentially expressed in IUGR placentas. Among these, lipoprotein receptors (VLDL-ApoE), lipoprotein lipase (LPL), fatty acid-binding protein 4 (FABP4) and intracellular lipase activities are involved in the transport of fatty acids from the maternal circulation across the placenta. These changes might therefore modify the placental delivery of fatty acids to the fetus in a critical period when fat is deposited exponentially. This is in agreement with the different profile of polyunsaturated fatty acids that has been reported in IUGR [23].

sVEGFR has also been found to be upregulated in cases of IUGR. Soluble VEGFR has a higher affinity for VEGF than VEGFR and it has been hypothesized that it may function as an inhibitor of the cellular VEGF response [24]. This study also identified caveolins as potential factors implicated in IUGR.

Further investigations are needed to detail the significance and the role of the differentially expressed genes identified by the array analysis. In particular, two main issues are under discussion: technical relevance and placental function. The first issue challenges the reproducibility, sensitivity and accuracy of microarray results. One major concern is the variability of tissue biopsies, which is intrinsic to tissue heterogeneity but also results from sampling procedures. This is particularly relevant for the human placenta at term because of its size and composite structure. Hence, multiple variables have to be taken into account when comparing microarray results either from intra-experiments as well as results from different investigators. Phenotype classification and analytical strategy should also be taken into account since they determine the final number of genes eligible for comparison, a variable that happens to vary tremendously (from less than ten to several hundred genes) depending on investigators, experimental settings and procedures.

The second main issue is regarding the functional significance of the genes whose expression is reported to be modified in placenta of growth-restricted fetuses. Three of these genes are involved in the transport of fatty acids from the maternal circulation across the placenta. Since the changes suggest an increased supply of fatty acids to the fetus in spite of low fetal adiposity and despite results in vivo demonstrating reduced feto-maternal relationships for the long-chain polyunsaturated fatty acids [23], the question arises whether this could represent an adaptation of placental transfer function to compensate for poor fetal growth. Whether compensatory mechanisms should be regarded as tentative explanations when experimental results are contrary to physiological/biochemical expectations is a matter of debate.

CONCLUSIONS

In the last decades, thanks to new technologies like ultrasound and fetal blood sampling we have learned a lot more about development of the fetus and abnormalities of its growth trajectory leading to IUGR. Moreover, the placental mechanisms involved in this pathology are starting to be understood. However, although this progress has led to improvements in diagnosis and understanding, we are still in the situation of no reasonable therapy during pregnancy, optimal timing of delivery still representing the only available intervention in order to reduce adverse pregnancy outcomes. Further efforts are needed to utilize this body of new information in the development of novel therapeutic strategies.

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