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SYMPOSIUM ON 'NUTRITION AND EARLY DEVELOPMENT'

Nutrition of the conceptus: aspects of its regulation

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Viewed simplistically, nutrition of the developing conceptus can be seen as an added cost on the nutrient requirements of the pregnant animal. However, although the additional nutritional cost for maintenance and tissue accretion in the conceptus represents a moderately-small toll on the nutritional economy of the mother for much of gestation, provision of adequate nutrients to support growth of the conceptus is clearly more complex and involves considerable adaptive responses on the part of the mother throughout gestation. Without doubt maternal nutritional state itself affects growth of the developing conceptus. Severe maternal undernutrition at all stages of gestation, but particularly in late pregnancy, significantly reduces fetal growth and the birth weight of infants (Rosso, 1981; Parr *et al.* 1982; Mellor, 1983; Robinson, 1983). Even where nutrition is apparently not seriously inadequate, it is evident that substantial utilization of maternal tissue protein and fat stores occurs to support anabolic activity within the conceptus (Robinson *et al.* 1980).

Of the adaptive responses in the mother, probably the most important are those concerned with ensuring adequate blood and nutrient delivery to the maternal–fetal interface in the placenta. Haemodynamic changes resulting in substantially enlarged blood volume, increased cardiac output and redistribution of blood to the uteroplacenta are well recognized (Rosenfeld, 1984), as are the endocrine and metabolic changes which permit diversion of substrates, particularly glucose, from maternal tissues to the uterus for transport to the developing conceptus (Freinkel & Metzger, 1979; Kalkhoff & Kim, 1979; Freinkel, 1980). Effects of severe maternal undernutrition on the haemodynamic changes are also well recognized (Morriss *et al.* 1980; Rosso & Kava, 1980; Ahokas *et al.* 1983, 1984).

Knowledge of the quantitative nutritional requirements of the developing conceptus has been greatly advanced by the work of Battaglia and associates (Battaglia & Meschia, 1978; Sparks *et al.* 1983; Battaglia, 1984). However, their interest has been focused primarily on the nutritional physiology of the fetal body separate from that of the placenta, rather than to that of the conceptus as a whole, because of the ready morphological identification of the developing fetus and its relatively easy physical isolation from the extra-corporeal tissue and fluid compartments of the conceptus for experimental observations (Battaglia & Meschia, 1978; Sparks *et al.* 1983; Battaglia, 1984). This is despite the fact that 45–50% of the combined ventricular output from the fetal heart is supplied to these extra-corporeal structures (Mott & Walker, 1983), the very high rate of oxidative metabolism in placental tissue (Meschia *et al.* 1980) and the total dependence of fetal somatic tissues on the placenta for nutrient and gaseous exchange. Accordingly, a principal purpose of the present paper is to re-emphasize the nutritional and developmental integration of extra-corporeal and somatic tissues in the conceptus, rather than their separation.

Nutrition and the relative growth of tissues in the conceptus

Studies on a wide range of species have directed attention to the close relation between placental and fetal size (Alexander, 1978; Fletcher *et al.* 1982; Mellor, 1983; Michael *et al.* 1983) and to the consequences of restricted placental development on subsequent growth of fetal somatic tissues (Mellor, 1983). There are also dramatically different effects of nutritional restriction on the growth of individual tissues within the fetus (Lafeber *et al.* 1979; Jones & Robinson, 1979). More recently, attention has been focused on the close relation between placental size and maternal blood flow to the placenta, especially in small polytoccus species (Duncan & Lewis, 1969; Bruce & Abdul-Karim, 1973; Myers *et al.* 1982; Jones & Parer, 1983), leading to considerable speculation that maternal effects on placental size directly determine fetal growth. However, though it may be correct that placental size limits future growth of the conceptus through its limitation of the area for exchange with maternal blood, it should not be concluded that regulation of placental growth is outside the control of the conceptus. Observations in our laboratory (J. M. Bassett, J. M. Fletcher, D. Grimwade and T. Warner, unpublished results) on the size, relative to fetal body-weight, of the placenta and other organs in individual fetal rabbits at 22–30 d gestation suggest that growth of the placenta is co-ordinated with that of other organs and tissues in the conceptus and is determined by nutrient availability to the conceptus as a whole. The change in placental weight relative to body-weight, within age-groups, is greater than the change in any other tissue examined especially between 22 and 26 d gestation, whereas the relative change in brain weight with change in body-weight is less than that of any other tissues.

These observations seem to imply that brain growth in size during this period of intrauterine life is highly conserved and influenced far less by nutrient availability than is the growth of other organs, perhaps because of the preferential streaming of

Table 1. *Regression coefficients for the relation of organ weights to body-weight in individual fetal rabbits* during the last week of gestation*

(Organ weights in individual fetuses were expressed as a percentage of the mean weight for the organ in all fetuses at 30 d gestation before calculation of regression coefficients and are mean values with their standard errors)

		Fetal placenta		Liver		Carcass		Heart		Kidney		Brain	
Gestational age (d)	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
22	28	10·36	2·21	5·21	0·62	2·58	0·02	1·80	0·77	2·16	0·46	1·20	0·83
26	18	5·02	0·65	4·14	0·49	2·22	0·09	2·44	0·52	2·94	0·45	1·25	0·27
30	81	2·84	0·22	3·42	0·14	2·28	0·06	2·26	0·17	2·86	0·16	0·96	0·11
Pooled values†	197	1·52	0·08	2·44	0·06	2·35	0·01	2·28	0·04	2·56	0·04	1·70	0·04

*From Fletcher *et al.* (1982) and J. M. Bassett, J. M. Fletcher, D. Grimwade and T. Warner, unpublished results.

†Includes observations on twenty-two fetuses at 24 d and forty-nine fetuses at 28 d gestation as well as values for groups given here.

ductus venosus blood to this organ (Edelstone & Rudolph, 1979), whilst growth of the placenta is extremely susceptible to such variation, despite the consequences for future nutrition of the conceptus. Interestingly, fetal liver weight also varied far more relative to body-weight than did that of heart, kidneys or carcass. The slopes of the relations between body-weight and placental or liver weight, within age-groups, were much greater than the slopes of the pooled relation ignoring age (Table 1), suggesting that age is not an important determinant of their growth, whereas the converse is true in the case of the fetal brain. Over a wide range of fetal size in each age-group, the results demonstrate a substantial degree of consistency in the size of organs relative to total body-weight and suggest that the marked differences among organs noted in studies of intrauterine growth retardation (Jones & Robinson, 1979; Lafeber *et al.* 1979) largely reflect differences in their responsiveness to restricted nutrient availability. Observations on fetal pigs at a range of fetal ages demonstrate relations between placental and body-weights which are remarkably similar to those observed in the rabbit (Michael *et al.* 1983).

Our observations have also shown a close positive association at each of these ages of fetal plasma insulin with both fetal body and placental weights (Fletcher *et al.* 1982). Although such correlations cannot be taken to imply causality, they do suggest endocrine integration of tissue responses to varying nutrient availability. It is perhaps significant that large insulin receptor populations have been reported on both fetal liver and placenta (Neufeld *et al.* 1980; Potau *et al.* 1981) and it has been shown that injections or infusions of insulin into fetal monkeys (Susa *et al.* 1979) or rabbits (Bassett & Fletcher, 1982) increase placental weight. Insulin also increases protein synthesis in this tissue (Horn *et al.* 1983). The involvement of endocrine systems in growth regulation during prenatal undernutrition has been discussed in more detail elsewhere (Jones & Robinson, 1979; Bassett & Fletcher, 1982).

While maternal blood flow to the placenta appears to be determined by placental size (Myers *et al.* 1982; Jones & Parer, 1983), the nature of the signal (presumably generated by the conceptus) regulating maternal blood flow remains unknown. If its production is related to placental size and metabolic activity, it is evident that greater nutrient uptake by larger placentas will, through a positive feedback effect within the conceptus, tend to increase placental growth, perfusion and nutrient uptake further and so improve the competitive position of the conceptus. However, if there is control by the conceptus of maternal flow to each placenta or placentome (the compensatory increases in fetal coteledonary tissue attached to each maternal caruncle in multiple pregnancies or after surgical reduction in caruncle number in sheep (Alexander, 1978; Mellor, 1983) suggest this) it must also be envisaged that this placental tissue is nourished and regulated by the umbilical arterial vasculature and not directly by nutrients traversing the placenta from the mother.

The source of nutrients used by the placental tissue of the conceptus

The source of nutrients used by placental tissue is also a question of more general relevance, for tracer studies of metabolite kinetics in the conceptus are

dependent on the assumption that all tissues use material from the central well-mixed pool of the metabolite in the conceptus. Tracer methods appear ideally suited to quantitative definition of nutrient utilization in the conceptus and measurement of fluxes between mother and conceptus because of the impossibility of access to the connections between them. However, such methods would be invalidated if there were extensive utilization of metabolites in transit from the maternal pool before mixing with the central pool of the conceptus. Despite this, radiotracer methods have been widely used without such validation in attempts to quantify glucose, lactate and amino acid metabolism within the conceptus.

Fortunately, although both lactate and fructose are important products of placental glucose metabolism in sheep, the permeability of the ovine placenta to both is very limited. Consequently, the source of glucose utilized by maternal and 'fetal' portions of the placenta can be determined by measurement of ^{14}C:^{3}H in these metabolites under steady-state conditions during infusion of [^{3}H]glucose and [^{14}C]glucose to mother and fetus respectively. Determination in our laboratory of ^{14}C:^{3}H in fetal plasma lactate and fructose shows that they are statistically indistinguishable from that of fetal plasma glucose in the same fetus during dual-label glucose infusion experiments on both fed and fasted ewes (Bassett *et al.* 1985). Ratios in fetuses not infused with labelled glucose were identical to those in maternal glucose and differed greatly from those in the infused fetus (Table 2). This implies that glucose utilized for metabolic activity within the 'fetal' placenta

Table 2. *Mean steady-state* ^{14}C:^{3}H in maternal plasma glucose and in fetal plasma glucose, lactate and fructose during intravenous infusion of [6-^{3}H]glucose into the ewe and [U-^{14}C]glucose into one fetus (modified from Bassett* et al. 1985)

(Mean values and standard deviations)

	Glucose			Lactate			Fructose		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Fetal plasma:									
[U-^{14}C]glucose-infused									
Fed†	1·30	0·47	14	1·18NS	0·57	9	1·24NS	0·43	14
Fasted	1·08	0·53	6	0·83NS	0·27	5	0·98NS	0·53	6
Uninfused twin									
Fed	0·12	0·05	8	0·11	0·06	8	0·16	0·11	8
Fasted	0·15	0·06	5	0·17	0·10	5	0·15	0·06	5
Maternal plasma:									
[6-^{3}H]glucose-infused									
Fed	0·15	0·05	14		—			—	
Fasted	0·14	0·1	6		—			—	

NS, not significantly different from value for glucose (paired *t* test).

*The value for each experiment was the mean of six samples 90–240 min after starting the primed infusion of glucose.

†Studies were carried out 12–18 h after offering food (fed) or after a 48 h fast following undernutrition for 3 d (fasted).

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comes from the umbilical arterial blood perfusing the tissue and that maternal glucose taken up by the placenta enters the venous drainage to mix with glucose in the central vascular compartment of the conceptus before significant utilization.

Mother and conceptus can therefore be considered as isolable, but interconnected systems for purposes of modelling glucose kinetics. Dual-labelling techniques together with two-pool models or their derivatives can therefore be used for measurement of glucose utilization by mother and conceptus as well as fluxes between them, subject to the constraints set by recycling of tracers, but without blood flow measurements or the need for isolation of a third uteroplacental compartment as proposed by Hay *et al.* (1984).

Since placenta and membranes are important contributors to overall glucose consumption within the conceptus their weights should be taken into account in calculations of utilization on a tissue-weight basis, although there may be problems in doing so because of the difficulty of separating maternal and fetal placental tissue in the cotyledons (Alexander, 1978). Use of the term conceptus, rather than fetus, is also desirable as ambiguity on this score has already led to confusion in the literature in comparisons among studies (Hay *et al.* 1981). Despite arguments against this approach (Hay *et al.* 1981), Hay *et al.* (1984) and Jones *et al.* (1983) have recently used it to demonstrate extensive use by the placenta of glucose from the vascular pool of the fetus.

Recognition that 'fetal' placental tissue uses glucose from the umbilical circulation, rather than glucose in transit from the mother, should improve definition of the tissues responsible for the high rate of uteroplacental glucose consumption (Meschia *et al.* 1980; Sparks *et al.* 1983). Detailed comparison of the values reported by Sparks *et al.* (1983) for uteroplacental and fetal glucose consumption with values for glucose consumption calculated in other studies on the basis of a two-pool model (Anand *et al.* 1979; Hodgson *et al.* 1980) is not possible because of the variability of the values and differences in indices reported. However, since the ovine placenta is only poorly permeable to lactate, the finding that 60% of placental lactate production is transferred to the fetus (Sparks *et al.* 1983) suggests that half or more of the glucose consumed by the uteroplacenta may be metabolized by the fetal portion of the tissue, assuming that the fraction converted to lactate is similar on the two sides of the placenta.

More importantly though, our finding (Bassett *et al.* 1985) shows that the lactate transferred to the fetus from the placenta does not represent an exogenous source of lactate as generally stated (Battaglia & Meschia, 1978; Meschia *et al.* 1980; Sparks *et al.* 1982), but reflects the operation of a substrate cycle within the conceptus between the placenta and body of the fetus. The conversion of lactate to glucose within the conceptus remains controversial:lactate is probably oxidized rather than converted to glucose (Hay *et al.* 1983) and the liver, which apparently oxidizes virtually no glucose, is a potential site for this (Bristow *et al.* 1983). Other similar substrate cycles between the placenta and fetal body may also exist. Indeed, it has been suggested (Bassett, 1980) that the uptake of glutamate by the placenta might reflect the operation of an inter-organ substrate cycle rather than a means of

excreting ammonia as proposed by Lemons *et al.* (1976). The recent reports on placental handling of glutamate and glutamine (Pell *et al.* 1983*a*,*b*) support this suggestion.

The recognition that the placental tissue of the conceptus uses glucose from its own central pool, and not glucose in transit from the mother, suggests that the umbilical arterial blood is the source of all its metabolic substrates and possibly oxygen too. This view is entirely consistent with the views expressed earlier that placental growth in size is determined centrally within the conceptus and that the fetal placental tissue determines in some way the maternal vascular supply to the tissue.

This conclusion and its implications are also important for the validation of tracer studies on metabolites other than glucose. Similar or related 'two-pool' models have been used for assessment of lactate and amino acid metabolism in the 'fetus' without any apparent account having been taken of the metabolic activity of the placenta and other membrane structures of the conceptus or their interrelations with the fetal body (Kitts & Krishnamurti, 1982; Schaefer & Krishnamurti, 1982, 1984).

Nutrient exchange between amniotic fluid and fetal blood

In most reported studies of metabolite turnover in the conceptus, little or no account has been taken of the equilibration of tracers with the large fluid compartments bounded by the amnion and allantoic membranes. In the case of glucose this may matter little for studies on fetal sheep, as the glucose concentration in amniotic fluid is very low during the latter part of gestation. Amniotic concentrations of lactate and amino acids, by contrast, are not negligible in the sheep (Mellor & Slater, 1971, 1972) so the turnover of metabolites in these compartments cannot be ignored. However, while this question has not been examined in relation to quantification of fetal lactate or amino acid utilization, the amniotic fluid has received some attention as a possible route for nutritional supplementation of the fetus (Charlton, 1984*a*,*b*). Observations on both women and sheep show that 'nutrient' quantities of glucose and amino acids added to the amniotic fluid disappear rapidly: indeed when a 20 g dose of either glucose or a mixture of amino acids is added, substantial increases in fetal plasma glucose or α-amino-nitrogen concentrations are observed (Charlton, 1984*b*); these occur far too rapidly to be explained by fetal swallowing and absorption from the gut. Studies in our own laboratory (J. M. Bassett, A. H. Burks and D. H. Levine unpublished results) have confirmed that labelled glucose, glutamine and cysteine injected into the amniotic fluid appear in the plasma of the ovine conceptus within 30 min of injection. The rate of removal of labelled glucose or 1 g glucose loads from the amniotic fluid (half time ($t_{\frac{1}{2}}$) 185 (SE 32) min, n 16) was considerably faster than that for labelled glutamine or 1 g glutamine loads ($t_{\frac{1}{2}}$ 514 (SE 142) min, n 11).

Half-times for disappearance of [^{35}S]cysteine radioactivity from the amniotic fluid were also substantially longer than those for glucose and plateau

concentrations in fetal plasma were reached more slowly. Nevertheless, large amounts of cysteine radioactivity were incorporated into the perchloric-acid-precipitable fraction of plasma and wool from injected fetuses. Cysteine radioactivity was also found at post-mortem to have accumulated in allantoic fluid. On the other hand, continuous infusion into the amniotic fluid of L-amino acid mixtures, providing the amounts reported by Lemons *et al.* (1976) to be taken up across the umbilical circulation daily, failed to alter plasma α-amino-acid-N concentration in five fetal sheep (values were 9·30 (SE 0·26) *v.* 9·93 (SE 0·49) mmol/l respectively during control and 4 d infusion periods) and had no consistent effects on fetal plasma insulin or glucagon concentrations, even though amniotic fluid concentrations increased from 8·18 (SE 0·55) to 55·8 (range 39·3–87·8) mmol α-amino-acid-N/l. Studies by Charlton (1984*a*,*b*) indicate that nutritionally significant amounts of amino acids can be administered to fetal sheep by either the amniotic or gastric routes. Our observations are not entirely consistent with this view. They do, however, indicate that significant exchange of amino acids takes place between the plasma and amniotic fluid pools of the ovine conceptus with equilibrium between them taking many hours to establish.

Concluding remarks

Advances in chronic cannulation procedures have permitted considerable advances in knowledge about the quantitative nutrition of the conceptus and its regulation in utero. However, the extreme difficulty of quantifying metabolite and gaseous transfer between mother and conceptus at the placental interface has tended to divert attention from the essential integrity of the somatic and extra-corporeal tissues of the developing infant. To study nutritional physiology of the fetus yet ignore that of the placenta is rather like studying the nutrition of eviscerated experimental animals. It is to be hoped that recognition of the interdependence and integration of glucose and amino acid metabolism in the placenta and fetal body will lead to more adequate quantification of their utilization within the developing conceptus. Clearly, lactate should be removed from the balance sheet of nutrients supplied to the conceptus and replaced by glucose, restoring this metabolite to its former position as the major substrate for oxidative metabolism during intrauterine life.

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