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Whole-body fuel selection: 'reproduction'

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Sélection des substrats énergétiques de l'organisme entier: reproduction

RÉSUMÉ

La sélection des substrats énergétiques au cours de la reproduction chez les mammifères a des caractéristiques très différentes de celles qui apparaissent après la naissance. Les oocytes fertilisés et les embryons jusqu'au stade de huit cellules utilisent le pyruvate et passent à l'utilisation du glucose au stade morula ou blastocyte. Au cours des toutes premières divisions cellulaires, le métabolisme de la glutamine fournit également l'énergie cellulaire et protège probablement contre les effets néfastes des radicaux d'oxygène. Après l'élongation du trophoblaste, et pendant l'implantation, aussi bien l'embryon que les membranes extra-embryonnaires sont actifs dans l'utilisation glycolytique et oxydative du glucose. Exprimés par unités de tissu sec, ces processus métaboliques diminuent à mesure que le conceptus se développe. Bien que l'acétate ne soit pas utilisé par les embryons au stade précoce du clivage, il est métabolisé par le trophoblaste, la membrane vitelline et l'allantoïde du conceptus de mouton de 19 jours quand l'incorporation d'acétate-C dans le trophoblaste est double de celle du glucose. La nature quantitativement minime, et pourtant hautement spécifique des besoins des embryons au stade du clivage en nutriments évolue progressivement vers des demandes métaboliques d'ensemble pour la gestation qui montrent une énorme diversité entre les espèces. La généralisation de ces demandes par allométrie donne une dépense d'énergie quotidienne moyenne de 134 kJ/kg de poids de naissance pour la production des nouveau-nés de mammifères. En comparaison avec l'enthalpie de combustion du nouveau-né, ce chiffre est extrêmement élevé et explique les faibles efficacités (à peu près 14%) de l'utilisation de l'énergie métabolisable pour la croissance du conceptus. Les tentatives de répartir l'augmentation de chaleur entre ses sources de production suggèrent que les tissus utéro-placentaires sont un fournisseur majeur à la moitié de la gestation, et même tard dans la gestation ils fournissent encore de 30–50% de l'O_2 total utilisé par l'utérus gravide. L'augmentation de la chaleur non imputée aux tissus foetal et utéro-placentaire est également substantielle, mais on ne connaît pas la quantité fournie par les augmentations du métabolisme des organes maternels, du développement de la glande mammaire et de la production de colostrum, qui sont dues à la gestation. Tard dans la gestation, les taux élevés de consommation de glucose utéro-placentaire nécessaire au maintien de l'état satisfaisant du foetus proviennent de la circulation ombilicale. La concentration de glucose dans le sang du foetus dicte ses taux de transfert

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net par une diffusion facilitée entre le sang de la mère et celui du foetus. A la différence du glucose, les acides aminés sont transportés asymétriquement à travers le placenta par une grande variété de transporteurs. L'absorption ombilicale des acides aminés excède normalement celle requise pour la synthèse des protéines du foetus, et même les acides aminés essentiels sont oxydés par le foetus pour fournir de l'énergie métabolique. Les niveaux d'insuline et d'IGF-1 du foetus sont tous les deux positivement corrélés avec le poids du placenta et du foetus, et régulent l'absorption ombilicale et l'efficacité de l'utilisation des acides aminés par le foetus. A la naissance, la consommation d'O_2 augmente rapidement, et les augmentations concomitantes des catécholamines et des hormones thyroïdiennes stimulent une réponse thermogénique augmentée par une thermogénèse non frissonnante, et chez les espèces dépourvues de tissu adipeux brun, par une thermogénèse frissonnante. Les questions importantes qui restent sans réponse sont: (a) quels sont les mécanismes impliqués dans la programmation embryonnaire nutritionnelle de la croissance du foetus et de sa taille à la naissance? (b) avec une meilleure connaissance de l'absorption et du métabolisme des acides aminés, les acides aminés spécifiques peuvent-ils jouer un rôle pour atténuer le retard de croissance intra-utérin? (c) comment la malnutrition prénatale entraîne-t-elle l'hypertension de l'adulte?

Fuel selection during mammalian reproduction is characterized by features that are quite different from those employed postnatally. From a cluster of undifferentiated dividing cells within the zona pellucida, the recently fertilized mammalian ovum develops, in a matter of a few days, into a blastocyst differentiated into trophectoderm and inner cell mass. In so doing, it exhibits degrees of control over gene expression and cellular differentiation that dwarf those required by adult tissues for functional competence (Rieger, 1992). Development beyond this point is equally complex. Recent studies on the preferences of the embryo during organogenesis and of the rapidly expanding trophoblast during implantation for specific metabolic substrates illustrate this point (Wales & Du, 1993; Wales & Waugh, 1993*a*). Even when organogenesis is complete the requirement for metabolic fuels per unit dry weight of fetus is much higher than later on in pregnancy (Bell, 1992). These examples illustrate the diverse nature of the developmental processes involved in mammalian reproduction and provide a convenient framework for reviewing knowledge on fuel selection from fertilized ovum to newborn.

THE PRE-IMPLANTATION EMBRYO

Recent studies show that exposure of early cleavage-stage sheep and cattle embryos to different culture conditions affects their subsequent fetal growth and, therefore, size at birth (Walker *et al.* 1992; Bishonga *et al.* 1994; Farin *et al.* 1994; Thompson *et al.* 1994). Although the mechanisms involved in this embryonic programming of the fetal growth trajectory are still unknown, the importance of the observation is bound to focus greater emphasis on nutritional factors that alter metabolism and gene expression in the pre-implantation embryo.

Rapid progress is now being made in identifying the substrates and metabolic pathways involved in fuelling the energy requirements of the pre-implantation embryo (Leese, 1991; Rieger, 1992; Hardy, 1993). Although there are species differences some

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broad generalizations can be made. During the early cleavage stages, pyruvate and lactate are major substrates and glucose plays a very minor role. At this early stage glutamine metabolism also provides cellular energy, while at the same time probably acting to protect the embryo from the damaging effects of oxygen radicals. Following the activation of the embryonic genome, glucose utilization increases rapidly and thereafter is essential for normal development. In quantitative terms, glucose uptake by the blastocyst-stage (day 7 for sheep and cattle) embryo is about 500 pmol/d (Leese, 1991), i.e. about thirtyfold greater than that observed at fertilization. Over the same period of development there is a fifteenfold increase in metabolism via the pentose phosphate pathway (Rieger, 1992). Interestingly, glucose-6-phosphate dehydrogenase (*EC* 3.1.3.9), which is the rate-limiting enzyme in the pentose phosphate pathway, is linked to the X-chromosome. Thus, there is a doubling of the activity of this pathway in the glucose metabolism of female embryos compared with male embryos during early embryonic development when both X-chromosomes are expressed. Despite the enhanced activity of this pathway in female embryos, overall glucose metabolism is higher for male embryos than female embryos, a finding consistent with their more rapid rate of development to the blastocyst stage (Avery *et al.* 1991).

THE EMBRYO AND EXTRA-EMBRYONIC MEMBRANES

Wales & Waugh (1993*a*) make a strong case for using the 13–19-d-old sheep conceptus as a suitable model for *in vitro* studies on the preferences of both the embryo and the extra-embryonic membranes for metabolic fuels during the initiation of fetal organogenesis. In comparison with rodent embryos which implant soon after their differentiation into trophectoderm and inner cell mass, the sheep conceptus is still dependent on histotrophic nutrition during organogenesis. Wales & Waugh's (1993*a*) estimates for the amounts of ATP produced by each of the components of the conceptus from the metabolism of glucose are presented in Fig. 1. While trophoblastic energy production from glucose decreased with age, production by the embryo was highest on day 15 and decreased thereafter, perhaps reflecting the need for a major reduction in oxidative metabolism (70% in this study) in order to avoid any deleterious effects on organogenesis which begins at this time.

Clearly absolute values for conceptus substrate utilization obtained from *in vitro* culture may not be truly representative of those pertaining *in vivo*. The dissection of the tissues for culture may disturb the anabolic effects (Simmen *et al.* 1993) of insulin and the insulin-like growth factors (IGF) and may also isolate the conceptus from the uterine cytokines that enhance the production of trophoblastic proteins (Imakawa *et al.* 1993). Indeed, based on values (Wales & Cuneo, 1989) for the rates of RNA and DNA synthesis by 13–19-d-old sheep conceptuses, Wales & Du (1993) concluded that their *in vitro* observations for the contributions of the pentose phosphate pathway to the provision of pentose sugars for nucleotide and nucleic acid synthesis were only 39, 28, 13 and 15% for the embryo, trophoblast, yolk sac and allantois respectively. In attempting to explain these low contributions, Wales & Du (1993) point out that pentose production could have gone undetected in the methods used if embryos, like adult tissues, are able to generate pentose from fructose-6-phosphate and glyceraldehyde-3-phosphate via a reversal of the non-oxidative portion of the pentose phosphate pathway. Whether or not they can is not yet known.

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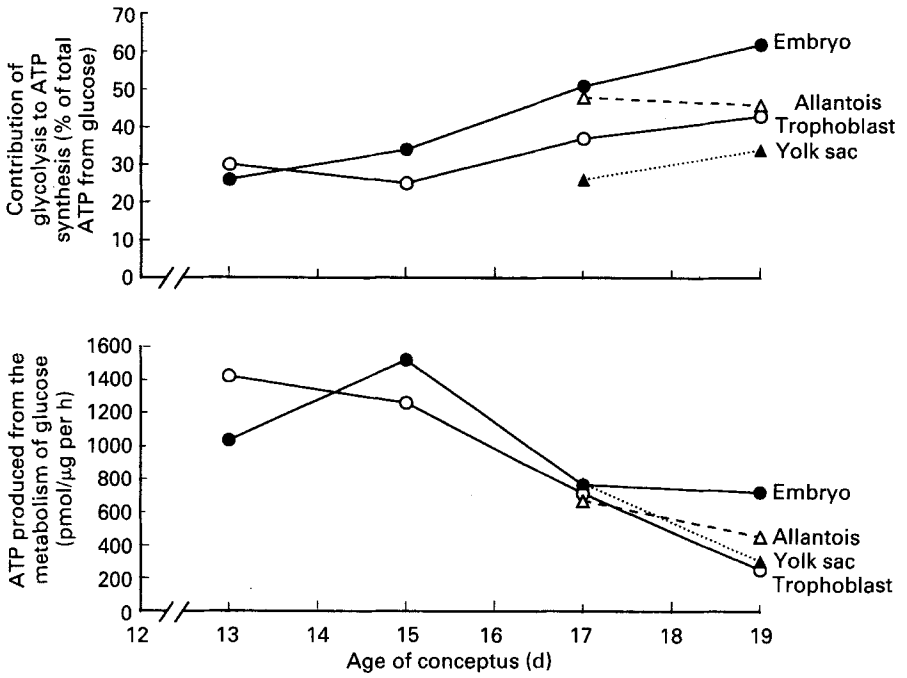

Fig. 1. Estimates of the amounts of ATP synthesized from the metabolism of glucose and the contribution of glycolysis to ATP synthesis in the components of 13–19-d-old sheep conceptuses. (Data from Wales & Waugh, 1993*a*.)

In terms of other metabolic fuels used by the sheep conceptus, Wales & Waugh (1993*b*) and Waugh & Wales (1993) investigated the role of acetate. Although acetate is not used by early-cleavage-stage embryos it is metabolized by the 13–19-d-old sheep conceptus. Relative to the yields of ATP from glucose, those from acetate are very small for the embryo (<4·7% on day 13, falling to <2% by day 19). In contrast values for the trophoblast increase sharply from <1% on days 13 and 15 to 11 and 27% respectively on days 17 and 19. Similar sharp increases in the contribution of ATP from acetate occur for the yolk sac and allantois between days 17 and 19, implying the initiation of a competent Krebs' cycle in extra-embryonic tissues at about this time. These increases are also reflected in the increasing amounts of acetate-C incorporated into extra-embryonic tissues. For the 19 d trophoblast this is twice that from glucose (Waugh & Wales, 1993).

THE ENERGY COSTS OF PREGNANCY

The relative uniformity in the morphology of early-cleavage-stage embryos across the mammalian species coupled with their metabolic demands, which are quantitatively minute yet highly specific in qualitative and temporal expression, gradually evolve into overall metabolic demands for pregnancy that show enormous interspecies diversity. Obvious factors contributing to this diversity are maternal size, gestation length and size of offspring at birth; less obvious is the energy stored in the offspring approximated in

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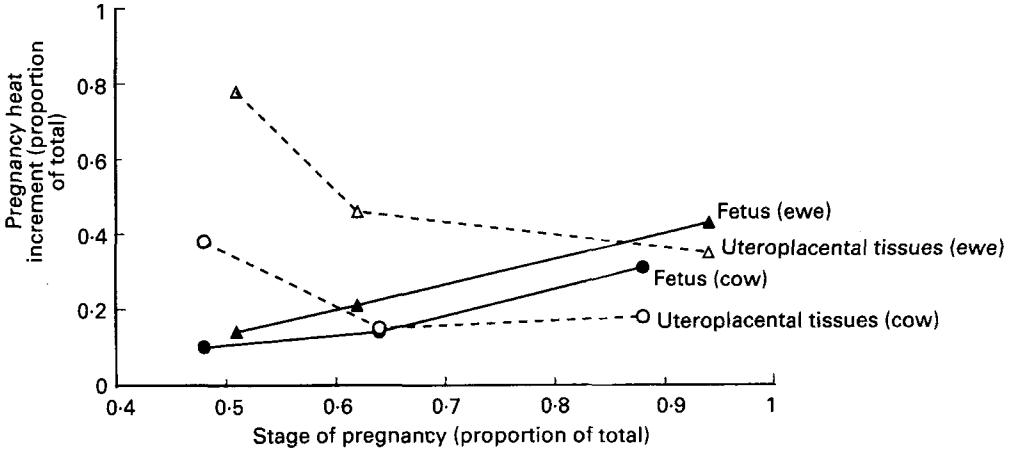

Fig. 2. Estimates for the proportion of the heat increment of pregnancy contributed by the fetus (▲, ●) and uteroplacental tissues (△, ○) in the ewe and cow at different stages of pregnancy. (From data presented by Bell, 1986.)

terms of enthalpy of combustion (MJ/kg birth weight) which varies from 3–4 for the rat and rabbit to 5–6 for sheep and cattle and 8–9 for the human baby (Blaxter, 1989). Nonetheless, attempts have been made to account for these interspecies differences and, thus, provide a common estimate for the energy needed to produce a newborn mammal. For example, Rahn (1982) divided Brody's (1945) interspecies equation, here expressed in SI units, $E = 17{\cdot}13W_L^{1{\cdot}24}$, in which E is the heat increment of gestation (MJ) and W_L is birth weight (kg) by Sacher & Staffeldt's (1974) equation for gestation length (G; d) $G = 130W_L^{0{\cdot}299}$, to obtain an average daily heat increment of gestation (MJ) of $0{\cdot}132W_L^{0{\cdot}94}$. When approximated to 0·132W MJ this provides an estimate for the rate of energy expenditure in the production of the newborn of 132 kJ/kg birth weight per d. While this exercise is valuable in placing the overall energy costs of mammalian reproduction in context, it ignores the enormous variation in the burden of pregnancy that exists within a species and is so vividly exemplified in the domestic sheep in which total birth weight increases by a factor of 2·8 as litter size increases from one to four (Robinson *et al.* 1977). It also fails to provide information on the important temporal changes in energy needs imposed by the exponential growth of the fetus. For example, half the enthalpy of combustion of the newborn lamb (about 12 MJ for a birth weight of 5 kg) accrues during the final 3 weeks of the 21-week pregnancy. With a production energetic efficiency of only 13·5% (Robinson *et al.* 1980) this represents over a 40% increase in the metabolizable energy (ME) requirement of the singleton-bearing ewe in late pregnancy.

Implicit in the low efficiency of utilization of ME for fetal growth is a high heat increment of pregnancy. In apportioning this to its sources of production, Bell (1986) combined observations from a range of experiments on ewes and cows and produced estimates for the contribution from the fetus, uteroplacental and maternal tissues in both species. Those for the fetus and uteroplacental tissues are plotted in Fig. 2 and demonstrate that by mid-pregnancy the fetal contribution is only 10–15%; even in late pregnancy it is still well below 50%. In contrast, the uteroplacental tissues are a major

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contributor in mid-pregnancy. Although their relative contribution declines as pregnancy progresses, they remain a major source of metabolic activity and heat production throughout gestation, accounting for one-third to half the total O_2 used by the gravid uterus in late pregnancy.

Within the uteroplacental tissues the placenta is the major site of metabolic activity. While the maternal component of the placenta is perfused by blood from the uterine artery the source for the fetal component is the umbilical artery, thus assigning control of the metabolic activity of the fetal placenta to the fetus (Bassett, 1995). This concept that the fetus plays an important role in placental metabolism is now making a major contribution to understanding the mechanisms controlling nutrient utilization by the conceptus during both normal and deranged growth.

FETAL–PLACENTAL INTERACTIONS

Under most conditions the fetus develops in an environment in which it is well protected from adverse changes in metabolic homeostasis. The magnitude of this protection is primarily dependent on the mother's ability to maintain fetal nutrient supply in conjunction with the placenta's capacity to maximize nutrient flow to the fetus. In the sheep, for example, placental growth is normally complete soon after mid-gestation (Alexander, 1964), with the primary factors determining its growth being maternal weight (Robinson *et al.* 1994) and nutrition (McCrabb *et al.* 1991). Because the ewe has a cotyledonary placenta, the number of placentomes play a major part in determining overall fetal growth, with the number of cotyledons per fetus decreasing as litter size increases, the decrease not being fully compensated for by increases in the weight of individual placentomes (Rhind *et al.* 1980). The important role that placental growth has in determining birth weight and, therefore, the demand for fetal fuel, is indicated from studies by Mellor (1983) in which irrespective of nutrient intake, almost two-thirds of the variance in fetal weight at term is accounted for by changes in placental weight. Between mid- and late gestation it is not unusual for the weight of the placenta to decrease by 50%, although dry weight remains constant (Vatnick & Bell, 1992). In contrast to the metabolic rate of the whole fetus and many of its organs, which decrease by 30–50% (when expressed on a dry weight basis; Bell *et al.* 1987), O_2 consumption by the fetal component of the placenta remains constant (Vatnick & Bell, 1992).

The stage at which placental growth reaches a maximum (just after mid-gestation in the ewe), corresponds to the stage of gestation at which fetal metabolic rate (per kg fresh weight) is greatest (Bell *et al.* 1986). This is also the time in fetal development at which the greatest proportion of fetal weight consists of metabolically active organs (i.e. brain, heart, kidneys, liver and lung; Table 1). In addition, the physiological functions plus interaction between placenta and fetus appear to be near maximal, as umbilical blood flow, heart rate and incidence of fetal breathing movements all decrease over the final 45 d of gestation (Table 1 and Fig. 3) but can be increased following maternal hyperoxia (Bekedam *et al.* 1991). Over this period the partitioning of available energy between growth and oxidation remains constant, but there is a 37% decline in energy requirements per kg fetal tissue (Table 1). Despite the magnitude of these changes in fetal metabolism, as long as maternal metabolism or placental function are not compromised the energy requirements for fetal maintenance and growth are met in equal parts by fetal uptake of glucose and amino acids (Lemons & Schreiner, 1983). Even essential amino

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Table 1. *Effect of fetal age on the metabolic requirements for growth and maintenance of the fetal lamb*

	Mid-gestation (75–90 d)	Late gestation (120–140 d)
Fetal wt* (g)	205	3200
Fetal V_{O_2}* (ml/min per kg dry wt)	91	40
Placental wt† (g)	635	330
Placental V_{O_2} (μl/min per kg dry wt)		
Fetal†	37	37
Maternal†	53	31
Umbilical blood flow‡§ (ml/min per kg)	468	260
Energy requirement‡‖ (kJ/d per kg wet wt)		
Oxidation	320	240
Growth	190	130
Organ**:Fetal wt‡¶	0·19	0·08

* Bell *et al.* (1987).
† Vatnick & Bell (1992).
‡ Bell *et al.* (1986).
§ Lemons & Schreiner (1983).
‖ Battaglia & Meschia (1978).
¶ Symonds *et al.* (1994*a*).
** Combined weight of brain, heart, kidney, liver and lung.

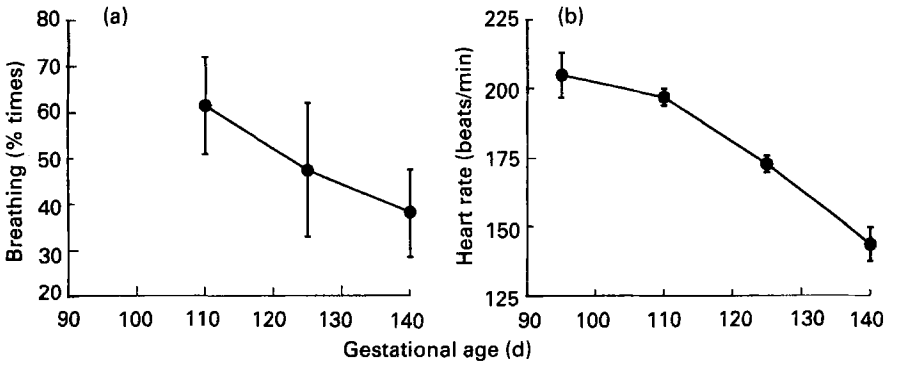

Fig. 3. (a) Incidence of fetal breathing movements and (b) changes in fetal heart rate during late gestation in the ovine fetus. (Adapted from Szeto *et al.* 1992 and Walker *et al.* 1987.)

acids are used extensively as a source of fetal fuel. For example, Battaglia (1992) reported that 20–25% of the [1-^{14}C]leucine infused into the fetal lamb was accounted for as $^{14}CO_2$ produced within the fetus and delivered into the placenta. Furthermore, the ovine placenta appears to provide the fetal requirement for glycine by conversion from fetal serine (Battaglia, 1992). This is particularly interesting in view of the finding by Robinson *et al.* (1985) that the normal diets fed to prolific ewes during late pregnancy are unlikely to provide sufficient glycine for fetal accretion.

The placenta is highly permeable to glucose which is transferred by facilitated diffusion

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(see Boyd *et al.* 1994), the concentration of glucose in maternal plasma being the primary determinant of fetal glucose supply (Hay *et al.* 1990). Moreover, the rate of placental perfusion by glucose is maintained at a sufficiently high level to ensure that it rarely limits glucose exchange between mother and fetus (Simmons *et al.* 1979). Studies on the molecular basis of placental glucose transfer are still in their infancy, but Zhou & Bondy (1993) provide evidence to suggest that in the rat glucose transporters GLUT1 and GLUT3 may be important for glucose transfer to placental and fetal tissues respectively. In contrast, amino acids are asymmetrically transported across the placenta by a range of transporters that are largely Na dependent (Boyd *et al.* 1994). The extent to which fetal amino acid supply may be regulated by alterations in placental transport has received little attention. Artificial manipulation of the amino acid composition in maternal plasma only appears to increase the concentration of those amino acids in fetal plasma which share the 'L' placental transport system (i.e. leucine, isoleucine, valine and phenylalanine; MacMahon *et al.* 1990). Fetal amino acid metabolism is also altered following continuous insulin infusion into the fetus which decreases the plasma concentration of all amino acids (Wilkening *et al.* 1994). Effects on amino acid uptake are not uniform and are dependent on the extent to which insulin concentration is increased in fetal plasma (Phillips *et al.* 1990). If the insulin concentration remains below 100 μU/ml so that the plasma concentration of glucose is unchanged, then amino acid catabolism is reduced and umbilical uptake of amino acids which are transported via the 'A' transport system is reduced (i.e. glycine, alanine, threonine, proline and serine). In contrast, if the insulin concentration in fetal plasma rises above 100 μU/ml, then glucose concentrations decrease by 45% and a modest increase in umbilical uptake of the majority of amino acids is observed (Phillips *et al.* 1990). Hyperinsulinaemia in conjunction with an artificially maintained euglycaemia does not, however, alter the total N uptake across the fetal hind limb tissues yet the uptakes of alanine, glycine, isoleucine, methionine and tyrosine are all enhanced (Wilkening *et al.* 1994).

MATERNAL NUTRITION AND FETAL GROWTH

The largest changes in fetal metabolism are observed following maternal food deprivation which results in both maternal and fetal hypoglycaemia (Hay *et al.* 1984) and in some cases this is associated with a complete cessation of fetal growth (Mellor & Matheson, 1979). Glucose uptake can decrease by up to 30% following a 5 d period of starvation in late-gestation sheep (Lemons & Schreiner, 1983). Amino acid uptake remains unchanged but much greater proportions of amino acids are oxidized and this leads to a doubling in urea-N production. The net result is impaired growth of skeletal muscle. Amino acid catabolism is also enhanced during chronic maternal undernutrition and this too is usually associated with a reduction in fetal growth. If, however, the level of undernutrition is not too severe, placental weight may be enhanced (Faichney & White, 1987). Further evidence that a decrease in nutrient availability determines fetal growth following maternal feed restriction or placental growth retardation is provided from studies in which fetal growth was restored following intravenous or intragastric infusion of glucose and amino acid mixtures directly into the fetus (Charlton & Johengen, 1985, 1987).

The full extent to which nutrient supplementation can benefit the growth-retarded fetus remains in doubt (Harding *et al.* 1992). This is partly because fetal growth

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retardation may not only result from a reduction in energy supply, but may also be linked to hypoxia and acidosis (Soothill *et al.* 1987). Fetal glucose supplementation, therefore, can exacerbate an underlying acidotic condition (Nicolini *et al.* 1990), particularly if it is associated with ketoacidaemia (Miodovnik *et al.* 1986). Failure of fetal growth can also alter the flux of nutrients between the fetus and its placenta to the extent that amino acids are supplied to the placenta from the fetus, thus leading to a wasting of the fetal body (Owens, 1991). It would appear, however, that during periods of decreased delivery of glucose to the fetus, gluconeogenesis from fetal tissue amino acids comes secondary to glycogenolysis and the release of free fatty acids from fetal lipid stores (Milley, 1993). It has been suggested that maternal O_2 administration, which improves fetal oxygenation (Paulick *et al.* 1992), may be one way of treating fetal growth retardation (Battaglia *et al.* 1992), although associated effects on fetal glucose homeostasis have not been described. It is important to appreciate, however, that if fetal undernutrition is of sufficient duration then growth retardation can be of such magnitude as to be irreversible (Mellor & Murray, 1981).

The mother's ability or otherwise to maintain glucose homeostasis has a number of tissue-specific effects on fetal growth. For example, growth of the endocrine pancreas is enhanced in overweight infants of diabetic mothers and decreased in infants that are small-for-dates (Fowden, 1989). This response is likely to be mediated via changes in fetal glucose supply as glucose is a potent stimulator of pancreatic β-cell replication (Hellerstrom & Swenne, 1991), although it should be noted that the insulin secretory response to glucose is much lower during fetal than postnatal life (Bassett *et al.* 1973).

The fetus is capable of making a number of metabolic and physiological adaptations in response to maternal undernutrition. These include the initiation of hepatic gluconeogenesis (Dalinghaus *et al.* 1991), although this is still unlikely to prevent fetal hypoglycaemia (Liechty & Lemons, 1984). However, the fetus can decrease metabolic requirements by reducing both the incidence and amplitude of breathing movements (Fowden *et al.* 1989). These have been associated with a lower rate of lung liquid secretion and occurrence of fetal swallowing (Harding *et al.* 1984; Moessinger *et al.* 1990). Changes in fetal glucose homeostasis not only influence plasma concentrations of insulin (Bassett & Madill, 1974) but also concentrations of IGF. IGF-1 levels are positively correlated with placental and fetal weight and circulating levels of glucose and O_2 (Owens *et al.* 1994). Intrauterine growth retardation in humans is also associated with a pronounced reduction in plasma concentrations of IGF-1, but not IGF-II (Lassarre *et al.* 1991). In contrast, De Chiara *et al.* (1992) found that in mice the transmission of an inactivated IGF-II gene from male chimeras produced heterozygous offspring that exhibited a 40% reduction in fetal and placental size. With regard to the influence of IGF-1 on fetal growth, recent studies indicate that in contrast to chronic hyperinsulinaemia, which has variable effects on the growth of major fetal organs (Susa *et al.* 1984; Milley, 1986), long-term IGF-1 administration enhances the growth of fetal heart, kidney, liver and lung (Robinson *et al.* 1994). Its stimulatory effect on fetal growth is accompanied by a reduction in fetal amino acid oxidation, enhanced feto-placental amino acid and glucose uptake with no change in uterine or umbilical blood flow, thus leading Harding *et al.* (1994) to conclude that IGF-1 has anabolic effects on feto-placental protein and carbohydrate metabolism. Although IGF-1 increases the proportion and surface area of fetal trophectoderm in the placenta and, therefore, the potential for an enhanced rate of nutrient transfer across the placenta, no increase in

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either simple or facilitated placental diffusion was observed by Harding *et al.* (1994). Of course, the IGFs are bound to specific proteins which, depending on the circumstances, may either inhibit or enhance IGF action. Intrauterine growth retardation caused by pre-eclampsia in humans is accompanied by increases in maternal IGF-binding protein-1 (IGFBP-1) and in animal experiments the decreases in fetal IGF-1 that accompany maternal food restriction and fetal hypoxia arising from reduced uteroplacental blood flow are all associated with increases in IGFBP-1 (Chard, 1994).

MODIFIERS OF FETAL GROWTH AND FUEL NEEDS

In intrauterine growth retardation caused by either fetal crowding or environmental heat stress there is a decrease in uterine blood flow (Dreiling *et al.* 1991; Ferrell & Reynolds, 1992). This leads to a fall in the supply of O_2 and glucose, and these are general features of fetal growth retardation. In Britain and other Northern European countries, winter shearing of ewes that are kept indoors during pregnancy is a recognized method of alleviating heat stress, and leads to an increase in lamb birth weight of about 15%. Prolonged cold exposure results in chronic maternal metabolic and hormonal adaptations, which prevent the usual catabolism of fetal tissues that occurs in undernourished ewes in the final days of gestation (Symonds *et al.* 1994*b*). Unlike the well-nourished situation this beneficial effect of cold exposure does not appear to be mediated by an increased rate of maternal glucose supply to the fetus. Fetal responses to alterations in maternal activity also vary with nutritional status in that maternal exercise enhances umbilical glucose uptake and placental glucose transfer capacity in underfed but not well-fed ewes (Leury *et al.* 1990).

Hormone and drug administration to the maternal body during pregnancy can also influence fetal growth. For example, Spencer & Robinson (1993) observed 20 and 15% increases in fetal and placental size respectively in rats receiving exogenously-administered thyroxine (T4), yet there was no effect on fetal T4 concentrations. Feeding the β-adrenergic agonist, clenbuterol, to pregnant rats induces an anabolic response in the maternal muscles but reduces the weight, muscle mass and total muscle fibre numbers of the fetus (Maltin *et al.* 1990). It is not known whether this is a direct effect on the development of immature muscle or an indirect effect arising from the repartitioning of nutrients between the fetal and maternal bodies. The β_2-adrenergic agonists are used in human pregnancy to prevent premature labour but little is known about their metabolic effects on the fetus. In sheep experiments, Bassett *et al.* (1989) found marked disturbances in fetal metabolism in the form of elevated plasma glucose, insulin, lactate and free fatty acids as well as severe hypoxaemia when the commonly-used β_2-adrenergic agonist, ritodrine, was infused into the fetus. Prolonged infusion also resulted in severe depletion of the fetal lamb's brown adipose tissue (BAT; Bassett & Symonds, 1993).

MAMMARY GLAND DEVELOPMENT

Pregnancy is accompanied by major increases in the numbers of lobule-alveolar epithelial cells in the mammary gland. These complete their differentiation during pregnancy and commence secretion of milk components (precolostrum) with a timing that is very variable both between species and between secretory constituents within a species (Forsyth, 1986). In the ewe the weight of the mammary gland is positively

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correlated with total lamb birth weight (about 350 g/kg birth weight) with over 95% of its growth occurring during pregnancy (Robinson *et al.* 1978; Robinson, 1986). This compares with about 60, 70 and 80% for the rat, rabbit and mouse respectively, three species in which the sucking stimulus further enhances mammary gland growth. In women the increment in mammary gland weight during pregnancy is about 10% of infant weight (Prentice & Whitehead, 1987). These examples demonstrate the enormous species diversity in the magnitude of mammary growth during pregnancy; they also demonstrate that compared with the fuel needs of the gravid uterus, those for the mammary gland are relatively minor in most species. Yet, as the estimates presented in Fig. 2 for the heat increment of pregnancy in sheep and cattle indicate, the proportion of the heat increment not accounted for by the fetal and uteroplacental tissues and, therefore, assumed to be from maternal tissues, is still quite substantial in late pregnancy (22 and 51% of the total for sheep and cattle respectively). How much of this arises from pregnancy-induced increases in the metabolism of maternal organs such as the heart, liver, kidney and gastrointestinal tract as opposed to the mammary gland is unknown. Based on measurements of mammary blood flow, O_2 uptake and CO_2 output, Oddy *et al.* (1984) estimated that the sheep mammary gland uses energy to produce milk with an efficiency of 90%, thus implying that the production of colostrum *per se* may be a minor contributor to the heat increment of pregnancy.

BIRTH

The metabolic responses which occur during the transition from fetal to neonatal life represent a change from a quiescent state in which inhibitory stimuli dominate to one of near maximal rates of metabolic activity that are rarely matched again during postnatal or adult life. In the fetus at term metabolic activity is minimized by limited placental transfer of O_2, the presence of placental inhibitory factors, low concentrations of stimulatory factors and an immature development of BAT (Symonds *et al.* 1994*a*).

For the lamb, natural birth and concomitant exposure to the cold stimulus of the extrauterine environment are key stimuli for establishing both continuous breathing (Harned & Ferreiro, 1973) and thermogenesis. O_2 consumption rapidly rises to approximately 30 ml/min per kg body weight (Mercer *et al.* 1979). Changes in metabolism by BAT and the liver play a primary role in the initiation and maintenance of these responses as a consequence of post-birth increases in BAT thermogenic and iodothyronine 5′-deiodinase (*EC* 3.8.1.4) activities (Symonds, 1995). After birth, not only is heat production by BAT and hepatic triiodothyronine synthesis stimulated, but there is also a transition from net anabolism to catabolism as lipid and glycogen stores are mobilized in order to meet the dramatic increase in metabolic rate (Mellor & Cockburn, 1986).

Thermoregulatory maturity at birth largely determines metabolic adaptation to the extrauterine environment. For example, altricial species such as rodents, which are immature at birth, benefit from the protection of a higher local environmental temperature by huddling in a nest. In contrast precocious species, such as lambs, have to thermoregulate independently at birth. Differences in temperature control are reflected in BAT development which peaks several days after birth in rats (Nedergaard *et al.* 1986), compared with 1–2 h postpartum in sheep (Symonds *et al.* 1994*a*). Lambs born spontaneously at term are able to maintain a constant body temperature over the first

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0·5 h of life, irrespective of the level of maternal nutrition over the final month of gestation (Symonds *et al.* 1994*b*). In contrast, pigs are an example of a group of mammals that do not possess BAT and, therefore, are entirely dependent on shivering thermogenesis in order to increase heat production (Mellor & Cockburn, 1986). This is a comparatively inefficient mechanism by which to maintain body temperature as it increases air movement around the animal, thereby reducing external insulation (Alexander, 1979). In piglets the post-birth surge in plasma concentration of triiodothyronine is one-third (2 nM; Berthon *et al.* 1993) that observed in lambs (6·6 nM; Symonds *et al.* 1994*c*). Piglets exhibit an appreciable decrease in rectal temperature of 2° after birth, a response which is increased if fetal hypothyroidism is induced by feeding sows a diet high in glucosinolate rapeseed (Berthon *et al.* 1993). Lambs, therefore, have a distinct survival advantage in being able to recruit both non-shivering and shivering thermogenesis after birth; shivering alone is insufficient to maintain body temperature and only appears to be recruited when heat production by BAT is limited (Symonds *et al.* 1992).

A primary factor influencing metabolic adaptation by BAT at birth is the route of delivery, since caesarean-section delivery of full-term infants or lambs compromises the newborn's ability to thermoregulate (Christensson *et al.* 1993; Symonds *et al.* 1994*b*). This response appears to be linked to a reduced postpartum surge in thyroid hormones (Symonds *et al.* 1994*c*) and lower plasma concentrations of catecholamines (Irestedt *et al.* 1982). An additional factor which influences metabolic adaptation in lambs delivered by caesarean section is ambient temperature, which may induce differential effects on catecholamine and thyroid status (Clarke *et al.* 1994; Symonds *et al.* 1994*c*). Over the first 0·5 h of life, delivery into a warm ambient temperature is not associated with any change in the noradrenaline or adrenaline content of BAT. This contrasts with a halving in the noradrenaline and fourfold increase in adrenaline contents of BAT in lambs delivered into a cold environment (Symonds *et al.* 1994*c*). By 1 d after delivery into a cool ambient temperature (15°) there is an increase in the plasma concentrations of thyroid hormones. These are important in neonatal thermoregulation in that they stimulate an enhanced thermogenic response via shivering thermogenesis. In contrast, lambs delivered into a warm (30°) environment do not show this elevation in thyroid hormones (Clarke *et al.* 1994).

THE FUTURE

Despite enormous advances in our understanding of the metabolism of the embryo, fetus and newborn, numerous important questions remain unanswered. What, for example, are the mechanisms involved in the nutritionally-mediated embryonic programming of fetal growth and size at birth? In attempting to alleviate intrauterine growth retardation, without inducing some of the metabolic and respiratory complications associated with glucose or indeed O_2 supplementation (Phillips *et al.* 1984; Warburton *et al.* 1987), is there a role for manipulating fetal amino acid metabolism? Epidemiological studies suggest that intrauterine growth retardation leads to adult hypertension (Barker, 1992); is its cause maternal malnutrition or, as suggested by Edwards *et al.* (1993), increased fetal exposure to maternal glucocorticoids arising from a reduction in placental 11β-hydroxysteroid dehydrogenase (*EC* 1.1.1.146) activity? These are only a few of the many important questions that need further investigation.

https://doi.org/10.1079/PNS19950055 Published online by Cambridge University Press

REFERENCES

Alexander, G. (1964). Studies on the placenta of the sheep (*Ovis aries L.*). Placental size. *Journal of Reproduction and Fertility* **7,** 289–305.

Alexander, G. (1979). Cold thermogenesis. In *International Review of Physiology – Environmental Physiology III*, vol. 20, pp. 43–155 [D. Robertson, editor]. Baltimore: University Park Press.

Avery, B., Madison, V. & Greve, T. (1991). Sex and development in bovine *in vitro* fertilized embryos. *Theriogenology* **35,** 953–963.

Barker, D. J. P. (1992). The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. *Proceedings of the Nutrition Society* **51,** 135–144.

Bassett, J. M. (1995). Glucose and fetal growth derangement. In *Fetus and Neonate*, vol. 3 *Growth* [M. A. Hanson, J. A. D. Spencer and C. H. Rodeck, editors]. Cambridge: Cambridge University Press (In the Press).

Bassett, J. M., Hanson, C. & Weeding, C. M. (1989). Metabolic and cardiovascular changes during prolonged ritodrine infusion in fetal lambs. *Obstetrics and Gynaecology* **73,** 117–122.

Bassett, J. M. & Madill, D. (1974). Influence of prolonged glucose infusions on plasma insulin and growth hormone concentrations of foetal lambs. *Journal of Endocrinology* **62,** 299–309.

Bassett, J. M., Madill, D., Nicol, D. H. & Thorburn, G. D. (1973). Further studies on the regulation of insulin release in foetal and post-natal lambs: the role of glucose as a physiological regulator of insulin release *in utero*. In *Foetal and Neonatal Physiology, Sir Joseph Barcroft Centenary Symposium*, pp. 351–359 [R. S. Comline, K. W. Cross, G. S. Dawes and P. W. Nathanielsz, editors]. Cambridge: Cambridge University Press.

Bassett, J. M. & Symonds, M. E. (1993). Effects of prolonged ritodrine or adrenaline administration on brown adipose tissue development in fetal lambs. *Journal of Physiology* **459,** 325P.

Battaglia, F. C. (1992). New concepts in fetal and placental amino acid metabolism. *Journal of Animal Science* **70,** 3258–3263.

Battaglia, C., Artini, P. G., D'Ambrogio, G., Galli, P. A., Segre, A. & Genazzani, A. R. (1992). Maternal hyperoxygenation in the treatment of intrauterine growth retardation. *American Journal of Obstetrics and Gynecology* **167,** 430–435.

Battaglia, F. C. & Meschia, G. (1978). Principal substrates of fetal metabolism. *Physiological Reviews* **58,** 499–527.

Bekedam, D. J., Mulder, E. J. H., Snijders, R. J. M. & Visser, G. H. A. (1991). The effects of maternal hyperoxia on fetal breathing movements, body movements and heart rate variation in growth retarded fetuses. *Early Human Development* **27,** 223–232.

Bell, A. W. (1986). Efficiency of prenatal growth and the heat increment of pregnancy. *Proceedings of the Cornell Nutrition Conference for Feed Manufacturers*, pp. 64–67. New York: Cornell University.

Bell, A. W. (1992). Foetal growth and its influence on postnatal growth and development. In *The Control of Fat and Lean Deposition*, pp. 111–127 [K. N. Boorman, P. J. Buttery and D. B. Lindsay, editors]. Oxford: Butterworth-Heinemann Ltd.

Bell, A. W., Battaglia, F. C. & Meschia, G. (1987). Relation between metabolic rate and body size in the ovine fetus. *Journal of Nutrition* **117,** 1181–1186.

Bell, A. W., Kennaugh, J. M., Battaglia, F. C., Makowski, E. L. & Meschia, G. (1986). Metabolic and circulating studies of fetal lamb at mid-gestation. *American Journal of Physiology* **250,** E538–E544.

Berthon, D., Herpin, P., Duchamp, C., Dauncey, M. J. & Le Dividich, J. (1993). Modification of thermogenic capacity in neonatal pigs by changes in thyroid status during late gestation. *Journal of Developmental Physiology* **13,** 253–261.

Bishonga, C., Robinson, J. J., McEvoy, T. G., Aitken, R. P., Findlay, P. A. & Robertson, I. (1994). The effects of excess rumen degradable protein in ewes on ovulation rate, fertilization and embryo survival *in vivo* and during *in vitro* culture. *British Society of Animal Production Jubilee Winter Meeting*, Paper no. 81. Edinburgh: British Society of Animal Production.

Blaxter, K. L. (1989). *Energy Metabolism in Animals and Man.* Cambridge: Cambridge University Press.

Boyd, R. D. H., D'Souza, S. W. & Sibley, C. P. (1994). Placental transfer. In *Early Fetal Growth and Development*, pp. 211–221 [R. H. T. Ward, S. K. Smith and D. Donnai, editors]. London: RCOG Press.

Brody, S. (1945). *Bioenergetics and Growth.* New York: Reinhold.

Chard, T. (1994). Insulin-like growth factors and their binding proteins in the control of human fetal growth. In *Early Fetal Growth and Development*, pp. 293–308 [R. H. T. Ward, S. K. Smith and D. Donnai, editors]. London: RCOG Press.

https://doi.org/10.1079/PNS19950055 Published online by Cambridge University Press

Charlton, V. & Johengen, M. (1985). Effects of intra-uterine nutritional supplementation on fetal growth retardation. *Biology of the Neonate* **48,** 125–142.

Charlton, V. & Johengen, M. (1987). Fetal intravenous nutritional supplementation ameliorates the development of embolization induced growth retardation in sheep. *Pediatric Research* **22,** 55–61.

Christensson, K., Siles, C., Cabrera, T., Belaustequi, A., De La Fuente, P., Lagercrantz, H., Puyol, P. & Winberg, J. (1993). Lower body temperatures in infants delivered by caesarean section than vaginally delivered infants. *Acta Paediatrica* **82,** 128–131.

Clarke, L., Darby, C. J., Lomax, M. A. & Symonds, M. E. (1994). Effect of ambient temperature during the first day of life on thermoregulation in lambs delivered by cesarean section. *Journal of Applied Physiology* **76,** 1481–1488.

Dalinghaus, M., Rudolph, C. D. & Rudolph, A. M. (1991). Effects of maternal fasting on hepatic gluconeogenesis and glucose metabolism in fetal lambs. *Journal of Developmental Physiology* **16,** 267–275.

De Chiara, T. M., Efstratiadis, A. & Robertson, E. J. (1992). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345,** 78–80.

Dreiling, C. E., Carman, F. S. III & Brown, D. E. (1991). Maternal endocrine and fetal metabolic responses to heat stress. *Journal of Dairy Science* **74,** 312–327.

Edwards, C. R. W., Benediktsson, R., Lindsay, R. S. & Seckl, J. R. (1993). Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet* **341,** 355–357.

Faichney, G. J. & White, G. A. (1987). Effects of maternal nutritional status on fetal and placental growth and on fetal urea synthesis in sheep. *Australian Journal of Biological Sciences* **40,** 365–377.

Farin, P. W., Farin, C. E. & Yang, L. (1994). *In vitro* production of bovine embryos is associated with altered fetal development. *Theriogenology* **41,** 193.

Ferrell, C. L. & Reynolds, L. P. (1992). Uterine and umbilical blood flows and net nutrient uptake by fetuses and uteroplacental tissues of cows gravid with either single or twin fetuses. *Journal of Animal Science* **70,** 426–433.

Forsyth, I. A. (1986). Variation among species in the endocrine control of mammary growth and function: the roles of prolactin, growth hormone, and placental lactogen. *Journal of Dairy Science* **69,** 886–903.

Fowden, A. L. (1989). The role of insulin in prenatal growth. *Journal of Developmental Physiology* **12,** 173–182.

Fowden, A. L., Harding, R., Ralph, M. M. & Thorburn, G. D. (1989). Nutritional control of respiratory and other muscular activities in relation to prostaglandin E in the fetal sheep. *Journal of Developmental Physiology* **11,** 253–262.

Harding, J. E., Liu, L., Evans, P. C. & Gluckman, P. D. (1994). Insulin-like growth factor-1 alters feto-placental protein and carbohydrate metabolism in fetal sheep. *Endocrinology* **134,** 1509–1514.

Harding, J. E., Owens, J. A. & Robinson, J. S. (1992). Should we try to supplement the growth retarded fetus? A cautionary tale. *British Journal of Obstetrics and Gynaecology* **99,** 707–710.

Harding, R., Snigger, J. N., Poore, E. R. & Johnson, P. (1984). Ingestion in fetal sheep and its relation to sleep states and breathing movements. *Quarterly Journal of Experimental Physiology* **69,** 477–486.

Hardy, K. (1993). Development of human blastocysts. In *Preimplantation Embryo Development*, pp. 184–199 [B. D. Bavister, editor]. New York: Springer-Verlag.

Harned, H. S. & Ferreiro, J. (1973). Initiation of breathing by cold stimulation. Effects of change in ambient temperature on respiratory activity of the full-term lamb. *Pediatrics* **83,** 663–669.

Hay, W. W. Jr, Molina, R. A., DiGiacomo, J. E. & Meschia, G. (1990). Model of placental glucose consumption and glucose transfer. *American Journal of Physiology* **258,** R569–R577.

Hay, W. W. Jr, Sparks, J. W., Wilkening, R. B., Battaglia, F. C. & Meschia, G. (1984). Fetal glucose uptake and utilization as functions of maternal glucose concentration. *American Journal of Physiology* **246,** E237–E242.

Hellerstrom, C. & Swenne, I. (1991). Functional maturation and proliferation of fetal pancreatic β-cells. *Diabetes* **40,** Suppl. 2, 89–93.

Imakawa, K., Helmer, S. D., Nephew, K. P., Meka, C. S. R. & Christenson, R. K. (1993). A novel role for GM-CSF: Enhancement of pregnancy specific interferon production, ovine trophoblast protein-1. *Endocrinology* **132,** 1869–1871.

Irestedt, L., Lagercrantz, H., Hjemdahl, P. & Belfrage, P. (1982). Fetal and maternal plasma catecholamine levels at elective caesarean section under general or epidural anaesthesia versus vaginal delivery. *American Journal of Obstetrics and Gynecology* **142,** 1004–1010.

https://doi.org/10.1079/PNS19950055 Published online by Cambridge University Press

Lassarre, C., Hardouin, S., Daffox, F., Forestier, F., Frankienne, F. & Binoux, M. (1991). Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. *Pediatric Research* **29,** 219–225.

Leese, H. J. (1991). Metabolism of the preimplantation mammalian embryo. In *Oxford Reviews of Reproductive Biology*, pp. 35–72 [S. R. Milligan, editor]. Oxford: Oxford University Press.

Lemons, J. A. & Schreiner, R. L. (1983). Amino acid metabolism in the ovine fetus. *American Journal of Physiology* **244,** E459–E466.

Leury, B. J., Bird, A. R., Chandler, K. D. & Bell, A. W. (1990). Glucose partitioning in the pregnant ewe: effects of undernutrition and exercise. *British Journal of Nutrition* **64,** 449–462.

Liechty, E. A. & Lemons, J. A. (1984). Changes in ovine fetal hindlimb amino acid metabolism during maternal fasting. *American Journal of Physiology* **246,** E430–E435.

McCrabb, G. J., Egan, A. R. & Hosking, B. J. (1991). Maternal undernutrition during mid pregnancy in sheep. Placental size and its relationship to calcium transfer during late pregnancy. *British Journal of Nutrition* **65,** 157–168.

MacMahon, R. A., Frampton, R. J. & Yardley, R. W. (1990). Effect on the fetus of infusing a commercial amino acid preparation into a pregnant sheep. *Biology of the Neonate* **57,** 231–237.

Maltin, C. A., Delday, M. I. & Hay, S. M. (1990). The effect of clenbuterol administration *in utero* and throughout lactation on pre- and post-natal muscle development in the rat. *Growth, Development and Aging* **54,** 143–150.

Mellor, D. J. (1983). Nutritional and placental determinants of fetal growth rate in sheep and consequences for the newborn lamb. *British Veterinary Journal* **139,** 307–324.

Mellor, D. J. & Cockburn, F. (1986). A comparison of energy metabolism in the newborn infant, piglet and lamb. *Quarterly Journal of Experimental Physiology* **71,** 361–379.

Mellor, D. J. & Matheson, I. C. (1979). Daily changes in the curved crown-rump length of individual fetuses during the last 60 days of pregnancy and effects of different levels of maternal nutrition. *Quarterly Journal of Experimental Physiology* **64,** 119–131.

Mellor, D. J. & Murray, L. (1981). Effects of placental weight and maternal nutrition on the growth rates of individual fetuses in twin bearing ewes during late pregnancy. *Research in Veterinary Science* **30,** 198–204.

Mercer, J. B., Andrews, D. C. & Szekely, M. (1979). Thermoregulatory responses in newborn lambs during the first thirty-six hours of life. *Journal of Thermal Biology* **4,** 239–245.

Milley, J. R. (1986). The effect of chronic hyperinsulinemia on ovine fetal growth. *Growth* **50,** 390–401.

Milley, J. R. (1993). Ovine fetal protein metabolism during decreased glucose delivery. *American Journal of Physiology* **265,** E525–E531.

Miodovnik, M., Skillman, C. A., Hertzberg, V., Harrington, D. J. & Clark, K. E. (1986). Effect of maternal hyperketonemia in hyperglycemic pregnant ewes and their fetuses. *American Journal of Obstetrics and Gynecology* **154,** 394–401.

Moessinger, A. C., Harding, R., Adamson, T. M., Singh, M. & Kin, G. T. (1990). Role of lung liquid volume in growth and maturation of the fetal sheep lung. *Journal of Clinical Investigation* **86,** 1270–1277.

Nedergaard, J., Connolly, E. & Cannon, B. (1986). Brown adipose tissue in the mammalian neonate. In *Brown Adipose Tissue*, pp. 152–213 [P. Trayhurn, editor]. London: Edward Arnold.

Nicolini, V., Hubinont, C., Santolaya, J., Fisk, N. M. & Rodeck, C. H. (1990). Effects of intravenous glucose challenge in normal and growth retarded fetuses. *Hormone and Metabolic Research* **22,** 426–430.

Oddy, V. H., Gooden, J. M. & Annison, E. F. (1984). Partitioning of nutrients in Merino ewes. 1. Contribution of skeletal muscle, the pregnant uterus and the lactating mammary gland to total energy expenditure. *Australian Journal of Biological Sciences* **37,** 375–388.

Owens, J. A. (1991). Endocrine and substrate control of fetal growth: placental and maternal influences and insulin-like growth factors. *Reproduction, Fertility and Development* **3,** 501–517.

Owens, J. A., Kind, L., Robinson, J. & Owens, P. C. (1994). Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology* **140,** 5–13.

Paulick, R. P., Meyers, R. J. & Rudolph, A. M. (1992). Effect of maternal oxygen administration on fetal oxygenation during graded reduction of umbilical or uterine blood flow in fetal sheep. *American Journal of Obstetrics and Gynecology* **167,** 233–239.

Phillips, A. F., Porte, P. J., Stabinsky, S., Rosenkrantz, T. S. & Raye, J. R. (1984). Effects of chronic fetal hyperglycemia upon oxygen consumption in the ovine fetus and conceptus. *Journal of Clinical Investigation* **74,** 279–286.

https://doi.org/10.1079/PNS19950055 Published online by Cambridge University Press

Phillips, A. F., Rosenkrantz, T. S., Lemons, J. A., Knox, I., Porte, P. J. & Raye, J. R. (1990). Insulin-induced alterations in amino acid metabolism in the fetal lamb. *Journal of Developmental Physiology* **13,** 251–259.

Prentice, A. M. & Whitehead, R. G. (1987). The energetics of human reproduction. In *Reproductive Energetics in Mammals, Zoological Society of London Symposia* 57, pp. 275–304 [A. S. I. Loudon and P. A. Racey, editors]. Oxford: Clarendon Press.

Rahn, H. (1982). Comparison of embryonic development in birds and mammals: birth weight, time and cost. In *A Companion to Animal Physiology*, pp. 124–137 [C. R. Taylor, K. Johansen and L. Bolis, editors]. Cambridge: Cambridge University Press.

Rhind, S. M., Robinson, J. J. & McDonald, I. (1980). Relationships among uterine and placental factors in prolific ewes and their relevance to variations in foetal weight. *Animal Production* **30,** 115–124.

Rieger, D. (1992). Relationships between energy metabolism and development of early mammalian embryos. *Theriogenology* **37,** 75–93.

Robinson, J. J. (1986). Changes in body composition during pregnancy and lactation. *Proceedings of the Nutrition Society* **45,** 71–80.

Robinson, J. J., McDonald, I., Brown, D. S. & Fraser, C. (1985). Studies on reproduction in prolific ewes. 8. The concentrations and rates of accretion of amino acids in the foetuses. *Journal of Agricultural Science, Cambridge* **105,** 21–26.

Robinson, J. J., McDonald, I., Fraser, C. & Crofts, R. M. J. (1977). Studies on reproduction in prolific ewes. 1. Growth of the products of conception. *Journal of Agricultural Science, Cambridge* **88,** 539–552.

Robinson, J. J., McDonald, I., Fraser, C. & Gordon, J. G. (1980). Studies on reproduction in prolific ewes. 6. The efficiency of energy utilization for conceptus growth. *Journal of Agricultural Science, Cambridge* **94,** 331–338.

Robinson, J. J., McDonald, I., McHattie, I. & Pennie, K. (1978). Studies on reproduction in prolific ewes. 4. Sequential changes in the maternal body during pregnancy. *Journal of Agricultural Science, Cambridge* **91,** 291–304.

Robinson, J. S., Owens, J. A., DeBarro, T., Lok, F. & Chidzanja, S. (1994). Maternal nutrition and fetal growth. In *Early Fetal Growth and Development*, pp. 317–329 [R. H. T. Ward, S. K. Smith and D. Donnai, editors]. London: RCOG Press.

Sacher, G. A. & Staffeldt, E. F. (1974). Relation of gestation time to brain weight for placental mammals: Implications for the theory of vertebrate growth. *American Naturalist* **108,** 593–615.

Simmen, R. C. M., Ko, Y. & Simmen, F. A. (1993). Insulin-like growth factors and blastocyst development. *Theriogenology* **39,** 163–175.

Simmons, M. A., Battaglia, F. C. & Meschia, G. (1979). Placental transfer of glucose. *Journal of Developmental Physiology* **1,** 227–243.

Soothill, P. W., Nicolaides, K. H. & Campbell, S. (1987). Prenatal asphyxia, hyperlacticaemia, hypoglycaemia and erythroblastosis in growth retarded fetuses. *British Medical Journal* **294,** 1051–1053.

Spencer, G. S. G. & Robinson, G. M. (1993). Stimulation of placental, fetal and neonatal growth by thyroxine administration to pregnant rats. *Journal of Endocrinology* **139,** 275–279.

Susa, J. B., Neave, C., Sehgal, P., Singer, D. B., Zeller, P. & Schwartz, R. (1984). Chronic hyperinsulinemia in the fetal rhesus monkey. *Diabetes* **33,** 656–660.

Symonds, M. E. (1995). Metabolism and growth during neonatal and postnatal development. In *The Fetus and Neonate*, vol. 3 *Growth* [M. A. Hanson, J. A. D. Spencer and C. H. Rodeck, editors]. Cambridge: Cambridge University Press (In the Press).

Symonds, M. E., Bird, J. A., Clarke, L., Darby, C. J., Gate, J. J. & Lomax, M. A. (1994*a*). Manipulation of brown adipose tissue development in neonatal and postnatal lambs. In *Temperature Regulation*, pp. 309–314 [A. S. Milton, editor]. Switzerland: Birkhauser.

Symonds, M. E., Bird, J. A., Clarke, L., Gate, J. J. & Lomax, M. A. (1994*b*). Nutrition, temperature and homeostasis during perinatal development. *Journal of Developmental Physiology* (In the Press).

Symonds, M. E., Bryant, M. J., Clarke, L., Darby, C. J. & Lomax, M. A. (1992). Effect of maternal cold exposure on brown adipose tissue and thermogenesis in the neonatal lamb. *Journal of Physiology* **455,** 487–502.

Symonds, M. E., Clarke, L. & Lomax, M. A. (1994*c*). The regulation of neonatal metabolism and growth. In *Early Fetal Growth and Development*, pp. 407–419 [R. H. T. Ward, S. K. Smith and D. Donnai, editors]. London: RCOG Press.

Szeto, H. H., Cheng, P. Y., Decena, J. A., Wu, D. L., Cheng, Y. & Dwyer, G. (1992). Developmental changes in continuity and stability of breathing in the fetal lamb. *American Journal of Physiology* **262,** R452–R458.

https://doi.org/10.1079/PNS19950055 Published online by Cambridge University Press

Thompson, J. G., Gardiner, D. K., Pugh, P. A., McMillan, W. H. & Tervit, H. R. (1994). Lamb birthweight following transfer is affected by the culture system used for pre-elongation development of embryos. *Journal of Reproduction and Fertility, Abstract Series* no. 13, Abstr. 69.

Vatnick, I. & Bell, A. W. (1992). Ontogeny of fetal hepatic and placental growth and metabolism in sheep. *American Journal of Physiology* **263,** R619–R623.

Wales, R. G. & Cuneo, C. L. (1989). Morphology and chemical analysis of the sheep conceptus from the 13th to the 19th day of pregnancy. *Reproduction, Fertility and Development* **1,** 31–39.

Wales, R. G. & Du, Z. F. (1993). Contribution of the pentose phosphate pathway to glucose utilization by preimplantation sheep embryos. *Reproduction, Fertility and Development* **5,** 329–340.

Wales, R. G. & Waugh, E. E. (1993*a*). Catabolic utilization of glucose by the sheep conceptus between days 13 and 19 of pregnancy. *Reproduction, Fertility and Development* **5,** 111–122.

Wales, R. G. & Waugh, E. E. (1993*b*). Oxidation of [U-^{14}C]acetate by the sheep conceptus between days 13 and 19 of pregnancy. *Reproduction, Fertility and Development* **5,** 201–208.

Walker, A. M., Cannata, J., Dowling, M. H., Ritchie, B. & Maloney, J. E. (1987). Sympathetic and parasympathetic control of heart rate in unanaesthetized fetal and newborn lambs. *Biology of the Neonate* **33,** 135–143.

Walker, S. K., Heard, T. M. & Seamark, R. F. (1992). *In vitro* culture of sheep embryos without co-culture: successes and perspectives. *Theriogenology* **37,** 111–126.

Warburton, D., Parton, L., Buckley, D., Cosico, L. & Saluna, T. (1987). Effects of glucose infusion on surfactant and glycogen regulation in fetal lamb lung. *Journal of Applied Physiology* **63,** 1750–1756.

Waugh, E. E. & Wales, R. G. (1993). Incorporation of substrate carbon from [U-^{14}C]acetate by the sheep conceptus recovered from the uterus on days 13 to 19 of pregnancy. *Reproduction, Fertility and Development* **5,** 209–217.

Wilkening, R. B., Boyle, D. W., Teng, C., Meschia, G. & Battaglia, F. C. (1994). Amino acid uptake by the ovine hindlimb under normal and euglycemic hyperinsulinemic states. *American Journal of Physiology* **266,** E72–E78.

Zhou, J. & Bondy, C. A. (1993). Placental glucose transporter gene expression and metabolism in the rat. *Journal of Clinical Investigation* **91,** 845–852.

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