[Organogenesis 4:3, 170-175; July/August/September 2008]; ©2008 Landes Bioscience

Review Series: Fetal Programming

# Early developmental influences on hepatic organogenesis

Melanie A. Hyatt, Helen Budge and Michael E. Symonds*

Centre for Reproduction and Early Life; Institute of Clinical Research; University of Nottingham UK

**Abbreviatioins:** SEC, sinusoidal endothelial cells; HSC, hepatic storage cells; PEPCK, phosphoenolpyruvate carboxykinase; G-6-Pase, glucose-6-phosphatase; HGF, hepatocyte growth factor; IGFs, insulin-like growth factors; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α; IL-6, interleukin 6; EGF, epidermal growth factor; FGF, fibroblast growth factor; GHR, growth hormone receptor; PRLR, prolactin receptor; GR, glucocorticoid receptor; IUGR, intrauterine growth restriction; LDL, low density lipoprotein; DOHaD, developmental origins of health and disease

**Key words:** liver, development, maternal constraint, nutrition, placental insufficiency, parity, growth factor

**The liver is the largest of the body's organs, with the greatest number of functions, playing a central role in coordinating metabolic homeostasis, nutrient processing and detoxification. The fetal liver forms during early gestation in response to a sequential array of distinct biological events, regulated by intrinsically programmed mechanisms and extracellular signals which instruct hepatic cells to either proliferate, differentiate or undergo apoptosis. A vast number of genes are involved in the initiation and control of liver development, many of which are sensitive to nutritional and hormonal regulation in utero. Moreover, liver mass is influenced by the gestational environment. Therefore, during periods of hepatic cell proliferation and differentiation, the developing fetal liver is sensitive to damage from both internal and external sources including teratogens, infection and nutritional deficiencies. For example, fetuses exposed to decreased materno-fetal nutrition during late gestation have a reduced liver mass, and/or perturbed liver function, which includes increased plasma LDL cholesterol and fibrinogen concentrations. These occur in conjunction with other risk factors present in the early stages of cardiovascular disease i.e. decreased glucose tolerance and insulin insensitivity in later life. Taken together, these findings suggest that liver mass, and later function, are essentially set in utero during fetal development—a process that is ultimately regulated by the intrauterine environment.**

## Introduction

Fetal liver development represents an array of distinct biological events i.e. foregut endoderm, liver primordium, mid-fetal liver, neonatal liver and adult liver, dependent upon the molecular and structural properties of liver tissue.[1] The intention of the present review is to focus on how the gestational environment may influence the latter stages of liver development that encompass a functional switch from fetal to adult liver. In order to determine the exact role of adequate placentation, prenatal nutrition and the endocrine environment upon hepatic organogenesis it is essential to understand the role of the liver in both fetal and postnatal life and the cellular and molecular events that regulate its development.

Both the location and structure of the liver are critical to its role within the body as a central regulator of metabolic homeostasis which may be compromized during liver injury.[2] The liver is a remarkable organ, in that it is responsible for maintaining overall metabolic homeostasis and detoxification. In addition, it performs an important endocrine role through the synthesis and secretion of hormones and growth factors which are essential for the promotion of postnatal growth.[3-5] Consequently, the successful development and functional maturation of the fetal liver is vital to fetal and neonatal survival as well as later health.[4,6]

Approximately 78% of the adult liver consists of parenchymal hepatocytes,[7] whilst the remaining liver is comprised of non-parenchymal cells including sinusoidal endothelial cells (SEC), Kupffer cells (hepatic macrophages) and hepatic stellate cells (HSC; fat storing cells).[8] Due to their high energy requirements, hepatocytes, unlike other endothelial cells, possess vast numbers of organelles.[9] Hepatocytes can contain as many as 2000 mitochondria, whereas relatively inactive cells such as lymphocytes have just a few mitochondria.[4] Hepatocytes are histochemically and metabolically heterogeneous with their primary function varying according to their location within the liver. Such hepatocyte heterogeneity, referred to as metabolic zonation, is determined by the distance of hepatocytes from the terminal portal and arterial branches which determine their nutritional and oxygen environment.[10-12] For example, periportal hepatocytes, supplied by the terminal afferent vessels, function in an environment rich in oxygen, substrates and hormones and are predominantly gluconeogenic. In contrast, perivenous hepatocytes, located around the central vein, are exposed to blood partially depleted of nutrients and oxygen and are, therefore, glycolytic.

Functional maturation of liver tissue is characterized according to the expression of liver- and stage-specific genes.[13,14] Alpha-fetoprotein, for example, decreases in abundance as the fetal liver develops.[15] Further markers of metabolic development include glucogenic proteins: Phosphoenolpyruvate carboxykinase (PEPCK)

*Correspondence to: Michael E. Symonds; Academic Division of Child Health; School of Human Development; University Hospital; Nottingham NG7 2UH UK; Tel.: +44.115.823.0611, Fax: +44.115.823.0626; Email: michael.symonds@nottingham.ac.uk

Submitted: 08/21/08; Accepted: 08/21/08

Previously published online as an *Organogenesis* E-publication: www.landesbioscience.com/journals/organogenesis/article/6849

and glucose-6-phosphatase (G-6-Pase). The fetus does not produce glucose until the prepartum cortisol surge in late gestation,[16,17] relying prior to that on placental uptake of maternal glucose into the fetal circulation. Coincident with the late gestational prepartum cortisol surge, there is a corresponding increase in hepatic PEPCK and G-6-Pase activity17 and a three- to-four- fold increase in glycogen content, which provides the major source of glucose for the newborn infant. However, when exposed to excess glucocorticoids and/or an inadequate nutritional supply, the compromised fetus can prematurely synthesize glucose in late gestation either by glycogenolysis or gluconeogenesis.[18,19]

## Liver Regeneration and Hepatic Growth Control Mechanisms

Hepatocytes are long-lived cells with a lifespan of approximately 150 days. In normal livers, parenchymal cells rarely divide.[4] However, upon resection or severe injury, hepatocytes proliferate rapidly to restore organ mass.[20] Liver regeneration studies provide a unique means of investigating, not only the cellular and molecular mechanisms that govern liver regeneration, but also organogenesis. Consequently, growth control mechanisms responsible for hepatocyte proliferation and apoptosis common to organogenesis, maintenance of liver size and regeneration have now been identified[21] and have been extensively reviewed.[1,22-26] Briefly, cross talk between the cell matrix, hepatocytes, and non-parenchymal cells regulate liver growth and encompasses both pro-mitogenic: Pro-inflammatory cytokines (TNFα and IL-6) and growth factors (HGF, EGF, FGF, TGFα),[27,28] and inhibitory apoptotic (TGF-β1 and Bcl-2) mechanisms.[1,3,29,30] Insulin, glucagon, noradrenalin, GH and thyroid hormones further regulate liver growth by enhancing the proliferative potential of mitogens, despite possessing little intrinsic mitogenic activity.[31,32]

Growth factors play a major role in the maturation of organ systems prior to birth.[17] For example, in the fetus, IGFs, synthesized by the placenta and fetal liver,[33] mediate growth[34] under the regulation of glucose and insulin.[35] The ontogenies of a number of genes that have a critical role in regulating both liver growth and development in utero, as well as postnatal growth and endocrine sensitivity,[36,37] have recently been determined in the sheep.[38] Key hepatic growth factors (with the exception of IGF-IIR and IGF-I) were found to increase throughout gestation reaching peak levels either during late gestation (PRLR) or at birth (GHR, IGF-IR and GR) and, thereafter, to decrease, signifying the perinatal period as an important time point in the regulation of liver growth and maturation.[38]

## Developmental Programming of Hepatic Organogenesis

Fetal growth and development is dependent on genetic, placental and maternal factors. In the presence of an adequate gestational environment, the fetus will grow to an appropriate size according to its inherent growth potential. However, in utero perturbations that may be intrinsic or extrinsic (see Table 1) can potentially affect the growth and development of the fetus and may play a critical role in the developmental programming of later disease susceptibility[39-41] and, in severe cases, may lead to intrauterine growth restriction (IUGR) and failure to thrive postnatally.[41,42]

Asymmetrically growth retarded fetuses, for example, are disproportionate in appearance and are characterized by a relative preservation of length and head circumference, whilst body weight is low (due mainly to a lower proportion of visceral and adipose tissue). Asymmetric IUGR is sometimes referred to as a 'wasted' as opposed to a 'stunted' (symmetrical) phenotype and may result from uteroplacental insufficiency and/or compromised nutritional supply during critical periods of development. These IUGR fetuses, if hypoxic, experience shunting of fetal blood flow, via the ductus venosus, to preserve the development of important organs, such as the brain, at the expense of the liver and kidneys which are hypoperfused.[43] This adaptation, known as fetal brain-sparing, may account for the disproportionate growth retardation and compromised liver growth leading to thinness/shortness at birth[39,44] and perturbed LDL cholesterol and fibrinogen metabolism in later life.[45,46]

Table 1 **Intrinsic and extrinsic factors influencing fetal growth and development**

| Factor | Reference |
|---|---|
| **Intrinsic** | |
| Genetics | 47–48 |
| Ethnicity | 49,50 |
| Fetal number | 51 |
| Hormones e.g. GH | 52 |
| Growth factors e.g. IGFs. | 53,54 |
| Infection | 55 |
| **Extrinsic** | |
| Pre-pregnancy weight | 56,57 |
| Maternal (uterine) size | 58,59 |
| Maternal age | 60-62 |
| Maternal nutrition | 63 |
| Maternal parity | 64 |
| Maternal body condition | 65 |
| Maternal smoking | 66–68 |
| Alcohol consumption | 69 |
| Socio-economic factors | 70,71 |
| Placental insufficiency | 72 |
| Placental vascularisation | 73 |
| Paternal height | 74 |

## Animal Models

Direct human research on potential regulators of fetal liver development is limited by ethical considerations. Consequently, animal models offer an alternative method of investigating the pathophysiologic aspects of the developmental origins of health and disease (DOHaD) hypothesis (for a review see refs. 75–77). The advantage of animal models over retrospective epidemiological studies, such as the Dutch famine,[39,78] is that the effect of nutrition, for example, can be largely assessed independently of confounding factors such as genetics and social status whilst controlling for fetal number, gender, parity and maternal body condition. Using animal models, it is possible to manipulate a number of aspects of fetal development including placental efficiency, maternal nutrition and hormonal balance at specific stages of gestation (e.g. embryonic, placental and fetal growth), thus allowing critical periods in hepatic organogenesis to be targeted. The most commonly used animal models of fetal programming are rats and sheep. The pregnant rat is an established

animal model of fetal programming and is susceptible to long-term effects of maternal nutrient restriction which include altered growth and functional development.[79, 80] There are a number of advantages of using rodents as models for fetal programming including, for example, short gestation (rat: 21 days) allowing for a high turnover in, and the opportunity for, generational studies. However, there are also a number of important physiological limitations, which include their high specific growth and metabolic rates and the inability to cannulate the fetal rat, thus hindering fetal physiological analyses. In contrast, sheep are large, relatively domesticated animals that develop bonds with their routine handlers, thus allowing experimental implementations and physiological and biochemical readings to be easily taken in a relatively unstressed state. The major advantage, over the rat, of using the pregnant sheep as an animal model is its close parallels with humans. The sheep is a monotocus species and is of comparable birth weight, fetal to maternal body weight ratio, metabolic rate, organogenesis and growth rate to humans. Due to its long gestational period and tolerance for intrauterine surgery, catheterization and chronic instrumentation without premature delivery, the pregnant ewe is considered the best large animal model to investigate fetal programming and is routinely used to study fetal growth, metabolism and endocrinology despite species differences in maternal and placental metabolism. Furthermore, restriction of nutrient intake to a comparable energy intake to that experienced during the Dutch Hunger Winter in pregnant sheep (i.e. to 50–60% metabolisable energy requirements) has been shown to similarly perturb fetal development, resulting in increased offspring blood pressure and glucose intolerance in later life.[81-84]

Numerous sheep studies have reported a link between various maternal constraints upon pregnancy including placentation, body condition, parity, adolescence, ambient temperature, fetal number and nutrition on liver growth, maturation and function in the resultant offspring.[16,38,62,76,81,85-89] Such constraints upon fetal development may result in an increase or decrease in offspring liver mass depending upon the timing, duration and severity of the gestational insult. That said, hepatic cellular and molecular adaptations also occur in the presence of normal liver growth. Therefore, developmental regulation of hepatic organogenesis and later function is not limited to alterations in liver mass, per se.[86,90,91]

## Nutritional Regulation of Liver Development

Epidemiological studies in several countries have suggested that size at birth and/or placental development predict adult health.[6,39,92] The Dutch Hunger Winter (1944–1945) provides a prominent example of the importance of adequate nutrition during pregnancy for later health.[78] Offspring born to mothers exposed to famine during early gestation had a three-fold increase in cardiovascular disease,[93] raised fibrinogen and decreased factor VII levels (in response to impaired liver development),[94] increased risk of obesity (women only)[95] and a more atherogenic lipid profile.[96] Moreover, men born with a decreased waist circumference, a proxy for compromised liver growth, have deleterious alterations in LDL cholesterol and fibrinogen metabolism in association with a greater mortality from coronary heart disease.[6,45,46] Taken together, epidemiological data imply that hepatic organogenesis is susceptible to nutritional reprogramming and that impaired liver development in utero can have long lasting functional consequences on disease risk in later life.

In the sheep, fetal liver growth is sensitive to changes in the materno-fetal nutrient supply. However, the magnitude of hepatic adaptation is dependent upon the timing and duration of the nutritional insult and on fetal number. For example, fetal and adult liver growth is markedly impaired by severe[97] and/or prolonged (encompassing a number of critical windows)[16] periods of nutrient deprivation, respectively. Furthermore, liver mass is reduced in twin compared with singleton offspring and in singleton lambs exposed to maternal nutrient restriction during late gestation.[88]

Nutritional manipulation of liver growth is accompanied by a range of endocrine adaptations including increased plasma GH and decreased plasma IGF-I and IGF-II, glucose and insulin concentrations,[97,98] together with decreased expression of hepatic GH and IGF-II receptors.[16,38,86]

In contrast, maternal nutrient restriction between early to mid gestation, coincident with the period of maximal placental growth,[99] has no effect on liver mass in singleton fetuses,[38,91,100] although an increase in liver weight has been observed when both singleton and twin pregnancies were studied.[101] Absolute, but not relative, liver mass is also reduced in lambs born to adolescent mothers that were undernourished during mid-gestation.[85] Furthermore, maternal nutrient restriction, during early to mid-gestation, increases hepatic glucocorticoid sensitivity[100] which is associated with alterations in the balance between mitogenic (GHR, PRLR and IGF-IIR) and pro-apoptotic (Bax and VDAC) proteins.[38] These data, therefore, indicate that exposure to an adverse nutritional environment in early pregnancy has a long term impact on liver growth. However, additional studies are needed to ascertain whether reduced liver mass and expression of growth factors in the nutrient restricted sheep affect its function and, indeed, whether such responses contribute to the developmental programming of poor health in later life.

## Maternal Parity and Ambient Temperature Influences Liver Development

Maternal parity influences offspring birth weight and growth trajectories in a range of species.[64,102-107] At birth, offspring born to nulliparous mothers tend to be lighter and of asymmetric body proportion with a low ponderal index and head circumference. Furthermore, firstborn offspring have significantly reduced liver weight possibly in response to in utero fetal brain-sparing.[86] Importantly, epidemiological studies have shown asymmetric offspring experience 'catch-up' growth (for both weight and height) over the first twelve months of life[108] which places them at an increased risk of being overweight in later life and for the development of the metabolic syndrome.[109-112]

## Placental Insufficiency and Hepatic Organogenesis

The placenta forms an interface between the mother and her fetus providing a number of functions essential for fetal growth and survival. Placental efficiency is determined by a number of factors including surface area, vascularity and permeability, uterine and umbilical flow rates and substrate transfer.[73,113] Placental insufficiency with respect to reduced transfer of oxygen and substrate is a major cause of IUGR and is associated with increased morbidity and mortality in the perinatal period and onset of disease in later life.[114]

The role of the placenta in fetal development, IUGR and later disease has been extensively investigated in the sheep and recently

reviewed.[76] Placental restriction, induced via carunclectomy, embolization, hyperthermia or sub-optimal nutrition, impairs placental development by decreasing placental surface area, vascularity and permeability and/or by compromising uterine and umbilical flow rates. Placental dysfunction, regardless of the surgical/experimental intervention used, results in altered or reduced fetal growth, which may be accompanied by perturbed hepatic organogenesis and morphology. For example, in the sheep, four days of umbilical-placental embolization during late gestation significantly reduces the weight of the 118 day embolized fetus. Moreover, these fetuses have significantly smaller livers relative to their body weights.[40]

Birth weight, body conformation and liver mass are also susceptible to changes in the thermal environment. For example, in human pregnancies maternal fever or exposure to tropical temperatures are associated with IUGR and premature delivery.[115,116] Chronic exposure of the pregnant ewe to a hyperthermic environment, commencing around 40 days gestation (term = 147 days), reduces placental growth rates resulting in placental insufficiency and asymmetric growth retardation. Resultant late gestational fetuses are lighter and have smaller livers and increased brain: Liver weight ratio.[117,118] As a consequence of a reduced substrate supply, fetuses are also hypoxic and hypoglycaemic in late gestation.[117,119] The primary cause of perturbed fetal development in heat stressed pregnancies is related to the degree of placental restriction and associated reduction in substrate supply to the developing fetus which, in males, results in the premature activation of the hypothalamic-pituitary-adrenal (HPA) axis.[120] In contrast, chronic cold exposure, induced via late gestation shearing, increases newborn liver mass, in response to a reduction in glycogen utilization and an associated increase in hepatic glycogen content.[89] Taken together, these studies indicate that extreme variations in the ambient temperature of the environment to which the mother is chronically exposed to during pregnancy is an important regulator of both fetal and placental development and also hepatic organogenesis. Moreover, such adaptations may have resulted from, or indeed caused, a reduction in the number of haematopoietic cell clusters within the livers of placentally restricted fetuses,[40] which may predispose the offspring to altered liver function in fetal and/or later life.

## Summary

The fetal liver forms during early gestation in response to an array of intracellular mechanisms. A vast number of genes are involved in the initiation and control of liver development, many of which are sensitive to nutritional and hormonal regulation in utero. Consequently, liver mass and its later function are essentially set in utero during fetal development, a process that is ultimately regulated by the intrauterine environment. This review paper aimed to demonstrate some of the complexities of exposure to a sub-optimal gestational environment upon the developing fetal liver with respect to its mass and expression of key growth factors. Nevertheless, the precise mechanisms responsible for programming liver size remain unknown, although they may be mediated, for example, by nutritional alterations in both hepatic mitogenic and apoptotic factors.[16] Further studies are needed to confirm whether the livers of nutrient restricted offspring are altered as a consequence of impaired organogenesis (altered cell number) or hepatic hypertrophy (altered cell size). Whether one or both of these final pathways are influenced depends upon the timing and severity of the gestational insult.

**Acknowledgements**

The European Community—The Early Nutrition Programming Project (FOOD-CT-2005-007036) supported this work.

**References**

1. Kinoshita T, Miyajima A. Cytokine regulation of liver development. Biochim Biophys Acta 2002; 1592:303-12.
2. Hui AY, Friedman SL. Molecular basis of hepatic fibrosis. Expert Rev Mol Med 2003; 5:1-23.
3. Fausto N, Webber EM. Control of liver growth. Crit Rev Eukaryot Gene Expr 1993; 3:117-35.
4. Kmiec Z. Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol 2001; 161:1-151.
5. Jungermann K. Zonation of metabolism and gene expression in liver. Histochemistry 1995; 103:81-91.
6. Barker DJP, Martyn CN, Osmond C, Wield GA. Abnormal liver growth in utero and death from coronary heart disease. Br Med J 1995; 310:703-4.
7. Motta PM. The three-dimensional microanatomy of the liver. Arch Histol Cytol 1984; 47:1-30.
8. Sasse D, Spornitz UM, Maly IP. Liver architecture. Enzyme 1992; 46:8-32.
9. Blouin A, Bolender RP, Weibel ER. Distribution of organelles and membranes between hepatocytes and non-hepatocytes in the rat parenchyma. J Cell Biol 1977; 72:441-55.
10. Ikezawa Y, Yamatani K, Ogawa A, Ohnuma H, Igarashi M, Daimon M, et al. Effects of glucagon on glycogenolysis and gluconeogenesis are region-specific in periportal and perivenous hepatocytes. J Lab Clin Med 1998; 132:547-55.
11. Quistorff B. Gluconeogenesis in periportal and perivenous hepatocytes of rat liver, isolated by a new high-yield digitonin/collagenase perfusion technique. Biochem J 1985; 22:221-6.
12. Teutsch HF. Regionality of glucose-6-phosphate hydrolysis in the liver lobule of the rat: metabolic heterogeneity of "portal" and "septal" sinusoids. Hepatology 1998; 8:311-7.
13. Panduro A, Shalaby F, Shafritz DA. Changing patterns of transcriptional and post-transcriptional control of liver-specific gene expression during rat development. Genes Dev 1987; 1:1172-82.
14. Derman E, Krauter K, Walling L, Weinberger C, Ray M, Darnell JEJ. Transcriptional control in the production of liver-specific mRNAs. Cell 1981; 23:731-9.
15. Shiojiri N, Lemire JM, Fausto N. Cell lineages and oval cell progenitors in rat liver development. Cancer Res 1991; 51:2611-20.
16. Hyatt MA, Gopalakrishnan GS, Bispham J, Gentili S, McMillen IC, Rhind SM, et al. Maternal nutrient restriction in early pregnancy programs hepatic mRNA expression of growth-related genes and liver size in adult male sheep. J Endocrinol 2007; 192:87-97.
17. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proc Nutr Soc 1998; 57:113-22.
18. Fowden AL, Mijovic J, Silver M. The effects of cortisol on hepatic and renal gluconeogenic enzyme activities in the sheep fetus during late gestation. J Endocrinol 1993; 137:213-22.
19. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest 1998; 101:2174-81.
20. Palmes D, Spiegel HU. Animal models of liver regeneration. Biomaterials 2004; 25:1601-11.
21. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002; 22:137-44.
22. Lemaigre F, Zaret KS. Liver development update: new embryo models, cell lineage control, and morphogenesis. Curr Opin Genet Dev 2004; 14:582-90.
23. Hata S, Namae M, Nishina H. Liver development and regeneration: from laboratory study to clinical therapy. Dev Growth Differ 2007; 49:163-70.
24. Duncan SA. Mechanisms controlling early development of the liver. Mech Dev 2003; 120:19-33.
25. Zaret KS. Liver specification and early morphogenesis. Mech Dev 2000; 92:83-8.
26. Zhao R, Duncan SA. Embryonic development of the liver. Hepatology 2005; 41:956-67.
27. Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, et al. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 1995; 373:699-702.
28. Uehara Y, Mori C, Noda T, Shiota K, Kitamura N. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. Nature 1995; 373:702-5.
29. Alison MR. Regulation of hepatic growth. Physiol Rev 1986; 66:499-541.
30. Sanchez A, Alvarez AM, Lopez P, J.M., Roncero C, Benito M, Fabregat I. Apoptotic response to TGF-beta in fetal hepatocytes depends on their state of differentiation. Exp Cell Res 1999; 252:281-91.
31. Cruise JL, Knechtle SJ, Bollinger RR, Kuhn C, Michalopoulos GK. α1 Adrenergic effects and liver regeneration. Hepatology 1987; 7:1189.
32. Bucher NLR, Patel U, Cohen S. Hormonal factors concerned with liver regeneration. Hepatotrophic Factors Ciba Found Symp 1978; 55:95.

33. Anthony RV, Pratt SL, Liang R, Holland MD. Placental-fetal hormonal interactions: impact on fetal growth. J Anim Sci 1995; 73:1861-71.
34. Harding JE, Liu L, Evans PC, Gluckman PD. Insulin-like growth factor 1 alters feto-placental protein and carbohydrate metabolism in fetal sheep. Endocrinology 1994; 134:1509-14.
35. Gallaher BW, Oliver MH, Eichhorn K, Kessler U, Kiess W, Harding JE, et al. Circulating insulin-like growth factor II/mannose-6-phosphate receptor and insulin-like growth factor binding proteins in fetal sheep plasma are regulated by glucose and insulin. Eur J Endocrinol 1994; 131:398-404.
36. Waters MJ, Kaye PL. The role of growth hormone in fetal development. Growth Horm IGF Res 2002; 12:137-46.
37. McMillen IC, Houghton DC, Phillips ID. Maturation of cytokine receptors in preparation for birth. Biochem Soc Trans 2001; 29:63-8.
38. Hyatt MA, Budge H, Walker D, Stephenson T, Symonds ME. Ontogeny and nutritional programming of the hepatic growth hormone-insulin-like growth factor-prolactin axis in the sheep. Endocrinology 2007; 148:4754-60.
39. Ravelli ACJ, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to the Dutch famine. Lancet 1998; 351:173-7.
40. Cheung CY, Bogic L, Gagnon R, Harding R, Brace RA. Morphologic alterations in ovine placenta and fetal liver following induced severe placental insufficiency. J Soc Gynecol Investig 2004; 11:521-8.
41. Ergaz Z, Avgil M, Ornoy A. Intrauterine growth restriction-eitology and consequences: what do we know about the human situation and experimental animal models? Reprod Toxicol 2005; 20:301-22.
42. Hales CN, Barker DJP. The thrifty phenotype hypothesis. Br Med Bull 2001; 60:5-20.
43. Nathanielsz PW, Hanson MA. The fetal dilemma: spare the brain and spoil the liver. J Physiol 2003; 548.2:333.
44. al-Ghazali W, Chita SK, Chapman MG, Allan LD. Evidence of redistribution of cardiac output in asymmetrical growth retardation. BJOG 1989; 96:697-704.
45. Barker DJP, Martyn CN, Osmond C, Hales CN, Fall CH. Growth in utero and serum cholesterol concentrations in adult life. Br Med J 1993; 307:1524-7.
46. Barker DJP, Meade TW, Fall CH, Lee A, Osmond C, Phipps K, et al. Relation of fetal and infant growth to plasma fibrinogen and factor VII concentrations in adult life. Br Med J 1992; 304:148-52.
47. Hannula K, Lipsanen-Nyman M, Kristo P, Kaitila I, Simola KO, Lenko HL, et al. Genetic screening for maternal uniparental disomy of chromosome 7 in prenatal and postnatal growth retardation of known cause. Pediatrics 2002; 109:441-8.
48. Cetin I, Foidart JM, Miozzo M, Raun T, Jansson T, Tsatsaris V, et al. Fetal growth restriction: a workshop report. Placenta 2004; 25:753-7.
49. Wilcox M, Gardosi J, Mongelli M, Ray C, Johnson I. Birth weight from pregnancies dated by ultrasonography in a multicultural British population. Br Med J 1993; 307:588-91.
50. Johar R, Rayburn W, Weir D, Eggert L. Birthweights in term infants. J Reprod Med 1988; 33:813-6.
51. Garite TJ, Clark RH, Elliott JP, Thorp JA. Twins and triplets: the effect of plurality and growth on neonatal outcome compared with singleton infants. Am J Obstet Gynecol 2004; 191:700-7.
52. Gluckman PD, Gunn AJ, Wray A, Cutfield WS, Chatelain PG, Guilbaud O, et al. Congenital idiopathic growth hormone deficiency associated with prenatal and early postnatal growth failure. The International Board of the Kabi Pharmacia International Growth Study. J Pediatr 1992; 121:920-3.
53. Woods KA, Camacho-Hubner C, Barter D, Clark AJ, Savage MO. Insulin-like growth factor gene deletion causing intrauterine growth retardation and severe short stature. Acta Paediatr Suppl 1996; 423:39-45.
54. Holmes R, Montemagno R, Jones J, Preece M, Rodeck C, Soothill P. Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. Early Hum Dev 1997; 49:7-17.
55. Kalanda BF, van Buuren S, Verhoeff FH, Brabin BJ. Anthropometry of fetal growth in rural Malawi in relation to maternal malaria and HIV status. Arch Dis Child Fetal Neonatal Ed 2005; 90:F161-F5.
56. Michlin R, Ottinger M, Odeh M, Koury S, Ophir E, Barak M, et al. Maternal obesity and pregnancy outcome. Isr Med Assoc J 2000; 2:10-3.
57. Clarke L, Yakubu DP, Symonds ME. Influence of maternal bodyweight on size, conformation and survival of newborn lambs. Reprod Fertil Dev 1997; 9:509-14.
58. Allen JC, Wilsher S, Turnbull C, Stewart F, Ousey J, Rossdale PD, et al. Influence of maternal size on placental, fetal and postnatal growth in the horse. Reproduction 2002; 123:445-53.
59. Walton A, Hammond J. The maternal effects on growth and conformation in the Shire horse-Shetland pony crosses. Proc Biol Sci 1938; 125:311-35.
60. Lenders CM, McElrath TF, Scholl TO. Nutrition in adolescent pregnancy. Curr Opin Pediatr 2000; 12:291-6.
61. Menacker F, Martin JA, MacDorman MF, Ventura SJ. Births to 10-14 year-old mothers, 1990-2002: Trends and health outcomes. National Vital Statistics Report 2004; 53:1-18.
62. Wallace JM, Aitken RP, Milne JS, Hay WWJ. Nutritionally mediated placental growth restriction in the growing adolescent: consequences for the fetus. Biol Reprod 2004; 71:1055-62.
63. Heasman L, Clarke L, Firth K, Stephenson T, Symonds ME. Influence of restricted maternal nutrition in early to mid gestation on placental and fetal development at term in sheep. Ped Res 1998; 44:546-51.
64. Lumey LH, Stein AD. Offspring birthweight after maternal intrauterine undernutrition: a comparison within sibships. Am J Epidemiol 1997; 146:810-9.
65. Wallace JM, Bourke DA, Aitken RP, Palmer RM, Da Silva P, Cruickshank MA. Relationship between nutritionally-mediated placental growth restriction and fetal growth, body composition and endocrine status during late gestation in adolescent sheep. Placenta 2000; 21:100-8.
66. Shah NR, Bracken MB. A systemic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol 2000; 182:465-72.
67. Vogt Isaksen C. Maternal smoking, intrauterine growth restriction, and placental apoptosis. Pediatr Dev Pathol 2004; 7:433-42.
68. Peacock JL. Smoking in pregnancy and fetal growth. Acta Paediatr 2002; 91:263-4.
69. Gauthier TW, Drews-Botsch C, Falek A, Coles C, Brown LA. Maternal alcohol abuse and neonatal infection. Alcohol Clin Exp Res 2005; 29:1035-43.
70. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001; 414:782-7.
71. Wynn SW, Wynn AH, Doyle W, Crawford MA. The association of maternal social class with maternal diet and the dimensions of babies in a population of London women. Nutr Health 1994; 9:303-15.
72. Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 1989; 161:996-1001.
73. Owens JA, Falconer J, Robinson JS. Restriction of placental size in sheep enhances efficiency of placental transfer of antipyrine, 3-O-methyl-D-glucose but not of urea. J Dev Physiol 1987; 9:457-64.
74. Nahum GG, Stanislaw H. Relationship of paternal factors to birth weight. J Reprod Med 2003; 48:963-8.
75. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 2005; 85:571-633.
76. Morrison JL. Sheep models of intrauterine growth restriction: fetal adaptations and consequences. Clin Exp Pharmacol Physiol 2008; 35:730-43.
77. Barry JS, Anthony RV. The pregnant sheep as a model for the human pregnancy. Theriogenology 2008; 69:55-67.
78. Painter RC, Roseboom TJ, Bossuyt PMM, Osmond C, Barker DJP, Bleker OP. Adult mortality at age 57 after prenatal exposure to the Dutch famine. Eur J Epidemiol 2005; 20:673-6.
79. Langley-Evans SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci 1994; 86:217-22.
80. Holemans K, Aerts L, Van Assche FA. Fetal growth and long-term consequences in animal models of growth retardation. Eur J Obstet Gynecol Reprod Biol 1998; 81:149-56.
81. Edwards LJ, McMillen IC. Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. Biol Reprod 2002; 66:1562-9.
82. Gardner DS, Pearce S, Dandrea J, Walker RM, Ramsey MM, Stephenson T, et al. Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. Hypertension 2004; 43:1290-6.
83. Gardner DS, Tingey K, Van Bon BWM, Ozanne SE, Wilson V, Dandrea J, et al. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. Am J Physiol 2005; 289:R947-R54.
84. Gopalakrishnan GS, Gardner DS, Rhind SM, Rae MT, Kyle CE, Brooks AN, et al. Programming of adult cardiovascular function after early maternal undernutrition in sheep. Am J Physiol 2004; 287:R12-R20.
85. Luther J, Aitken RP, Milne JS, Matsuzaki M, Reynolds LP, Redmer DA, et al. Maternal and fetal growth, body composition, endocrinology, and metabolic status in undernourished adolescent sheep. Biol Reprod 2007; 77:343-50.
86. Hyatt MA, Budge H, Walker D, Stephenson T, Symonds ME. Effects of maternal parity and late gestational nutrition on mRNA abundance for growth factors in the liver of postnatal sheep. Am J Physiol Regul Integr Comp Physiol 2007; 292:R1934-42.
87. Hyatt MA, Butt E, Budge H, Stephenson T, Symonds ME. Effects of maternal cold exposure and nutrient restriction on the ghrelin receptor, the growth hormone - insulin like growth factor axis and metabolic regulation in the postnatal ovine liver. Reproduction 2008; 135:723-32.
88. Budge H, Dandrea J, Mostyn A, Evens Y, Watkins R, Sullivan C, et al. Differential effects of fetal number and maternal nutrition in late gestation on prolactin receptor abundance and adipose tissue development in the neonatal lamb. Ped Res 2003; 53:302-8.
89. Clarke L, Bryant MJ, Lomax MA, Symonds ME. Maternal manipulation of brown adipose tissue and liver development in the ovine fetus during late gestation. Br J Nutr 1997; 77:871-83.
90. Zhou Y, Nijland M, Miller M, Ford S, Nathanielsz PW, Brenna JT. The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. Lipids 2008; 43:525-31.
91. Brameld JM, Mostyn A, Dandrea J, Stephenson TJ, Dawson JM, Buttery PJ, et al. Maternal nutrition alters the expression of insulin-like growth factors in fetal sheep liver and skeletal muscle. J Endocrinol 2000; 167:429-37.

92. Leon DA, Koupilova I, Lithell HO, Berglund L, Mohsen R, Vagero D, et al. Failure to realize growth potential in utero and adult obesity in relation to blood pressure in 50 year old Swedish men. Br Med J 1996; 312:401-6.
93. Roseboom TJ, van der Meulen JH, Osmund C, Barker DJP, Ravelli AC, Schroeder-Tanka JM, et al. Coronary heart disease after prenatal exposure to the Dutch famine. Heart 2000; 84:595-8.
94. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJP, Bleker OP. Plasma fibrinogen and factor VII concentrations in adults after prenatal exposure to famine. Br J Haematol 2000; 111:112-7.
95. Ravelli AC, Van der Meulen JH, Osmund C, Barker DJP, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr 1999; 70:811-6.
96. Roseboom TJ, van der Meulen JH, Osmond C, Barker DJP, Ravelli AC, Bleker OP. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. Am J Clin Nutr 2000; 72:1101-6.
97. Bauer M, Breier B, Harding J, Veldhuis J, Gluckman P. The fetal somatotropic axis during long term maternal undernutrition in sheep: evidence for nutritional regulation in utero. Endocrinology 1995; 136:1250-7.
98. Edwards LJ, Symonds ME, Warnes KE, Owens JA, Butler TG, Jurisevic A, et al. Responses of the fetal pituitary-adrenal axis to acute and chronic hypoglycemia during late gestation in the sheep. Endocrinology 2001; 142:1778-85.
99. Winick M. Cellular changes during placental and fetal growth. Am J Obstet Gynecol 1971; 109:166.
100. Whorwood CB, Firth KM, Budge H, Symonds ME. Maternal undernutrition during early to midgestation programs tissue-specific alterations in the expression of the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin II receptor in neonatal sheep. Endocrinology 2001; 142:2854-64.
101. Vonnahme KA, Hess BW, Hansen TR, McCormick RJ, Rule DC, Moss GE, et al. Maternal undernutrition from early- to mid-gestation leads to growth retardation, cardiac ventricular hypertrophy, and increased liver weight in the fetal sheep. Biol Reprod 2003; 69:133-40.
102. Magnus P, Berg K, Bjerkedal T. The association of parity and birthweight: testing the sensitisation hypothesis. Early Hum Dev 1985; 12:49-54.
103. Wilcox MA, Chang AM, Johnson IR. The effects of parity on birthweight using successive pregnancies. Acta Obstet Gynecol Scand 1996; 75:459-63.
104. Meikle A, Kulcsar M, Chilliard Y, Delavaud C, Cavestany D, Chilibroste P. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction 2004; 127:727-37.
105. Town SC, Patterson JL, Pereira CZ, Gourley G, Foxcroft GR. Embryonic and fetal development in a commercial dam-line genotype. Anim Reprod Sci 2005; 85:301-16.
106. Gardner DS, Buttery PJ, Daniel Z, Symonds ME. Factors affecting birth weight in sheep: maternal environment. Reproduction 2007; 133:297-307.
107. Bradford GE. The role of maternal effects in animal breeding. VII. Maternal effects in sheep. J Anim Sci 1972; 35:1324-34.
108. Ong KKL, Preece MA, Emmett PM, Ahmed ML, Dunger DB. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: Longitudinal birth cohort study and analysis. Ped Res 2002; 52:863-7.
109. Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: Longitudinal study. Br Med J 2001; 323:1331-5.
110. Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJP. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. Br Med J 1999; 319:1403-7.
111. Adair LS, Cole TJ. Rapid child growth raises blood pressure in adolescent boys who were thin at birth. Hypertension 2003; 41:451-6.
112. Khong TY, Adema ED, Erwich JJHM. On an anatomical basis for the increase in birth weight in second and subsequent born children. Placenta 2003; 24:348-53.
113. Owens JA, Falconer J, Robinson JS. Effect of restriction of placental growth on umbilical and uterine blood flows. Am J Physiol 1986; 250:R427-34.
114. Godfrey K, Robinson S, Barker DJP, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. Br Med J 1996; 312:410-4.
115. Edwards MJ, Walsh DA, Li Z. Hyperthermia, teratogenesis and the heat shock response in mammalian embryos in culture. Int J Dev Biol 1997; 41:354-8.
116. Eran E, Iddo ZBD, Yami S, Nilli D, Ruth A, Daniel S, et al. Acute Hepatitis A Infection in Pregnancy Is Associated With High Rates of Gestational Complications and Preterm Labor. Gastroenterology 2006; 130:1129-34.
117. Regnault TR, Orbus RJ, Battaglia FC, Wilkening RB, Anthony RV. Altered arterial concentrations of placental hormones during maximal placental growth in a model of placental insufficiency. J Endocrinol 1999; 162:433-42.
118. Dreiling CE, Carman FS, III, Brown DE. Maternal Endocrine and Fetal Metabolic Responses to Heat Stress. J Dairy Sci 1991; 74:312-27.
119. Thureen PJ, Trembler KA, Meschia G, Makowski EL, Wilkening RB. Placental glucose transport in heat-induced fetal growth retardation. Am J Physiol Regul Integr Comp Physiol 1992; 263:R578-85.
120. Wallace JM, Regnault TRH, Limesand SW, Hay WW, Jr., Anthony RV. Investigating the causes of low birth weight in contrasting ovine paradigms. J Physiol 2005; 565:19-26.