REPRODUCTION
REVIEW

Gene-nutrient interactions during fetal development

Christopher A Maloney and William D Rees

The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, Scotland

Correspondence should be addressed to W D Rees; Email: W.Rees@rowett.ac.uk

Abstract

Eukaryotic cells have evolved a complex series of nutrient sensors that protect them from damage caused by acute deficiencies and also mediate adaptive responses to prolonged excess or deficiency of particular nutrients. In adults gene expression is regulated by nutrients interacting with pathways involving mammalian target of rapamycin (mTOR), CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator activator proteins (PPARs). These systems are also present in key cells of the developing oocyte, embryo and fetus. In this review we will consider the role of interactions between genes and nutrients during reproduction with a particular emphasis on their possible involvement in the prenatal programming of glucose metabolism in the adult.

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Introduction

It has long been known that the maternal diet, and therefore the nutrient supply to the developing oocyte, embryo or fetus, is one of the principal environmental factors influencing the development of the offspring. A reliable and balanced supply of amino acids, lipids and carbohydrates is required to support the high rates of cell proliferation and the key developmental processes that take place during the embryonic (pre-implantation) and fetal (post-implantation) stages of life. Eukaryotic cells have evolved a complex series of nutrient sensors that are able to regulate gene expression in response to imbalances in the supply of nutrients. In adults these systems serve two purposes; firstly to protect the cell from damage caused by acute deficiencies and secondly to optimise homeostatic control to deal with a prolonged excess or deficiency of a particular nutrient. This second process may have a critical impact on the long term health of the offspring. It has been proposed that adverse nutritional conditions during fetal development lead to adaptive changes in metabolism that lead to a 'thrifty phenotype' in the offspring (Hales and Barker 1992). Poor nutrition in early life produces permanent changes in glucose-insulin metabolism, including a reduced capacity for insulin secretion and insulin resistance (Hales and Barker 2001). However, if this 'programming' of metabolism during embryonic and fetal development is inappropriate for the long term nutritional environment it may lead to adverse long term sequelae for the offspring (Sayer *et al.* 2004, Yajnik 2004, Barker 2004). The initiating factor(s) for fetal programming may be nutrient(s) interacting directly with genes and their regulatory elements at the cellular level, altering patterns of growth and gene expression. In this review we will concentrate on some of the direct interactions between genes and nutrients and their possible influence on fetal development.

Interactions between nutrients and gene expression in cells

The signals generated by growth factors and their receptors are modulated by macronutrient sensing pathways, which integrate developmental and environmental signals to regulate cellular growth and differentiation. Much of our understanding of these pathways has come from studies of cells *in vitro* and have been the subject of a number of excellent recent reviews (Ramji & Foka 2002, Fingar & Blenis 2004, Pegorier *et al.* 2004). Three interrelated systems change both the rate of mRNA translation and the transcription of genes in response to both transient and long-term imbalances in the nutrient supply (Fig 1).

Nutrient sensing protein kinases

The translation of mRNA into protein is regulated by a protein kinase known as the mammalian target of rapamycin (mTOR) which orchestrates an immediate response to disturbances in the amino acid or energy supply (Bruhat *et al.* 2002, Asnaghi *et al.* 2004). When amino acids, and in particular leucine, are plentiful mTOR forms a complex with other proteins to phosphorylate key components of the complex which translates mRNAs into proteins. These include the cap-binding initiation factor eIF4E, the

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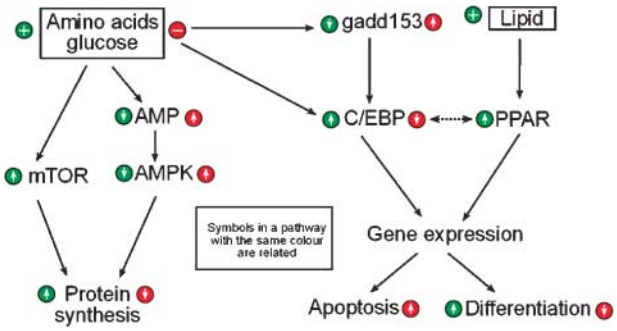

Figure 1 Mammalian cells possess three inter-related nutrient signalling systems that mediate the response to the availability of amino acids, glucose and lipids in the surrounding medium. Amino acid or glucose levels are sensed directly through the mTOR kinase or indirectly through the AMP activated kinase. Sufficient levels of these nutrients (+) stimulate mTOR (⇑) and activate protein synthesis (⇑). Conversely low levels of glucose and amino acids (−) increase intracellular AMP (⇑) activating the AMP dependent kinase which inhibits protein synthesis (⇓). The availability of glucose and amino acids also influences the gadd153 and C/EBP transcriptional activators through signals generated either by the accumulation of proteins in the endoplasmic reticulum or through the mTOR and AMPK kinases. When glucose and amino acids are low (−) the levels of gadd153 mRNA rise (⇑) interfering with the normal action of C/EBP and changing patterns of gene expression. Lipids are sensed through binding to the PPAR subfamily of nuclear hormone receptors. Binding of ligands (⇑) increases PPAR activity inducing gene expression. Interactions between gadd153, C/EBP and PPAR transcription factors modulate gene expression in the cell determining whether a cell undergoes apoptosis or differentiation.

ribosomal protein S6 kinases (p70 S6 kinase), and elongation factor eEF2 (Fingar & Blenis 2004, Hay & Sonenberg 2004). Depletion of intracellular amino acid pools or removal of amino acids from the extracellular medium inhibits the mTOR kinase, suppressing protein synthesis. The absence of mTOR signalling also prevents the activation of cyclin dependent kinases (CDKs) and accelerates the turnover of cyclin D1, leading to a deficiency of active CDK4/cyclin D1 complexes, arresting cell growth in the G1-phase of the cell cycle (Panwalkar *et al.* 2004). The response to low glucose or hypoxia is regulated by a parallel protein kinase activated by increased intracellular AMP concentrations. A reduction in glycolysis or oxidative metabolism depletes intracellular ATP and increases AMP. The AMP activated protein kinase pathway senses this accumulation of AMP and inhibits the activation of p70 S6 kinase (Tokunaga *et al.* 2004, Meijer & Dubbelhuis 2004). This pathway also exerts effects on transcriptional regulators responsible for mitochondrial biogenesis and lipid synthesis in adult tissues.

Leucine zipper proteins

Cells respond to nutrient deficiency with the appearance of mRNAs normally found at low levels in rapidly dividing cells. The CHOP-10/gadd153 (C/EBP homologous protein 10/growth arrest and DNA damage gene 153) mRNA is upregulated within 4 hours of cells being exposed to media deficient in glucose, amino acids (Jousse *et al.* 2004) or other key components such as choline (van der Sanden *et al.* 2004). The expression of CHOP-10/gadd153 is regulated by the mTOR pathway (Entingh *et al.* 2001) and also by transcription factors from the activating transcription factor (ATF) family (Averous *et al.* 2004). The ATF-4 factors are produced in response to the accumulation of unfolded proteins in the endoplasmic reticulum through premature termination of the peptide chain, a failure of glycosylation or other defects in the export process. Unfolded proteins activate a series of protein kinases known as the unfolded protein response (UPR) (Zhang & Kaufman 2004). These kinases phosphorylate the alpha subunit of translation initiation factor 2 (eIF2-alpha), one of the proteins that control the binding of ribosomes to mRNA. Phosphorylation of eIF2-alpha inhibits the translation of most mRNAs and suppresses total protein synthesis (Harding *et al.* 2000). However, some mRNAs, including that for the transcription factor ATF-4, are still able to associate with ribosomes. Therefore the UPR promotes translation of ATF-4 and stimulates CHOP-10/gadd153 transcription (Harding *et al.* 2003).

The product of the CHOP-10/gadd153 mRNA is a nuclear protein related to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. All of these proteins have a leucine rich region (leucine zipper) and a DNA binding domain (Ramji and Foka 2002). Two C/EBP molecules bind together through the leucine zipper to form a dimer, which activates transcription from specific DNA sequences in the promoters of target genes. Homo- and hetero- dimers of the C/EBP transcription factors are involved in a well characterised cascade of gene expression which leads to the terminal differentiation of a number of cell types including hepatocytes and adipocytes (Fig. 2) (Lane *et al.* 1999). One of the key components in this cascade is C/EBP-beta which is expressed during the process of cell commitment (Ramji and Foka 2002). When the CHOP-10/gadd153 protein is induced, it can form dimers with other members of the C/EBP family of transcription factors including C/EBP-beta. This disrupts the normal pattern of C/EBP-beta mediated gene transcription and initiates the expression of a new series of mRNAs (Wang *et al.* 1998). Under extreme circumstances the changes in gene expression mediated by CHOP-10/gadd153 leads to the death of the cell through apoptosis (Zinszner *et al.* 1998).

The C/EBP family of transcription factors are themselves subject to transcriptional regulation and postranslational modification by the nutrient supply. The UPR influences the C/EBP-beta mRNA levels through an element upstream of the promoter (Chen *et al.* 2004). In the liver several N-terminally truncated isoforms of C/EBP-alpha and C/EBP-beta are produced by initiation of translation from alternative start sites. The C/EBP-beta protein occurs in three alternative translation products; the full length 35 kDa protein, liver activating protein (LAP; a 32 kDa

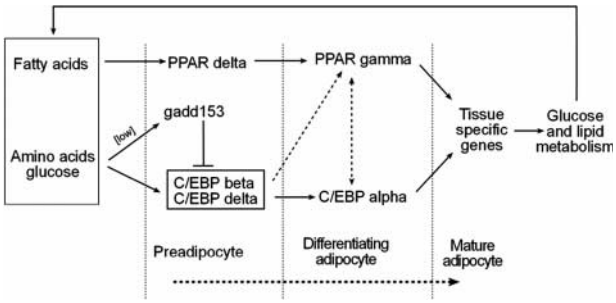

Figure 2 Temporal changes in nutrient sensitive gene expression during the differentiation of pre-adipocytes into mature adipocytes. Following the induction of cell differentiation the C/EBP and PPAR genes form a nutrient sensitive feedback loop to regulate the differentiation process. When fats are abundant long chain fatty acids interact with PPAR-delta and PPAR-gamma to promote adipogenesis. Similarly amino acids and glucose interact with C/EBP-delta and C/EBP-beta. Low levels of glucose induce gadd153, which forms inactive dimers with C/EBP-beta inhibiting the progress of adipocyte development. Interactions between the PPAR and C/EBP transcriptional activators (dotted lines) control the expression of tissue specific genes leading to and maintaining the mature phenotype. The numbers of mature adipocytes influence whole body glucose and lipid metabolism to create a feedback loop, which influences the subsequent differentiation of the next lineage of preadipose cells. Thus the availability of nutrients influences the development of adipose tissue depots. A similar feedback loop involving PPARs and C/EBPs also regulates the development of hepatocytes and pancreatic beta cells.

active isoform of C/EBP-beta) and liver inhibitory protein (LIP; a 20 kDa inhibitory isoform of C/EBP-beta) (Descombes and Schibler 1991). LIP retains the leucine zipper but lacks the activation domain found in the active isoform LAP. In the adult prolonged hyperglycemia increases the LAP/LIP ratio and alters phosphoenol pyruvate carboxy kinase (PEPCK) expression (Shao *et al.* 2005). The function of C/EBP-beta is also regulated by a protease induced by C/EBP-alpha following terminal differentiation of cells. This protease generates a truncated form of the C/EBP-beta protein and represses the activity of the full length protein (Welm *et al.* 1999). This range of control mechanisms enables the C/EBP proteins to respond to nutrients and act in concert with other factors such as CHOP-10/gadd153 or members of the peroxisome proliferator-activated receptor (PPAR) family (described below) to modify the process of cell commitment and terminal differentiation.

Nuclear hormone receptor super family

Fatty acids and other lipophilic molecules control the expression of a network of target genes through interactions with members of the nuclear hormone receptor super family (Olefsky 2001). These receptors can be broadly categorised according to their ligands. For example, one family of these receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR) bind retinoic acid (derived from vitamin A) and play an essential role in regulating the differentiation of numerous cell types (Zile 2001). The nuclear receptor superfamily also includes receptors for thyroid hormones, vitamin D and liver X receptor (LXR). Another group of receptors from this family associated with cellular responses to fatty acids are the PPARs (Kota *et al.* 2005). These receptors modulate lipid and glucose metabolism by acting as transcriptional activators following the binding of fatty acids or fatty acid derived molecules such as prostaglandin J2 (Ferre 2004, Evans *et al.* 2004). The PPAR family comprises three isoforms that are expressed at different stages of development and in different tissues (van Bilsen *et al.* 2002). In the adult PPAR-alpha is expressed in liver, heart and muscle, PPAR-gamma is highly expressed in adipose tissue and the third isoform PPAR-delta (also known as PPAR-beta) is ubiquitously expressed in many tissues.

Members of the superfamily can form hetero-dimers with one another, thus for example; PPARs can form dimers with the retinoic acid receptors, the thyroid hormone receptor and the vitamin D receptor. Heterodimers of LXR/RXR play a particularly important role as the dominant activators of the sterol regulatory element-binding proteins (SREBPs). These membrane-bound transcription factors from the basic helix-loop-helix leucine zipper family are highly expressed in liver and adipose tissue where they are key regulators of cholesterol and fatty acid synthesis (Yoshikawa *et al.* 2001, Yoshikawa *et al.* 2003). In adults the SREBP-1c isoform responds to dietary glucose and controls hepatic lipogenesis through its close links to the signalling cascade downstream of the insulin receptor (Osborne 2000). Another isoform, SREBP-2 is a key regulator of cholesterol biosynthesis. Through their impact on factors such as SREBPs the nuclear hormone receptors are essential in the nutrient mediated regulation of lipid metabolism.

The nuclear receptors share common structural motifs, which includes areas for DNA recognition, ligand binding and cofactor interactions. The DNA recognition domain binds to specific nucleotide response elements in enhancer or promoter regions of the target genes and is able to activate transcription after ligand binding (Privalsky 2004). The co-factor binding sites enable the dimers to form complexes with co-repressor proteins which catalyse post-translational modifications of histones and other proteins to maintain the local chromatin structure and facilitate gene transcription. These modifications of chromatin may be an important factor in prenatal metabolic programming.

Gene nutrient interactions during development

Human and animal studies suggest that the developing fetus is susceptible to metabolic programming throughout gestation. Imbalances in the nutrient supply induce both responsive and adaptive changes in development. The mTOR kinase has a central role in responding to adverse environmental conditions, protecting cells by regulating

protein synthesis. Under more extreme conditions induction of CHOP-10/gadd153 invokes stress responses to eliminate damaged cells through apoptosis. The C/EBP and PPAR factors play a more adaptive role during tissue development. By sensing the availability of carbohydrate and lipids these factors regulate differentiation of progenitor cells, adapting tissue development in response to the nutrient supply. Studies of animals with specific defects suggest that both the stress response and adaptive responses to nutrients are essential in the normal development of key cell types involved in metabolic homeostasis. A number of potential mechanisms for the long term programming of metabolism involving the direct effects of nutrients on gene expression are becoming apparent. Current experimental data support each of these mechanisms, however it does not preclude the possibility that they may all be operating as part of a combined process which ultimately leads to the programming of metabolism in the offspring.

Changes in the growth of the developing oocyte or embryo

Disturbances in pre-implantation development have been reported to produce a number of different phenotypes including obesity (Sjoblom *et al.* 2005) and hypertension (Kwong *et al.* 2000) in the offspring. Disrupting the growth of the embryo *in vivo* prior to implantation modifies cell allocation in the blastocyst (Fleming *et al.* 2004). Embryo culture *in vitro* is also reported to restrict fetal growth and modify placental morphogenesis (Sjoblom *et al.* 2005). These studies suggest that suboptimal pre-implantation or early post-implantation growth alter the ratio of placental to fetal tissue mass. Perturbation of early placental growth cannot be reversed, permanently compromising the nutrient supply to developing fetus. Studies of embryo development *in vitro* and subsequent embryo transfer have shown that early development is dependent on a number of different factors, including oocyte maturation and blastocyst growth.

Oocyte maturation

The early stages of development post-fertilisation depend on the quality of the oocyte. Data from oocytes matured *in vitro* suggests that several nutritional and endocrine factors influence follicle development (Rodriguez & Farin 2004). In particular the successful maturation of the oocyte is critically dependent on interactions with the surrounding granulosa cells (Kim *et al.* 1997). There is evidence that mTOR, C/EBPs and PPARs are all involved in regulating granulosa cell function. These may provide a mechanism to suppress ovulation in response to severe nutritional imbalances such as those induced by famine or anorexia. However, it is also possible that inappropriate oocyte maturation may lead to subtle changes in post-fertilisation development.

Cell proliferation following stimulation with follicle stimulating hormone is dependent on the mTOR pathway to promote the phosphoinositol 3-kinase-dependent activation of p70 S6 kinase (Alam *et al.* 2004). In addition to mTOR, follicular differentiation is also dependent on the C/EBP transcription factors. Deletion of the C/EBP-beta gene leads to failure of follicular development as the granulosa cells are unable to respond to luteinising hormone (Sterneck *et al.* 1997). There is also an important role for C/EBP-alpha and C/EBP-beta during the transition period from follicle to corpus luteum (Gillio-Meina *et al.* 2005). The mRNA of all three PPAR isoforms is expressed in the rat ovary, with PPAR-gamma the principal form found in the granulosa cells. Mice with a targeted deletion of the PPAR-gamma gene in oocytes and granulosa cells had a normal population of follicles, were able to ovulate and develop corpora lutea (Cui *et al.* 2002). This suggests that PPAR-gamma is not essential for follicular functions, however, the animals had a reduced implantation rate. It has been suggested that PPAR-gamma plays a part in regulating oocyte maturation by acting as a negative regulator of follicular growth and differentiation (Lovekamp-Swan & Chaffin 2005). All of these studies demonstrate that granulosa cells possess mechanisms to regulate oocyte maturation in response to the nutritional environment. However, at present it is not clear if changes in the maternal diet can modify oocyte maturation through these gene nutrient interactions.

Blastocyst growth

Mouse blastocyst outgrowth *in vitro* and probably implantation *in vivo* require amino acid signalling via the mTOR pathway. The presence of amino acids, and particularly leucine, in the extracellular medium stimulates mTOR activity in the blastocyst, suggesting that this pathway regulates blastocyst growth and implantation in response to the amino acid supply (Martin *et al.* 2003). When the kinase domain of mTOR is disrupted by homologous recombination, homozygous mutant embryos die shortly after implantation due to impaired cell proliferation showing that this is a critical developmental checkpoint for implantation (Gangloff *et al.* 2004, Murakami *et al.* 2004).

Prolonged exposure to genotoxic stress (and probably amino acid deficiency) induces the CHOP-10/gadd153 mRNA and initiates apoptosis in the blastocyst (Fontanier-Razzaq *et al.* 2001). This response to nutritional and environmental stress probably removes damaged cells and prevents them from proliferating through the developing fetus. The C/EBP transcription factors appear to become important following implantation where they are expressed alongside the CHOP-10/gadd153 mRNA during placental development. Mouse embryos with targeted deletions of both C/EBP-alpha and C/EBP-beta die between embryonic days 10 and 11 because of defects in the labyrinth layer of their placentas (Begay *et al.* 2004). However, a single copy of either

C/EBP-beta, or C/EBP-alpha is sufficient to support development. This suggests that although both transcription factors function during early embryonic development there is a degree of redundancy. A targeted deletion of the PPAR-gamma gene also affects the ability to establish a labyrinth layer in the placenta (Asami-Miyagishi *et al.* 2004). The placenta acts as the interface between the fetus and the maternal circulation where it serves an important role in regulating fetal nutrient supply. These studies of animals with targeted deletions suggest that the C/EBP proteins regulate placental development in concert with PPARs. However, it is not known to what degree these nutrient sensitive genes have a physiological role in buffering and adapting placental development to optimise the nutrient supply to the developing fetus.

Epigenetic modifications of chromatin structure

A number of disease states in humans are the result of subtle changes in gene expression caused by alterations in chromatin structure (epigenetic modifications; Robertson & Wolffe 2000). Epigenetic marks such as the covalent methylation of cytosine residues in DNA are set in the chromatin during development and determine the accessibility of a particular gene to the transcriptional machinery (Spiegelman & Heinrich 2004). By regulating gene expression through changes in the promoter region, these epigenetic modifications represent another mechanism for the nutritional regulation of gene expression.

Epigenetic modifications may result from the direct effects of changes in metabolism as the process of chromatin remodelling depends on a number of products derived from intermediary metabolism such as S-adenosyl methionine (SAM), acetyl CoA and nicotinamide adenine dinucleotide ($NAD^{(+)}$). The modification of cytosine residues with methyl groups derived from SAM serves two purposes, transcriptional repression and genome defence (Grace Goll & Bestor 2005). There is a growing body of evidence showing that transcriptional repression and genome defence is perturbed by changes in metabolism. Embryo culture techniques in farm animals frequently expose the preimplantation embryo to an inappropriate nutritional environment *in vitro*, leading to defective epigenetic programming and a number of developmental abnormalities collectively known as the large offspring syndrome (Young *et al.* 2001). Studies have revealed that the expression of a number of key genes is altered in these large offspring (Young & Beaujean 2004). Defects in epigenetic programming can also affect metabolism, the surviving offspring of mice produced by nuclear transfer (where the transferred nucleus undergoes extensive epigenetic reprogramming) exhibit an obese phenotype (Tamashiro *et al.* 2002). Many of the cytosine residues modified by methylation reside within parasitic DNA elements or retrotransposons, such as endogenous retroviruses. One of the best-studied examples is the Agouti mouse (Wolff *et al.* 1998). In this animal an endogenous retrovirus-like transposon sequence is inserted close to the gene coding for the Agouti protein. Normally a cryptic promoter within the retrotransposon is silenced by methylation allowing normal tissue-specific and regulated agouti expression. However, if this site is undermethylated the promoter is active and drives constitutive ectopic expression of the agouti gene, leading to yellow coat colour and obesity. It has been shown that the methylation status of these inserted viral DNA sequences can be modified by the methionine, folic acid and choline content of the maternal diet, illustrating that the extent of methylation is determined by events occurring *in utero* (Cooney *et al.* 2002). Adding additional methyl donors to the maternal diet increases methylation of the retrotransposon, suppresses ectopic gene expression and improves the outcome for the offspring. However, the wider relevance of this model still unclear and it is not known whether nutritional regulation of similar retrotransposon sequences is responsible for disease in humans.

The methylation of cytosine bases in DNA is tightly associated with modification of histone tails and other changes in chromosome structure (reviewed by Santos & Dean 2004). DNA is assembled on core histones; the tails of which are exposed on the nucleosome surface, where they are subject to a variety of enzyme-catalysed, post-translational modifications. The acetylation and methylation of histones, mediated by histone acetyl transferases and histone methyl transferases, changes the structure of the nucleosome and allows other proteins access to the DNA (Hermanson *et al.* 2002). A group of histone deacetylases reverses the acetylation of histones in a reaction that requires $NAD^{(+)}$ (Blander & Guarente 2004). These processes are essential for the differentiation of cells such as hepatocytes and adipocytes. It has been suggested that methylation and acetylation of histones introduces a 'histone code' which acts as a form of cellular memory influencing the subsequent binding and therefore function of transcriptional activators responsible for the differentiated phenotype (Turner 2002).

Chromatin remodelling is particularly important in the oocyte and early embryo. In these cells the pattern of co-activator expression is different from that observed in somatic cells, reflecting the specialised events occurring during early development (Zheng *et al.* 2005). In particular the modification of chromatin with poly ADP-ribose (derived from $NAD^{(+)}$) is essential for normal preimplantation development (Imamura *et al.* 2004). It is interesting to speculate that this requirement for $NAD^{(+)}$ during the early stages of development may be associated with the dependence of the pre-implantation embryo on pyruvate metabolism (Houghton & Leese 2004). The $NAD^{(+)}$ dependent deacetylases are also essential during the later stages of fetal development where loss of activity leads to reduced fetal growth and defects in the lung and pancreas (McBurney *et al.* 2003). In the liver of rat fetuses, the nutritional stresses produced by uteroplacental insufficiency produce specific changes in the association of

acetylated histones with the promoters of genes dependent on PPAR (Fu *et al.* 2004). Nutrient sensitive transcriptional activators, such as the C/EBPs and PPARs, are able to determine local chromatin structure through interactions with co-activator proteins. This provides a second indirect route for nutritionally mediated epigenetic programming of gene expression (Jia *et al.* 2004).

Post-implantation development and organogenesis

After implantation a number of discrete metabolic compartments are established within the fetus as the organs are formed. The feedback loops that regulate adult metabolism begin to develop during this period, presenting another opportunity for developmental programming. In the case of the insulin axis this involves the beta-cells of the pancreas which produce insulin and the insulin sensitive tissues which include the liver, muscle and adipose tissue (Fig 3). Key roles for mTOR, C/EBP and PPARs have been described in the development of tissues involved in maintaining metabolic homeostasis and in the adaptive response of adult animals to obesogenic diets. However less is known about the role of these gene nutrient interactions during fetal development.

Cells of the liver and pancreas originate from a common pool of progenitor cells in the ventral foregut endoderm. This close relationship is not surprising, as these tissues are responsible for processing the nutrients from ingested food and maintaining lipid and glucose concentrations in the blood. The proliferation of adult pancreatic beta cells is mediated by physiological concentrations of leucine and glucose *in vitro.* These nutrients stimulate the mTOR pathway which increases p70 S6 kinase phosphorylation (Xu *et al.* 2001) and DNA synthesis (Kwon *et al.* 2004). The mTOR pathway also plays a key role in regulating the proliferation of primary hepatocyte cultures (Coutant *et al.* 2002). However, studies of fetal livers during late gestation suggests that proliferation may be mTOR and insulin independent (Anand *et al.* 2002). Therefore the role of this signalling pathway during fetal development of both pancreas and liver remains unclear.

In contrast there is evidence that mTOR is involved in the regulation of fetal muscle development. In the adult, muscle is one of the main organs involved in the clearance of glucose and amino acids from the circulation and thus changes in muscle mass or insulin sensitivity greatly affect whole body glucose metabolism. For example, in sheep the development of muscles and skeleton was retarded in fetuses of nutrient-restricted ewes. The levels of mTOR and ribosomal protein S6 proteins were unchanged, but the phosphorylation of both proteins was reduced in muscle from nutrient-restricted fetuses. It is possible that the decrease in mTOR signalling reduces myoblast proliferation and reduces the number of secondary myofibers (Zhu *et al.* 2004).

The CHOP-10/gadd153 gene is not essential for the process of organogenesis as animals with a targeted deletion of the gene develop normally. This is despite it being widely expressed in fetal tissues. Data from adult animals show that the unfolded protein response, mediated though CHOP-10/gadd153, is important in regulating the apoptosis of beta cells (Oyadomari *et al.* 2002). The presence of misfolded insulin molecules induces the unfolded protein response (UPR) in the beta cell and leads to its destruction by apoptosis. These data suggest that this pathway is concerned with the response to stress rather than normal development. It will be interesting to see if animals with targeted deletions of CHOP-10/gadd153 show abnormal fetal development when they are exposed to nutritional stresses during gestation.

The CHOP-10/gadd153 and C/EBP genes play a pivotal role in the differentiation and final maturation of the beta cells, hepatocytes and adipocytes *in vitro* (Fig 2). Studies of animals with targeted deletions of the C/EBP alpha gene show that it is not essential for the morphological development of the liver *in vivo.* However the expression of C/EBP-alpha is required for the expression of genes involved in the normal metabolic functions of adult liver. Animals lacking C/EBP-alpha do not express glycogen synthase and die at the neonatal stage due to hypoglycaemia (Ramji & Foka 2002). In contrast deletion of the C/EBP-beta gene leads to early embryonic lethality because it is required for the development of the placenta. The C/EBP-beta gene also plays an important part in the

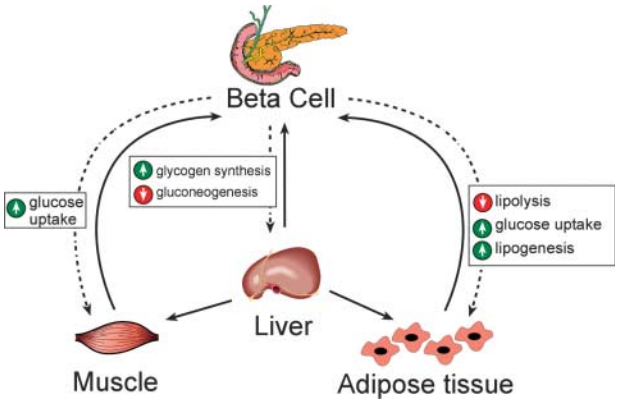

Figure 3 The feedback loops of the insulin axis involve a number of different tissues. These tissues learn to communicate with each other during the late fetal and early postnatal stages of development. Signalling from the beta cells of the pancreas is via insulin (dotted lines). This acts on liver, muscle and adipose tissue to change levels of glucose in the circulation (solid lines). The development of liver, muscle and adipose tissue is regulated by gene nutrient interactions. If the nutritional balance is perturbed by changes in the maternal diet, the development of tissues is also changed altering the characteristics of this feedback loop. The communication between these organs is a complex web where subtle changes in just one component can influence the behaviour of the whole system. For example, decreased maternal nutrition may suppress beta cell development resulting in fewer fetal beta cells, resulting in reduced insulin release, requiring a corresponding increase in insulin sensitivity of fetal liver, muscle or adipose tissue to maintain glucose homeostasis.

regulation of islet development (Lu *et al.* 1997). Analysis of C/EBP expression in the livers of fetuses exposed to a low protein diet fail to show any changes either in C/EBP or CHOP-10/gadd153 mRNA levels suggesting that maternal protein deficiency does not influence transcription (Maloney *et al.* 2005). However, other nutritional imbalances, e.g. hyperglycaemia, may induce different patterns of metabolic stress which lead to changes in CHOP-10/gadd153 expression or to changes in the C/EBP isoforms.

The role of PPARs in modulating the insulin axis has been highlighted by the development of pharmacological agonists for these receptors. Treatment with PPAR-gamma agonists improves whole body insulin sensitivity by redirecting lipid from non-adipose tissue towards adipocytes. This is accomplished by altering the expression of genes involved in lipid metabolism such as the aP2 fatty acid binding protein, the CD36 lipoprotein receptor, lipoprotein lipase as well as adipose signalling molecules such as leptin and resistin. In adults fed a high fat diet PPAR-alpha modulates islet insulin secretion and it has been suggested that it may act as a general sensor of overall tissue lipid supply (Sugden & Holness 2004). Animals with a targeted deletion of PPAR-alpha are fertile but adults have severe metabolic defects suggesting that PPAR-alpha plays a role later in development. In contrast deletion of the PPAR-gamma gene is lethal because the placenta fails to develop. When these embryos are rescued by forming chimeras, embryos with a deletion of PPAR-gamma survived showing that expression is not essential for organogenesis. However, the offspring are devoid of adipose tissue and die soon after birth (Barak *et al.* 1999). A targeted deletion of PPAR-gamma in the beta cells leads to islet hyperplasia in mice, however, the increase in islet mass in response to high-fat feeding does not occur (Rosen *et al.* 2003). Analysis of PPAR gene expression in the liver suggests that the expression of these mRNAs is low during the embryonic period (Balasubramaniyan *et al.* 2005). It will be interesting to see if nutritional imbalances, such as those induced by feeding high fat diets during gestation have any impact on gene expression.

The gene deletion experiments highlight the essential role of PPAR-gamma in adipogenesis. During development immature pre-adipocytes are held in an undifferentiated state through inhibition of C/EBP and PPAR expression by Wnt signalling (Ross *et al.* 2000, Bennett *et al.* 2002). In response to adipogenic stimuli pre adipose cells express C/EBP-beta and PPAR-delta. These transcriptional activators in turn initiate the expression of PPAR-gamma and C/EBP-alpha which then transcribe the genes involved in creating the complete adipocyte phenotype. Adipogenesis is controlled by a positive feedback loop, which is sensitive to long-chain, saturated, and polyunsaturated fatty acids through PPAR-gamma and to glucose through CHOP-10/gadd153 and C/EBP. Although this system is well understood in the adult there is little information on the regulation of adipogenesis during fetal development. The proliferation and differentiation of pre-adipocytes isolated from rat fetuses is not sensitive to the level of protein in the maternal diet (Bieswal *et al.* 2004). The available evidence suggests that changes in adiposity and fat deposition in animal models result from a change in the endocrine environment of the offspring. However, these studies do not rule out the possibility of changes in the numbers of pre adipose cells available for differentiation through the regulation of C/EBP and PPAR function.

Conclusions

It is becoming apparent that embryonic and fetal cells have a complex system to integrate nutritional signals from their environment and adapt their development accordingly to ensure survival. Human diets are comprised of complex mixtures of protein, fats, carbohydrate and vitamins. The full impact of inappropriate programming of metabolic regulation is only just beginning to be appreciated. The available evidence suggests that nutrient sensing regulatory systems are present in many critical tissues during early development. It remains to be seen whether they play an important part in establishing homeostatic control mechanisms early in life.

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