

PEDIATRIC REVIEW

Effect of intrauterine growth retardation on liver and long-term metabolic risk

S Cianfarani^{1,9}, C Agostoni^{2,9}, G Bedogni^{3,9}, R Berni Canani^{4,9}, P Brambilla^{5,9}, V Nobili^{6,9} and A Pietrobelli^{7,8,9}

Intrauterine growth retardation predisposes toward long-term morbidity from type 2 diabetes and cardiovascular disease. To explain this association, the concept of *programming* was introduced to indicate a process whereby a stimulus or insult at a critical period of development has lasting or lifelong consequences on key endocrine and metabolic pathways. Subtle changes in cell composition of tissues, induced by suboptimal conditions *in utero*, can influence postnatal physiological functions. There is increasing evidence, suggesting that liver may represent one of the candidate organs targeted by programming, undergoing structural, functional and epigenetic changes following exposure to an unfavorable intrauterine environment. The aim of this review is to provide insights into the molecular mechanisms underlying liver programming that contribute to increase the cardiometabolic risk in subjects with intrauterine growth restriction.

International Journal of Obesity (2012) 36, 1270–1277; doi:10.1038/ijo.2012.54; published online 24 April 2012

Keywords: non-alcoholic fatty liver disease (NAFLD); type 2 diabetes; cardiometabolic disease; programming; intrauterine growth retardation (IUGR)

THE CONCEPT OF PROGRAMMING

Epidemiological studies have shown an association between low-birth weight and the risk of developing the cluster of disorders such as abdominal adiposity, hypertension, dyslipidemia, hyperinsulinemia, glucose intolerance, type 2 diabetes and cardiovascular disease (CVD) that together lead to cardiometabolic disease (CMD), that is, the association between CVD and type 2 diabetes.^{1,2} To explain this association the thrifty phenotype hypothesis was proposed.³ According to this hypothesis, when the fetus is exposed to malnutrition the organism diverts the limited nutrient supply for favoring survival of vital organs such as the brain at the expense of growth and other organs such as pancreas.⁴ Fetal malnutrition or, in general, suboptimal uterine environment may induce permanent anatomical and functional changes in various tissues and organs, ultimately leading to increased risk of metabolic and CVD. The process by which early insults at critical stages of development lead to permanent changes in tissue structure and function is known as intrauterine programming.⁵ The critical time windows at which intrauterine programming may occur are the periods characterized by a high rate of tissue differentiation or proliferation.⁵ As a consequence of programming adult disease may arise *in utero* as a result of changes in the development of key endocrine and metabolic pathways during suboptimal intrauterine conditions ('fetal origins' hypothesis).⁶

Programming represents an adaptive response of the organism to the surrounding environment. A detrimental environment induces a series of phenotype changes aimed at favoring survival

under the adverse circumstances. However, the following exposure to a different (sometimes opposite) environment in extrauterine life determines a mismatch eventually leading to disease.^{7,8} In this context, prenatal malnutrition followed by postnatal overnutrition represents a mismatch leading the organism programmed *in utero* for surviving in conditions of limited nutrient supply, to develop obesity, type 2 diabetes and CVD, and ultimately premature death.⁹

THE MECHANISMS OF PROGRAMMING

The ability of the organism to change structure and function in response to environmental signals is named 'developmental plasticity'.^{10,11} Such plasticity permits a range of phenotypes to develop from a single genotype and is finalized to allow the organism to match its environment.¹² The evidence for invoking developmental plasticity as a biological property, which may influence the risk of disease, stems from numerous studies in animals in which dietary or endocrine challenges at various times from conception until weaning have been shown to induce persistent changes in cardiovascular and metabolic function in the offspring. The most commonly used animal models involve a prenatal nutrient imbalance, which can be induced by a global reduction in overall maternal food intake or by protein restriction in an isocaloric diet, or glucocorticoid exposure. When environmental cues act during windows of developmental plasticity, which correspond to the early phases of life, they may induce permanent changes as a result of biological 'tradeoffs'.

¹Molecular Endocrinology Unit-DPUO, Bambino Gesù Children's Hospital – 'Rina Balducci' Center of Pediatric Endocrinology, Tor Vergata University, Rome, Italy; ²Institute of Pediatrics, Fondazione IRCCS Cà Granda – Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; ³Clinical Epidemiology Unit, Liver Research Center, Trieste, Italy; ⁴Department of Pediatrics and European Laboratory for the Investigation of Food Induced Diseases, University of Naples 'Federico II', Italy; ⁵ASL Milano 2, Milan, Italy; ⁶Metabolic and Autoimmunity Liver Unit, Bambino Gesù Children's Hospital, Rome, Italy; ⁷Pediatric Unit, Verona University Medical School, Verona, Italy and ⁸Pennington Biomedical Research Center, Baton Rouge, LA, USA. Correspondence: Professor S Cianfarani, Molecular Endocrinology Unit-DPUO, Bambino Gesù Children's Hospital – 'Rina Balducci' Center of Pediatric Endocrinology, Tor Vergata University, Room E-178, Via Montpellier 1, I-00133, Rome, Italy.

E-mail: stefano.cianfarani@uniroma2.it

⁹Italian Hobbitt Network on Pediatric Nutrition.

Received 7 November 2011; revised 27 February 2012; accepted 4 March 2012; published online 24 April 2012

In animal models, intrauterine exposure to maternal undernourishment affects the expression of multiple genes involved in different metabolic pathways.¹³ These changes alter the ability to coordinate fat and carbohydrate metabolism, favoring a shift to a preferential use of fatty acids as an energy source in order to adapt the organism to the *in utero*-reduced nutrient supply. This finding is consistent with our observation showing that the exposure of rats to uteroplacental insufficiency induces adaptations in hypothalamic lipid sensing mechanisms, ultimately affecting food intake and endogenous glucose production in postnatal life.¹⁴ Another example of permanent alteration induced by developmental plasticity is provided by the observation that adult nephron numbers are reduced both in humans and in animals exposed to suboptimal uterine environment that develop hypertension in adult life.^{15–18} This might represent the consequence of a biological trade-off to spare energy in response to *in utero* nutrient deprivation, having immediate but no long-term adaptive value.

A major mechanism underlying the adaptive response leading to programming is the epigenetic modification of gene promoters involved in the control of key metabolic pathways.^{19,20} The epigenetic control of gene expression is based on modulation of chromatin structure and accessibility to transcription factors. This type of control is achieved by multiple mechanisms such as methylation–demethylation of cytidine–guanosine (CpG) sequences in the promoter regions, acetylation–deacetylation of lysine residues of core histones in the nucleosome and the presence of microRNA molecules that bind to complementary sequences in the 3' end of mRNA and reduce the rate of protein synthesis.²¹ Intrauterine cues determine the phenotype of the offspring by inducing epigenetic changes finalized to match the organism to its environment. However, if the emerging phenotype is not appropriately matched with the later (postnatal) environment, the risk of disease is increased.⁷ Multiple functions such as growth, metabolism, appetite, body composition, circulation, brain function, stress response, reproduction and longevity may be affected by epigenetic programming whose consequences result in life-long changes in gene expression.⁷ There is evidence showing that early exposure to suboptimal environment induces epigenetic changes predisposing to type 2 diabetes (Table 1).^{22–25}

In rats, the exposure to uteroplacental insufficiency induces hepatic DNA hypomethylation and histone hyperacetylation of histone H3 on lysine 9 (H3K9), lysine 14 (H3K14) and lysine 18 (H3K18) at birth.²⁶ These changes persist up to day 21 of postnatal life, suggesting a permanent effect on hepatic gene expression. The hyperacetylation on histone H3 in the liver of IUGR rats occurs in association with decreased nuclear protein levels

of histone deacetylase 1 (HDAC1) and HDAC activity.²² These site-specific changes in histone H3 acetylation alter the histone association with the promoter regions of PPAR-gamma coactivator (*PGC-1*) and carnitine–palmitoyl transferase I (*CPT1*), two genes that are persistently altered in the rat with intrauterine growth retardation. It is noteworthy that *PGC-1* expression is increased, whereas *CPT1* expression is reduced in IUGR rats predisposed to develop diabetes.^{27,28} *PGC-1* is a transcriptional coactivator that mediates hepatic glucose production by controlling mRNA levels of key gluconeogenic enzymes, such as glucose-6-phosphatase, phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase.²⁹ *CPT1* is a rate-limiting transporter in mitochondrial fatty acid β oxidation.³⁰ Altered expression of these two genes characterizes the liver of newborn rats exposed to adverse intrauterine environment, and persists postnatally (Table 1). Unfortunately, most of these and similar epigenetic studies do not provide information on the effects of the observed epigenetic changes on gene expression and lack of a prolonged postnatal follow-up of the study animals.

Therefore, epigenetics provides a molecular link between prenatal environment, genes, cellular processes and subsequent susceptibility to disease. Any cellular component may be affected by programming: membrane and nuclear hormone receptors, signaling pathways, ion channels, nutrient transporters, protein synthesis structures, enzymes, and mitochondria.⁵ An inhibitory effect of intrauterine malnutrition on the establishment of stem cell reservoir in the tissues has also been proposed to explain the early exhaustion of organs such as pancreas later in life.³¹

EVIDENCE FOR PROGRAMMING

Intrauterine programming of postnatal physiological functions has been demonstrated experimentally in several species using a range of techniques to compromise the intrauterine environment and induce fetal growth retardation, such as maternal stress, hypoxia, glucocorticoid administration, dietary manipulation or utero–placental insufficiency. Two previous papers highlighted the major experimental findings in different species.^{32,33}

In humans, evidence that embryo–fetal exposure to unfavorable uterine environment may predispose to disease in adulthood stems from the finding that individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–1945 showed a higher risk of developing cardiovascular and metabolic diseases in adulthood.³⁴ Interestingly, the subjects exposed to famine periconceptionally showed, 60 years later, decreased methylation of IGF-II, whereas no change in the degree of IGF-II methylation was observed in those exposed to famine late in gestation.³⁵ The reduced methylation of IGF-II may represent the

Table 1. The table lists the different genes whose function is linked to the development of type 2 diabetes

Model	Tissue	Gene	Gene function	Epigenetic change
IUGR rats ²²	Liver	<i>PPAR-γ</i> coactivator	Transcriptional coactivator of key gluconeogenic enzymes	H3K9 hyperacetylation affecting association with gene promoter
IUGR rats ²²	Liver	<i>CPT-1</i>	Rate-limiting transporter in mitochondrial fatty acid β -oxidation	H3K9 hyperacetylation affecting association with gene promoter
IUGR rats ²³	Pancreatic islets	<i>PDX-1</i>	Transcription factor critical for β -cell function and development	H3 and H4 deacetylation, H3K4 demethylation, H3K9 methylation
IUGR rats ²⁴	Skeletal muscle	<i>GLUT4</i>	Glucose transporter	H3K14 de-acetylation; H3K9 methylation
IUGR rats ²⁵	Pancreatic islets	<i>CGH-1</i>	Role in endothelial dysfunction and β -cell development	CpG hypermethylation in intergenic sequences
IUGR rats ²⁵	Pancreatic islets	<i>FGFR-1</i>	Fibroblast growth factor receptor	CpG hypomethylation in intergenic sequences
IUGR rats ²⁵	Pancreatic islets	<i>PCSK-5</i>	Role in peptide processing and maturation	CpG hypermethylation in transcription start site
Humans ³⁵	Blood	<i>IGF-II</i>	Fetal growth	CpG hypomethylation

Abbreviations: CGH-1, GTP cyclohydrolase 1; CPT-1, carnitine–palmitoyl transferase I; FGFR-1, fibroblast growth factor receptor 1; IGF-II, insulin-like growth factor-II; IUGR, intrauterine growth-retarded; PCSK-5, proprotein convertase subtilisin/ketin type 5; PDX-1, pancreatic and duodenal homeobox 1; PPAR- γ coactivator, peroxisome proliferator-activated receptor-gamma coactivator.

consequence of intrauterine exposure to deficient methyl donors supply, such as the amino acid methionine, although additional contribution of other stress factors such as cold and emotional stress cannot be ruled out. Consistent with the potential role of methyl donors in determining *IGF-II* gene methylation status is the observation that periconceptual folic acid use of the mother is related to increased methylation of the *IGF-II* gene in the offspring.³⁶ The daughters of women exposed to the Dutch Hunger Winter showed a decreased birth weight and an increased risk of insulin resistance, and their daughters were born with a lower birth weight,^{37,38} suggesting that programming can be transmitted to the following generations.³⁹

Prenatal stress or exposure to excess glucocorticoids represent further programming events during intrauterine life, linking low-birth weight with subsequent development of cardiovascular risk factors and disease.⁴⁰ Both hypertension and insulin resistance have been associated with antenatal glucocorticoid administration.^{41,42} The exposure to dexamethasone during fetal life results in hypothalamic–pituitary–adrenal axis (HPAA) programming in non-human primate offspring.⁴³

In humans, low-birth weight is associated with increased levels of plasma cortisol in both childhood and adulthood, and hyperactivity of HPAA.^{44–47} HPAA activation may ultimately lead to the metabolic rearrangement.⁴⁸ Finally, maternal stress may affect fetal growth and development leading to intrauterine growth retardation as suggested by the finding of increased rate of intrauterine growth restriction in the offspring of mothers who were pregnant and present in zones near the World Trade Center on 11 September 2001.⁴⁹ Epigenetics may intervene in the regulation of HPAA during fetal life as suggested by the association between methylation of the glucocorticoid receptor gene in human placentas and birth weight.⁵⁰

Despite there is a very large body of experimental evidence in animal models relating altered fetal development to later metabolic risk, most of these results cannot be easily applied to humans. For instance, although there has been considerable progress in the definition of the specific insults leading to organ programming in the rat, there remains a lack of detailed information on the mechanisms affecting programming in different species, such as the human, sheep and pig. Moreover, unlike rodents, in humans, organ development occurs over longer periods of time, thus allowing progressive fetal adaptations to *in utero* insults.³³

EVIDENCE FOR LIVER PROGRAMMING

Animal models

Virtually all organs can be programmed during fetal life thereafter changing their morphology and function permanently. There is abundant evidence that liver is a target for programming. The exposure to uteroplacental insufficiency alters the expression of genes encoding enzymes involved with hepatic energy production,⁵¹ decreasing hepatic oxidative phosphorylation⁵² and affecting liver glucose transport.⁵³

The impaired suppression of endogenous hepatic glucose production is an important component of the peripheral insulin resistance associated with type 2 diabetes. Peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1) is a key regulator of the expression of genes such as glucose-6-phosphatase (*G-6-Pase*), phosphoenolpyruvate carboxykinase (*PEPCK*) and fructose-1,6-bisphosphatase (*FBPase*) closely involved in hepatic gluconeogenesis. Intrauterine growth restriction secondary to uteroplacental insufficiency has been found to be associated with increased expression of *PGC-1* and consequent *G-6-Pase*, *PEPCK* and *FBPase* gene expression in liver of growth restricted animals.²⁸ Using the same experimental model, an impairment of hepatic fatty acid metabolism was demonstrated.²⁷

To investigate the effects of uteroplacental insufficiency on metabolic pathways of different organs, we have used a rat model of intrauterine growth retardation induced by maternal uterine artery ligation during late pregnancy.^{14,54,55} More recently, the same model of Sprague–Dawley newborn rats born to dams undergone uterine artery ligation was used to study the effects of uteroplacental insufficiency on liver metabolic pathways. To quantify the degree of liver gene expression, we applied PCR array on six sham and six IUGR livers from different litters. Each array contained a panel of 96 primer sets for a thoroughly researched set of 84 pathway-focused genes, plus 5 housekeeping genes, 1 rat genomic DNA contamination, 3 reverse transcription control and 3 positive PCR quality controls. Comparison of the gene expression profile of the 84 genes constituting the array showed that a total of 26 genes were differentially expressed in the liver of ligated rats versus sham animal. In all, 15 were upregulated and 11 were downregulated representing 30.9% and 13%, respectively, of the total number of analyzed genes. The functional classification of these genes further revealed that most of them are involved in signal transduction and regulation of metabolic processes. The metabolism of glucose seems to be the most affected by uteroplacental insufficiency as indicated by the lower expression of *Fbp1*, *Gpd*, *Pklr* and the upregulation of *Gck*, *Hk2* and *Slc2a1*. *Fbp1* encodes fructose-1,6-bisphosphatase, a key enzyme in the regulation of gluconeogenesis.⁵⁶ *Gck* encodes glucokinase phosphorylates glucose as first step of glucose utilization in hepatocytes.⁵⁷ The role of glucokinase is to provide glucose 6-phosphate for the synthesis of glycogen and fatty acids. *Gpd1*, *Hk2* and *Pklr* encode for glycolytic enzymes. *Gpd1* encodes glycerol-3-phosphate dehydrogenase 1 that has an important role in the synthesis of triacylglycerol and in the transport of reducing equivalents from the cytosol to mitochondria.⁵⁸ *Hk2* encodes hexokinase 2 that phosphorylates glucose to produce glucose-6-phosphate, the preliminary step of intracellular glucose metabolism.⁵⁹ *Pklr* encodes pyruvate kinase that catalyzes the production of phosphoenolpyruvate from pyruvate and ATP.⁶⁰ *Slc2a1* (previously known as *GLUT1*) encodes glucose transporter 1.⁶¹ *Acox1* encodes peroxisomal acyl-coenzyme A oxidase 1, which is the first enzyme of the fatty acid β -oxidation pathway, that catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. It donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide.⁶² The other genes whose expression resulted affected by uterine ligation were those encoding signal transduction molecules, such as *Map2k1* and *Pik3cb*, and transcription factors, such as *Cebpb*, *Fos*, *Jun*, *Pparg* and *Srebf1* (unpublished results, Table 2). Our study was limited at birth and investigated gene expression only. Therefore, the present data do not permit to know whether the alterations in gene expression observed at birth are transient or permanent, or whether they compensate each other or in the long term may determine metabolic dysregulation, eventually leading to insulin resistance and type 2 diabetes in adulthood, as previously described in the same animal model.⁶³

Maternal protein restriction represents an alternative method to induce intrauterine programming in the offspring. Deprivation of proteins during pregnancy in rat dams induces structural and functional changes in liver of the offspring ultimately affecting glucose production and insulin sensitivity.^{64–66} Gene expression profile of liver obtained from young adult male rats exposed to maternal undernourishment during pregnancy shows 249 differentially expressed genes whose expression pattern reflects the propensity to develop adiposity and insulin resistance.¹³ These animals show downregulation of expression of several key genes affecting the entry of glucose into the cell and its metabolism via the glycolytic and tricarboxylic acid pathways, and upregulation of genes involved in the intracellular trafficking oxidation of fatty acids.¹³ Maternal protein restriction induces multiorgan transcriptional alterations in the offspring,⁶⁷ determining a

Table 2. Overview of gene expression changes in the liver of IUGR newborn Sprague–Dawley rats obtained by maternal uterine artery ligation on day 19 of gestation vs control animals

Gene name	Gene symbol	GenBank	Fold Change	P-value
<i>Metabolism</i>				
Acyl-Coenzyme A oxidase 1, palmitoyl	<i>Acox1</i>	NM_017340	– 2.36	0.0005
Fructose-1,6-biphosphatase 1	<i>Fbp1</i>	NM_012558	– 3.64	0.0000
Glucokinase	<i>Gck</i>	NM_012565	1.4	0.0394
Glycerol-3-phosphate dehydrogenase 1 (soluble)	<i>Gpd1</i>	NM_022215	– 2.95	0.0002
Hexokinase 2	<i>Hk2</i>	NM_012735	2.88	0.0001
Pyruvate kinase	<i>Pklr</i>	NM_012624	– 1.55	0.0030
Solute carrier family 2 (facilitated glucose transporter), member 1	<i>Slc2a1</i>	NM_138827	5.32	0.0000
<i>Signal transduction</i>				
CCAAT/enhancer-binding protein (C/EBP), beta	<i>Cebpb</i>	NM_024125	2.01	0.0001
Docking protein 2	<i>Dok2</i>	XM_224344	– 1.96	0.0142
Eukaryotic translation initiation factor 4E-binding protein 1	<i>Eif4ebp1</i>	NM_053857	1.42	0.0039
FBJ osteosarcoma oncogene	<i>Fos</i>	NM_022197	5.2	0.0063
Fibroblast growth factor receptor substrate 3	<i>Frs3</i>	NM_001017382	– 1.9	0.0377
GRB2-associated binding protein 1	<i>Gab1</i>	XM_341667	– 1.42	0.0465
Growth factor receptor bound protein 2	<i>Grb2</i>	NM_030846	1.73	0.0213
Harvey rat sarcoma virus oncogene	<i>Hras</i>	XM_001062236	1.6	0.0006
Jun oncogene	<i>Jun</i>	NM_021835	3.92	0.0046
Mitogen-activated protein kinase kinase 1	<i>Map2k1</i>	NM_031643	– 1.64	0.0067
Peroxisome proliferator-activated receptor gamma	<i>Pparg</i>	NM_013124	1.68	0.0120
Phosphoinositide-3-kinase, catalytic, beta polypeptide	<i>Pik3cb</i>	NM_053481	– 1.47	0.0140
Son of sevenless homolog 1	<i>Sos1</i>	XM_233820	1.39	0.0429
Sterol regulatory element binding transcription factor 1	<i>Srebf1</i>	XM_213329	– 2.91	0.0053
V-raf murine sarcoma viral oncogene homolog B1	<i>Braf</i>	XM_231692	1.21	0.0495
V-raf-leukemia viral oncogene 1	<i>Raf1</i>	NM_012639	– 1.47	0.0070
Bcl2-like 1	<i>Bcl2l1</i>	NM_031535	2.8	0.0000
Insulin-like growth factor 1 receptor	<i>Igf1r</i>	NM_052807	1.73	0.0213
Insulin-like growth factor binding protein 1	<i>Igfbp1</i>	NM_013144	2.07	0.0084

rearrangement of transcription factor-binding sites specifically in the liver.⁶⁷

Maternal hypoxia is another experimental condition that induces intrauterine growth retardation. It has recently been demonstrated that the exposure to hypoxia *in utero* inhibits the expression of hepatic phospho-Akt-1, Akt-2 and PKC ζ in adult offspring.⁶⁸

It is noteworthy that maternal overfeeding may also program liver metabolism.⁶⁹ Mice offspring of dams with high fat intake during pregnancy show insulin resistance, reduced glucose transporter-2 expression and hepatic steatosis.^{70,71} Consistently, *in utero* exposure to high fat diet programs the expression and epigenetics of hepatic phosphoenolpyruvate carboxykinase gene in offspring rats⁷² and affects hepatic energy sensing pathways in a porcine model.⁷³ Finally, in heterozygous leptin receptor-deficient mice, the exposure to maternal gestational diabetes mellitus induces hepatic insulin resistance in the adult offspring.^{74,75}

Humans

In humans, the impairment of growth during fetal and early postnatal life has been associated with increased plasma concentrations of fibrinogen and factor VII in adulthood.⁷⁶ The high plasma concentrations of the two hemostatic factors may predispose to thrombosis and increase the risk of CVD. The finding that middle aged men and women with small abdominal circumference at birth, a proxy for liver size, show raised serum concentrations of total and low density lipoprotein cholesterol and apolipoprotein B suggests an impaired liver function in subjects with intrauterine growth retardation, eventually leading to altered cholesterol metabolism.^{77,78} To explain these findings, a redistribution of blood flow in favor of vital organs, such as the brain, heart and adrenal glands, at the expense of liver has been

hypothesized. The reduced blood supply to liver would limit the organ growth, ultimately leading to altered organ function.^{79,80} There is evidence suggesting that among men with low-birth weight, those with either a reduced or a large abdominal circumference at birth are at higher risk of coronary heart disease.⁷⁸

Another mechanism that has been advocated to explain the reduced liver function and metabolic risk in infants born small for gestational age is the impaired passage of long-chain polyunsaturated fatty acids (LCPUFAs) from mother to fetus during pregnancy.^{80,81} There is evidence suggesting that high dietary intake of LCPUFAs of the *n*-3 series is associated with low prevalence of hypertension, coronary heart disease and type 2 diabetes.^{82–85} LCPUFAs have protective effects against inflammation, platelet aggregation, hypertension and hyperlipidemia.⁸⁶ A significant association between birth weight and serum levels of eicosapentenoic acid and docosahexenoic acid, which have been associated with cardiometabolic risk,^{87–89} has recently been described in a large European cohort of adolescents (HELENA study).⁹⁰ In this context, early nutrition has been proposed to influence the metabolic risk in later life. Breast milk is rich in LCPUFAs,⁹¹ and breast feeding has been associated with lower risk of developing CMD in adult life.^{92–95} Preterm infants randomly assigned human milk versus formula, for 4 weeks, showed marked benefits for lipid profile,⁹⁴ blood pressure⁹³ and insulin sensitivity.⁹⁶ Consistent with the key role of PUFAs in liver metabolism is the effectiveness of docosahexenoic acid supplementation in reducing fat content in children with non-alcoholic fatty liver disease (NAFLD).⁹⁷

A study aimed at investigating peripheral and hepatic insulin action as well as intracellular partitioning of glucose fluxes in a cohort of young adult men with low-birth weight showed an enhanced suppression of hepatic glucose production in response to high insulin concentrations.⁹⁸ To explain this finding, an altered

programming of the expression patterns of glucose transporters was proposed.⁹⁸

Finally, early feeding habits may have a crucial role in programming the long-term metabolic risk. Breast feeding has protective effects against the risk of obesity, hypertension, hypercholesterolemia and type 2 diabetes.^{99–102} There is preliminary evidence suggesting that breast feeding may also affect the later expression of NAFLD, protecting the liver from the development of non-alcoholic steatohepatitis and fibrosis.¹⁰³

CLINICAL IMPLICATIONS

People who experienced intrauterine growth retardation are at increased risk for the development of CMD, including both type 2 diabetes and CVD. Type 2 diabetes represents a worldwide growing disease epidemic.¹⁰⁴ The two major factors leading to type 2 diabetes and associated NAFLD are chronic fuel surfeit leading to obesity and genetic predisposition. However, many overnourished and obese individuals do not develop diabetes, thus showing the importance of other predisposing factors such as genetic and epigenetic background. There is increasing evidence indicating that part of diabetes susceptibility is acquired early in life, through intrauterine programming via epigenetic phenomena (Figure 1). Maternal and early childhood health might, therefore, be crucial for the development of effective prevention strategies. Maternal periconceptual and early pregnancy nutritional status, as well as adequate nutrient supply to fetus, seem to have a key role in determining the metabolic outcome.³²

The epigenetic changes induced by an unfavorable uterine environment may affect the structure and function of multiple organs, such as the brain, pancreas, kidney, endothelium, skeletal and cardiac muscle, adipose tissue and liver, ultimately leading to

a detrimental programming that favor the susceptibility to CMD. In this context, liver programming increases the risk of type 2 diabetes by raising the endogenous hepatic glucose production that leads to persistent hyperglycemia (Figure 1).

Type 2 diabetes is strongly associated with NAFLD, each being highly predictive of the other, and is a determinant of its severity and liver-related mortality.¹⁰⁵ It is noteworthy that NAFLD has been reported to be associated with intrauterine growth retardation, with low-birth weight children showing high prevalence of non-alcoholic steatohepatitis.¹⁰⁶ This finding suggests that intrauterine liver programming together with adipose tissue dysfunction may substantially contribute to the onset of NAFLD and subsequent liver dysfunction stages. Therefore, in the complex network of factors contributing to the cardiometabolic risk liver programming certainly has a role whose importance probably varies among different individuals according to their genetic and epigenetic predisposition. Besides the adverse affect exerted by the exposure to a suboptimal intrauterine environment, the growth trajectory in early childhood seems to have a key role in increasing the risk of CMD in adult life. Rapid weight gain during the first 2 weeks of life in premature infants is associated with insulin resistance in adolescence.⁹⁶ Consistent with this finding is the observation that fast weight gain in the first 3 months of life is inversely associated with insulin sensitivity and serum high-density lipoprotein cholesterol level, whereas is positively associated with waist circumference, acute insulin response, ratio of total cholesterol to high-density lipoprotein cholesterol and level of triglycerides in early adulthood.¹⁰⁷ Finally, a large study on adults with coronary events showed that the association of low-birth weight and rapid weight gain after the age of 2 years was associated with insulin resistance and risk of coronary events in later life.¹⁰⁸ These findings could be explained by the fact that infants who have experienced intrauterine growth

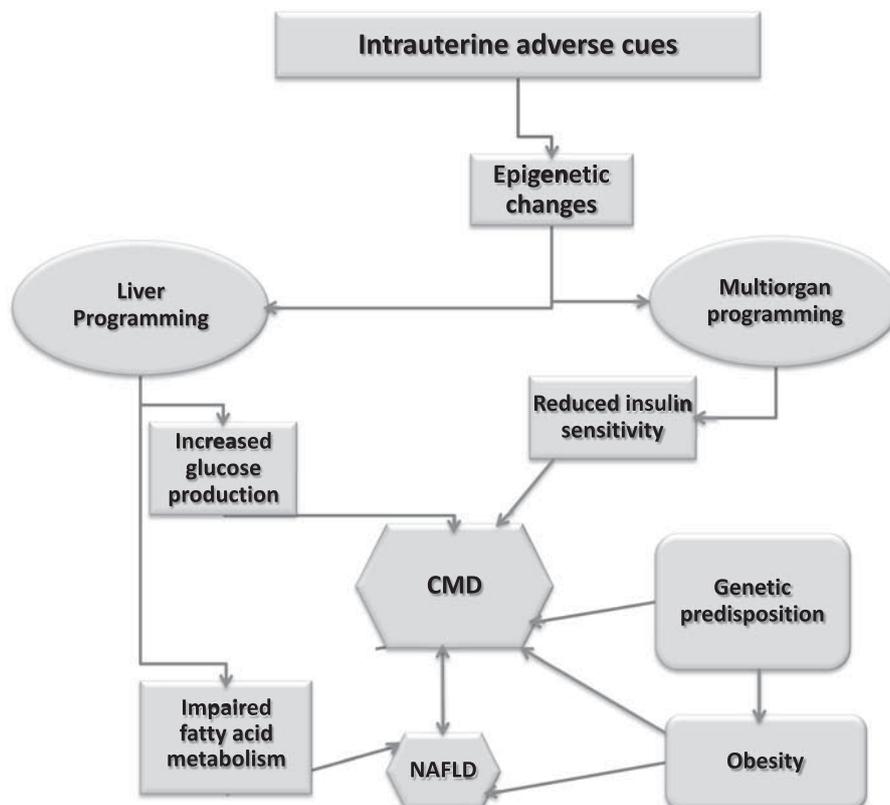


Figure 1. Interplay between liver programming and other mechanisms in the development of cardiometabolic disease (CMD), that is, the association between cardiovascular disease (CVD) and type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD).

retardation lack muscle mass at birth. This deficiency will persist into childhood and adulthood, as there is negligible muscle cell replication after birth.¹⁰⁹ Therefore, a rapid weight gain may lead to a disproportion between fat and muscle mass in favor of the former, ultimately leading to increased cardiometabolic risk.¹⁰⁸ These data indicate that any rational approach for preventing CMD should embrace a life-course perspective in order to promote maternal health from the periconceptual period, optimal fetal development and appropriate early postnatal nutrition and growth.

TOWARDS TARGETED INTERVENTIONS

Within the detrimental triad predisposing to CMD, including genetic predisposition, intrauterine programming and overweight, the latter two represent acquired susceptibility factors that are potentially preventable with strategies aimed at promoting maternal health before and during pregnancy and appropriate diet and exercise during childhood. Unfortunately, however, *in utero* programming is often independent of maternal health and nutritional status. Therefore, future research should take up the challenge to partially or totally reverse the established programming, taking advantage of the developmental plasticity still present in early postnatal life.¹¹⁰ The feasibility of this approach was demonstrated in rat by short-term administration of leptin in a specific time window of early postnatal life.¹¹¹ In another animal model, the supplementation of acid during the juvenile–pubertal period resulted effective in modifying the phenotype and epigenotype induced by prenatal nutritional constraint.¹¹² The rationale of a deprogramming intervention postponed to pubertal period was that the stability of epigenome is reduced during this phase, puberty representing another period of plasticity that can be exploited with targeted interventions.

Low-birth weight premature babies represent a population at risk of long-term metabolic disorders. They are usually fed with protein and calorie-enriched formulas to stimulate catch-up growth and brain development. However, the early exposure to high nutrient intake and rapid weight gain in infancy are associated with metabolic risk in adolescence and adulthood.^{96,107,113} Innovative feeding strategies should be finely balanced to guarantee appropriate brain development and catch-up growth, without exposing the infant to postnatal nutritional programming.¹¹³ Future research will test the feasibility and effectiveness of targeted nutritional interventions at different stages of development (from periconceptual time to puberty) in preventing or reversing the epigenetic programming.¹¹⁴

Finally, the knowledge of epigenomic markers, such as methylation patterns in specific gene promoters, may enable the identification of individuals who will have increased susceptibility to chronic disease in adulthood because of adverse factors in their early environment. The identification of such individuals may allow targeted prevention strategies, either by lifestyle modification or by active nutritional or pharmacological interventions.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Animal studies have provided compelling evidence for liver programming induced by intrauterine exposure to adverse cues. In humans, data on the hepatic involvement in the developmental origin of CMD are still limited. Further research in man is needed to elucidate whether and to what extent a suboptimal intrauterine environment may substantially alter liver structure and function, thus contributing to the susceptibility to fat accumulation and metabolic risk in later life. Long-term prospective studies from birth to adulthood or retrospective studies in well-characterized cohort of subjects with low-birth weight exposed to different diet regimens and life styles in postnatal life would ascertain whether

liver undergoes changes predisposing or accompanying the development of CMD.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

- 1 Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986; **1**: 1077–1081.
- 2 Barker D, Winter P, Osmond C, Margetts B, Simmonds S. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989; **2**: 577–580.
- 3 Hales C, Barker D. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992; **35**: 595–601.
- 4 Hales C, Barker D. The thrifty phenotype hypothesis. *Br Med Bull* 2001; **60**: 5–20.
- 5 Fowden A, Giussani D, Forhead A. Intrauterine programming of physiological systems: causes and consequences. *Physiology (Bethesda)* 2006; **21**: 29–37.
- 6 Barker DJ. The fetal and infant origins of adult disease. *BMJ* 1990; **301**: 1111.
- 7 Godfrey K, Lillycrop K, Burdge G, Gluckman P, Hanson M. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatr Res* 2007; **61**: 5R–10R.
- 8 Cianfarani S, Germani D, Branca F. Low birthweight and adult insulin resistance: the "catch-up growth" hypothesis. *Arch Dis Child Fetal Neonatal Ed* 1999; **81**: F71–F73.
- 9 Ozanne SE, Hales CN. Lifespan: catch-up growth and obesity in male mice. *Nature* 2004; **427**: 411–412.
- 10 Gluckman P, Hanson M. Living with the past: evolution, development, and patterns of disease. *Science* 2004; **305**: 1733–1736.
- 11 Canani RB, Di Costanzo M, Leone L, Bedogni G, Brambilla P, Cianfarani S *et al*. Epigenetic mechanisms elicited by nutrition in early life. *Nutr Res Rev* 2011 **1**–8.
- 12 Gluckman P, Hanson M, Bateson P, Beedle A, Law C, Bhutta Z *et al*. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet* 2009; **373**: 1654–1657.
- 13 Morris TJ, Vickers M, Gluckman P, Gilmour S, Affara N. Transcriptional profiling of rats subjected to gestational undernourishment: implications for the developmental variations in metabolic traits. *PLoS One* 2009; **4**: e7271.
- 14 Puglianiello A, Germani D, Antignani S, Tomba GS, Cianfarani S. Changes in the expression of hypothalamic lipid sensing genes in rat model of intrauterine growth retardation (IUGR). *Pediatr Res* 2007; **61**: 433–437.
- 15 Ritz E, Amann K, Koleganova N, Benz K. Prenatal programming-effects on blood pressure and renal function. *Nat Rev Nephrol* 2011; **7**: 137–144.
- 16 Moritz KM, Singh RR, Probyn ME, Denton KM. Developmental programming of a reduced nephron endowment: more than just a baby's birth weight. *Am J Physiol Renal Physiol* 2009; **296**: F1–F9.
- 17 Gingery A, Soldner EL, Heltemes A, Nelson A, Bozadjieva N. Developmental programming of the kidney: does sex matter? *J Physiol* 2009; **587**: 5521–5522.
- 18 Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat *Life Sci* 1999; **64**: 965–974.
- 19 Gluckman P, Hanson M, Buklijas T, Low F, Beedle A. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol* 2009; **5**: 401–408.
- 20 Pinney S, Simmons R. Epigenetic mechanisms in the development of type 2 diabetes. *Trends Endocrinol Metab* 2010; **21**: 223–229.
- 21 Goldberg A, Allis C, Bernstein E. Epigenetics: a landscape takes shape. *Cell* 2007; **128**: 635–638.
- 22 Fu Q, McKnight R, Yu X, Wang L, Callaway C, Lane R. Uteroplacental insufficiency induces site-specific changes in histone H3 covalent modifications and affects DNA-histone H3 positioning in day 0 IUGR rat liver. *Physiol Genomics* 2004; **20**: 108–116.
- 23 Park J, Stoffers D, Nicholls R, Simmons R. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. *J Clin Invest* 2008; **118**: 2316–2324.
- 24 Raychaudhuri N, Raychaudhuri S, Thamocharan M, Devaskar S. Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring. *J Biol Chem* 2008; **283**: 13611–13626.
- 25 Thompson R, Fazzari M, Niu H, Barzilai N, Simmons R, Grealley J. Experimental intrauterine growth restriction induces alterations in DNA methylation and gene expression in pancreatic islets of rats. *J Biol Chem* 2010; **285**: 15111–15118.
- 26 MacLennan N, James S, Melnyk S, Piroozzi A, Jernigan S, Hsu J *et al*. Uteroplacental insufficiency alters DNA methylation, one-carbon metabolism, and histone acetylation in IUGR rats. *Physiol Genomics* 2004; **18**: 43–50.
- 27 Lane R, Kelley D, Gruetzmacher E, Devaskar S. Uteroplacental insufficiency alters hepatic fatty acid-metabolizing enzymes in juvenile and adult rats. *Am J Physiol Regul Integr Comp Physiol* 2001; **280**: R183–R190.

- 28 Lane R, MacLennan N, Hsu J, Janke S, Pham T. Increased hepatic peroxisome proliferator-activated receptor-gamma coactivator-1 gene expression in a rat model of intrauterine growth retardation and subsequent insulin resistance. *Endocrinology* 2002; **143**: 2486–2490.
- 29 Yoon J, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001; **413**: 131–138.
- 30 McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system - from concept to molecular analysis. *Eur J Biochem* 1997; **244**: 1–14.
- 31 Cianfarani S. Foetal origins of adult diseases: just a matter of stem cell number? *Med Hypotheses* 2003; **61**: 401–404.
- 32 Fowden AL, Giussani DA, Forhead AJ. Endocrine and metabolic programming during intrauterine development. *Early Hum Dev* 2005; **81**: 723–734.
- 33 McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev* 2005; **85**: 571–633.
- 34 Lumey L, Stein A, Kahn H, van der Pal-de Bruin K, Blauw G, Zybert P et al. Cohort profile: the Dutch Hunger Winter families study. *Int J Epidemiol* 2007; **36**: 1196–1204.
- 35 Heijmans B, Tobi E, Stein A, Putter H, Blauw G, Susser E et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* 2008; **105**: 17046–17049.
- 36 Steegers-Theunissen R, Obermann-Borst S, Kremer D, Lindemans J, Siebel C, Steegers E et al. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child *PLoS One* 2009; **4**: e7845.
- 37 Stein A, Lumey L. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch Famine Birth Cohort Study. *Hum Biol* 2000; **72**: 641–654.
- 38 Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab* 2010; **21**: 214–222.
- 39 Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, Schallert T et al. Transgenerational epigenetic imprints on mate preference. *Proc Natl Acad Sci USA* 2007; **104**: 5942–5946.
- 40 Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nat Clin Pract Endocrinol Metab* 2007; **3**: 479–488.
- 41 Doyle LW, Ford GW, Davis NM, Callanan C. Antenatal corticosteroid therapy and blood pressure at 14 years of age in preterm children. *Clin Sci (Lond)* 2000; **98**: 137–142.
- 42 Dalziel SR, Walker NK, Parag V, Mantell C, Rea HH, Rodgers A et al. Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. *Lancet* 2005; **365**: 1856–1862.
- 43 de Vries A, Holmes MC, Heijnis A, Seier JV, Heerden J, Louw J et al. Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. *J Clin Invest* 2007; **117**: 1058–1067.
- 44 Cianfarani S, Geremia C, Scott CD, Growth Germani D. IGF system, and cortisol in children with intrauterine growth retardation: is catch-up growth affected by reprogramming of the hypothalamic-pituitary-adrenal axis? *Pediatr Res* 2002; **51**: 94–99.
- 45 Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ et al. Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? *J Clin Endocrinol Metab* 1998; **83**: 757–760.
- 46 Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C et al. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension* 2000; **35**: 1301–1306.
- 47 Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB et al. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab* 2001; **86**: 245–250.
- 48 Buhl ES, Neschen S, Yonemitsu S, Rossbacher J, Zhang D, Morino K et al. Increased hypothalamic-pituitary-adrenal axis activity and hepatic insulin resistance in low-birth-weight rats. *Am J Physiol Endocrinol Metab* 2007; **293**: E1451–E1458.
- 49 Berkowitz GS, Wolff MS, Janevic TM, Holzman IR, Yehuda R, Landrigan PJ. The World Trade Center disaster and intrauterine growth restriction. *JAMA* 2003; **290**: 595–596.
- 50 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE et al. Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. *Epigenetics* 2011; **6**: 566–572.
- 51 Lane RH, Flozak AS, Ogata ES, Bell GI, Simmons RA. Altered hepatic gene expression of enzymes involved in energy metabolism in the growth-retarded fetal rat. *Pediatr Res* 1996; **39**: 390–394.
- 52 Ogata ES, Swanson SL, Collins JW, Finley SL. Intrauterine growth retardation: altered hepatic energy and redox states in the fetal rat. *Pediatr Res* 1990; **27**: 56–63.
- 53 Lane RH, Crawford SE, Flozak AS, Simmons RA. Localization and quantification of glucose transporters in liver of growth-retarded fetal and neonatal rats. *Am J Physiol* 1999; **276**: E135–E142.
- 54 Germani D, Puglianiello A, Cianfarani S. Uteroplacental insufficiency down regulates insulin receptor and affects expression of key enzymes of long-chain fatty acid (LCFA) metabolism in skeletal muscle at birth. *Cardiovasc Diabetol* 2008; **7**: 14.
- 55 Puglianiello A, Germani D, Cianfarani S. Exposure to uteroplacental insufficiency reduces the expression of signal transducer and activator of transcription 3 and proopiomelanocortin in the hypothalamus of newborn rats. *Pediatr Res* 2009; **66**: 208–211.
- 56 Moon S, Kim JH, Han JH, Ko SH, Ahn YB, Yang SH et al. Novel compound heterozygous mutations in the fructose-1,6-bisphosphatase gene cause hypoglycemia and lactic acidosis. *Metabolism* 2011; **60**: 107–113.
- 57 Jiang MH, Fei J, Lan MS, Lu ZP, Liu M, Fan WW et al. Hypermethylation of hepatic Gck promoter in ageing rats contributes to diabetogenic potential. *Diabetologia* 2008; **51**: 1525–1533.
- 58 Harding JW, Pyeritz EA, Copeland ES, White HB. Role of glycerol 3-phosphate dehydrogenase in glyceride metabolism. Effect of diet on enzyme activities in chicken liver. *Biochem J* 1975; **146**: 223–229.
- 59 Moreno F, Herrero P. The hexokinase 2-dependent glucose signal transduction pathway of *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 2002; **26**: 83–90.
- 60 Manco L, Ribeiro ML. Novel human pathological mutations. Gene symbol: PKLR. Disease: pyruvate kinase deficiency. *Hum Genet* 2009; **125**: 343.
- 61 Olson AL, Pessin JE. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr* 1996; **16**: 235–256.
- 62 Huang J, Jia Y, Fu T, Viswakarma N, Bai L, Rao MS et al. Sustained activation of PPAR[alpha] by endogenous ligands increases hepatic fatty acid oxidation and prevents obesity in ob/ob mice. *FASEB J* 2011; **26**: 628–638.
- 63 Simmons R, Templeton L, Gertz S. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. *Diabetes* 2001; **50**: 2279–2286.
- 64 Burns SP, Desai M, Cohen RD, Hales CN, Iles RA, Germain JP et al. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *J Clin Invest* 1997; **100**: 1768–1774.
- 65 Desai M, Byrne CD, Zhang J, Petry CJ, Lucas A, Hales CN. Programming of hepatic insulin-sensitive enzymes in offspring of rat dams fed a protein-restricted diet. *Am J Physiol* 1997; **272**: G1083–G1090.
- 66 Desai M, Byrne CD, Meeran K, Martenz ND, Bloom SR, Hales CN. Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet. *Am J Physiol* 1997; **273**: G899–G904.
- 67 Vaiman D, Gascoin-Lachambre G, Boubred F, Mondon F, Feuerstein JM, Ligi I et al. The intensity of IUGR-induced transcriptome deregulations is inversely correlated with the onset of organ function in a rat model. *PLoS One* 2011; **6**: e21222.
- 68 Camm EJ, Martin-Gronert MS, Wright NL, Hansell JA, Ozanne SE, Giussani DA. Prenatal hypoxia independent of undernutrition promotes molecular markers of insulin resistance in adult offspring. *FASEB J* 2011; **25**: 420–427.
- 69 Drake AJ, Reynolds RM. Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction* 2010; **140**: 387–398.
- 70 Gregorio BM, Souza-Mello V, Carvalho JJ, Mandarim-de-Lacerda CA, Aguilá MB. Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/6 offspring. *Am J Obstet Gynecol* 2010; **203**: 495.e1–495.e8.
- 71 Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology* 2009; **50**: 1796–1808.
- 72 Strakovsky RS, Zhang X, Zhou D, Pan YX. Gestational high fat diet programs hepatic phosphoenolpyruvate carboxykinase (Pck) expression and histone modification in neonatal offspring rats. *J Physiol* 2011; **589**: 2707–2717.
- 73 Oster M, Murani E, Metges CC, Ponsuksili S, Wimmers K. A high protein diet during pregnancy affects hepatic gene expression of energy sensing pathways along ontogenesis in a porcine model. *PLoS One* 2011; **6**: e21691.
- 74 Yamashita H, Shao J, Qiao L, Pagliassotti M, Friedman JE. Effect of spontaneous gestational diabetes on fetal and postnatal hepatic insulin resistance in Lepr(db/+) mice. *Pediatr Res* 2003; **53**: 411–418.
- 75 Mulay S, Philip A, Solomon S. Influence of maternal diabetes on fetal rat development: alteration of insulin receptors in fetal liver and lung. *J Endocrinol* 1983; **98**: 401–410.
- 76 Barker DJ, 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. *BMJ* 1992; **304**: 148–152.

- 77 Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH. Growth in utero and serum cholesterol concentrations in adult life. *BMJ* 1993; **307**: 1524–1527.
- 78 Barker DJ, Martyn CN, Osmond C, Wield GA. Abnormal liver growth in utero and death from coronary heart disease. *BMJ* 1995; **310**: 703–704.
- 79 Barker DJ, Hanson MA. Altered regional blood flow in the fetus: the origins of cardiovascular disease? *Acta Paediatr* 2004; **93**: 1559–1560.
- 80 Latini G, De Mitri B, Del Vecchio A, Chitano G, De Felice C, Zetterström R. Foetal growth of kidneys, liver and spleen in intrauterine growth restriction: "programming" causing "metabolic syndrome" in adult age. *Acta Paediatr* 2004; **93**: 1635–1639.
- 81 Cetin I, Giovannini N, Alvino G, Agostoni C, Riva E, Giovannini M *et al*. Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatr Res* 2002; **52**: 750–755.
- 82 Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF, Beilin LJ. Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am J Clin Nutr* 1999; **70**: 817–825.
- 83 Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes Relat Metab Disord* 1997; **21**: 637–643.
- 84 Jelenik T, Rossmeisl M, Kuda O, Jilkova ZM, Medrikova D, Kus V *et al*. AMP-activated protein kinase $\alpha 2$ subunit is required for the preservation of hepatic insulin sensitivity by n-3 polyunsaturated fatty acids. *Diabetes* 2010; **59**: 2737–2746.
- 85 Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M *et al*. n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. *Diabetologia* 2009; **52**: 941–951.
- 86 Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. *J Am Diet Assoc* 2009; **109**: 668–679.
- 87 Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT, Hulley SB. Serum fatty acids and the risk of coronary heart disease. *Am J Epidemiol* 1995; **142**: 469–476.
- 88 Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC *et al*. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002; **346**: 1113–1118.
- 89 Lindqvist HM, Sandberg AS, Fagerberg B, Hulthe J. Plasma phospholipid EPA and DHA in relation to atherosclerosis in 61-year-old men. *Atherosclerosis* 2009; **205**: 574–578.
- 90 Labayen I, Moreno LA, Ruiz JR, Ortega FB, Sjostrom M, Huybrechts I *et al*. Associations of birth weight with serum long chain polyunsaturated fatty acids in adolescents; the HELENA study. *Atherosclerosis* 2011; **217**: 286–291.
- 91 Agostoni C. Role of long-chain polyunsaturated fatty acids in the first year of life. *J Pediatr Gastroenterol Nutr* 2008; **47**(Suppl 2): S41–S44.
- 92 Das UN. A perinatal strategy to prevent coronary heart disease. *Nutrition* 2003; **19**: 1022–1027.
- 93 Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet* 2001; **357**: 413–419.
- 94 Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet* 2004; **363**: 1571–1578.
- 95 Martin RM, Davey Smith G. Does having been breastfed in infancy influence lipid profile in later life?: A review of the literature. *Adv Exp Med Biol* 2009; **646**: 41–50.
- 96 Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* 2003; **361**: 1089–1097.
- 97 Nobili V, Bedogni G, Alisi A, Pietrobattista A, Risé P, Galli C *et al*. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. *Arch Dis Child* 2011; **96**: 350–353.
- 98 Jensen CB, Storgaard H, Dela F, Holst JJ, Madsbad S, Vaag AA. Early differential defects of insulin secretion and action in 19-year-old caucasian men who had low birth weight. *Diabetes* 2002; **51**: 1271–1280.
- 99 Arenz S, Ruckerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity—a systematic review. *Int J Obes Relat Metab Disord* 2004; **28**: 1247–1256.
- 100 Martin RM, Gunnell D, Smith GD. Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis. *Am J Epidemiol* 2005; **161**: 15–26.
- 101 Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *Am J Clin Nutr* 2006; **84**: 1043–1054.
- 102 Owen CG, Whincup PH, Kaye SJ, Martin RM, Davey Smith G, Cook DG *et al*. Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. *Am J Clin Nutr* 2008; **88**: 305–314.
- 103 Nobili V, Bedogni G, Alisi A, Pietrobattista A, Alterio A, Tiribelli C *et al*. A protective effect of breastfeeding on the progression of non-alcoholic fatty liver disease. *Arch Dis Child* 2009; **94**: 801–805.
- 104 Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* 2011; **378**: 169–181.
- 105 Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. *Nat Rev Endocrinol* 2011; **7**: 456–465.
- 106 Nobili V, Marcellini M, Marchesini G, Vanni E, Manco M, Villani A *et al*. Intrauterine growth retardation, insulin resistance, and nonalcoholic fatty liver disease in children. *Diabetes Care* 2007; **30**: 2638–2640.
- 107 Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009; **301**: 2234–2242.
- 108 Barker D, Osmond C, Forsén T, Kajantie E, Eriksson J. Trajectories of growth among children who have coronary events as adults. *N Engl J Med* 2005; **353**: 1802–1809.
- 109 Widdowson EM, Crabb DE, Milner RD. Cellular development of some human organs before birth. *Arch Dis Child* 1972; **47**: 652–655.
- 110 Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel JC *et al*. Child health, developmental plasticity, and epigenetic programming. *Endocr Rev* 2011; **32**: 159–224.
- 111 Vickers M, Gluckman P, Coveny A, Hofman P, Cutfield W, Gertler A *et al*. Neonatal leptin treatment reverses developmental programming. *Endocrinology* 2005; **146**: 4211–4216.
- 112 Burdge GC, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA, Hanson MA. Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *J Nutr* 2009; **139**: 1054–1060.
- 113 Wiedmeier JE, Joss-Moore LA, Lane RH, Neu J. Early postnatal nutrition and programming of the preterm neonate. *Nutr Rev* 2011; **69**: 76–82.
- 114 Choi SW, Friso S, Epigenetics A. New Bridge between nutrition and health. *Adv Nutr (Bethesda)* 2010; **1**: 8–16.