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PEDIATRIC REVIEW

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

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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.

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## 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'.

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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 (*CPTI*), two genes that are persistently altered in the rat with intrauterine growth retardation. It is noteworthy that *PGC-1* expression is increased, whereas *CPTI* 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] CPTI 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 | *PPAR-γ* coactivator | Transcriptional coactivator of key gluconeogenic enzymes | H3K9 hyperacetylation affecting association with gene promoter |
| IUGR rats[22] | Liver | *CPT-I* | Rate-limiting transporter in mitochondrial fatty acid β-oxidation | H3K9 hyperacetylation affecting association with gene promoter |
| IUGR rats[23] | Pancreatic islets | *PDX-1* | Transcription factor critical for β-cell function and development | H3 and H4 deacetylation, H3K4 demethylation, H3K9 methylation |
| IUGR rats[24] | Skeletal muscle | *GLUT4* | Glucose transporter | H3K14 de-acetylation; H3K9 methylation |
| IUGR rats[25] | Pancreatic islets | *CGH-1* | Role in endothelial dysfunction and β-cell development | CpG hypermethylation in intergenic sequences |
| IUGR rats[25] | Pancreatic islets | *FGFR-1* | Fibroblast growth factor receptor | CpG hypomethylation in intergenic sequences |
| IUGR rats[25] | Pancreatic islets | *PCSK-5* | Role in peptide processing and maturation | CpG hypermethylation in transcription start site |
| Humans[35] | Blood | *IGF-II* | Fetal growth | CpG hypomethylation |

Abbreviations: CGH-1, GTP cyclohydrolase 1; CPT-I, 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 periconceptional 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 phosphorylation[52] 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-$\gamma$ 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 $\beta$-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* |
|---|---|---|---|---|
| *Metabolism* | | | | |
| Acyl-Coenzyme A oxidase 1, palmitoyl | *Acox1* | NM_017340 | − 2.36 | 0.0005 |
| Fructose-1,6-biphosphatase 1 | *Fbp1* | NM_012558 | − 3.64 | 0.0000 |
| Glucokinase | *Gck* | NM_012565 | 1.4 | 0.0394 |
| Glycerol-3-phosphate dehydrogenase 1 (soluble) | *Gpd1* | NM_022215 | − 2.95 | 0.0002 |
| Hexokinase 2 | *Hk2* | NM_012735 | 2.88 | 0.0001 |
| Pyruvate kinase | *Pklr* | NM_012624 | − 1.55 | 0.0030 |
| Solute carrier family 2 (facilitated glucose transporter), member 1 | *Slc2a1* | NM_138827 | 5.32 | 0.0000 |
| *Signal transduction* | | | | |
| CCAAT/enhancer-binding protein (C/EBP), beta | *Cebpb* | NM_024125 | 2.01 | 0.0001 |
| Docking protein 2 | *Dok2* | XM_224344 | − 1.96 | 0.0142 |
| Eukaryotic translation initiation factor 4E-binding protein 1 | *Eif4ebp1* | NM_053857 | 1.42 | 0.0039 |
| FBJ osteosarcoma oncogene | *Fos* | NM_022197 | 5.2 | 0.0063 |
| Fibroblast growth factor receptor substrate 3 | *Frs3* | NM_001017382 | − 1.9 | 0.0377 |
| GRB2-associated binding protein 1 | *Gab1* | XM_341667 | − 1.42 | 0.0465 |
| Growth factor receptor bound protein 2 | *Grb2* | NM_030846 | 1.73 | 0.0213 |
| Harvey rat sarcoma virus oncogene | *Hras* | XM_001062236 | 1.6 | 0.0006 |
| Jun oncogene | *Jun* | NM_021835 | 3.92 | 0.0046 |
| Mitogen-activated protein kinase kinase 1 | *Map2k1* | NM_031643 | − 1.64 | 0.0067 |
| Peroxisome proliferator-activated receptor gamma | *Pparg* | NM_013124 | 1.68 | 0.0120 |
| Phosphoinositide-3-kinase, catalytic, beta polypeptide | *Pik3cb* | NM_053481 | − 1.47 | 0.0140 |
| Son of sevenless homolog 1 | *Sos1* | XM_233820 | 1.39 | 0.0429 |
| Sterol regulatory element binding transcription factor 1 | *Srebf1* | XM_213329 | − 2.91 | 0.0053 |
| V-raf murine sarcoma viral oncogene homolog B1 | *Braf* | XM_231692 | 1.21 | 0.0495 |
| V-raf-leukemia viral oncogene 1 | *Raf1* | NM_012639 | − 1.47 | 0.0070 |
| Bcl2-like 1 | *Bcl2l1* | NM_031535 | 2.8 | 0.0000 |
| Insulin-like growth factor 1 receptor | *Igf1r* | NM_052807 | 1.73 | 0.0213 |
| Insulin-like growth factor binding protein 1 | *Igfbp1* | 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 PKCζ 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 rats[72] 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 pressure[93] 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

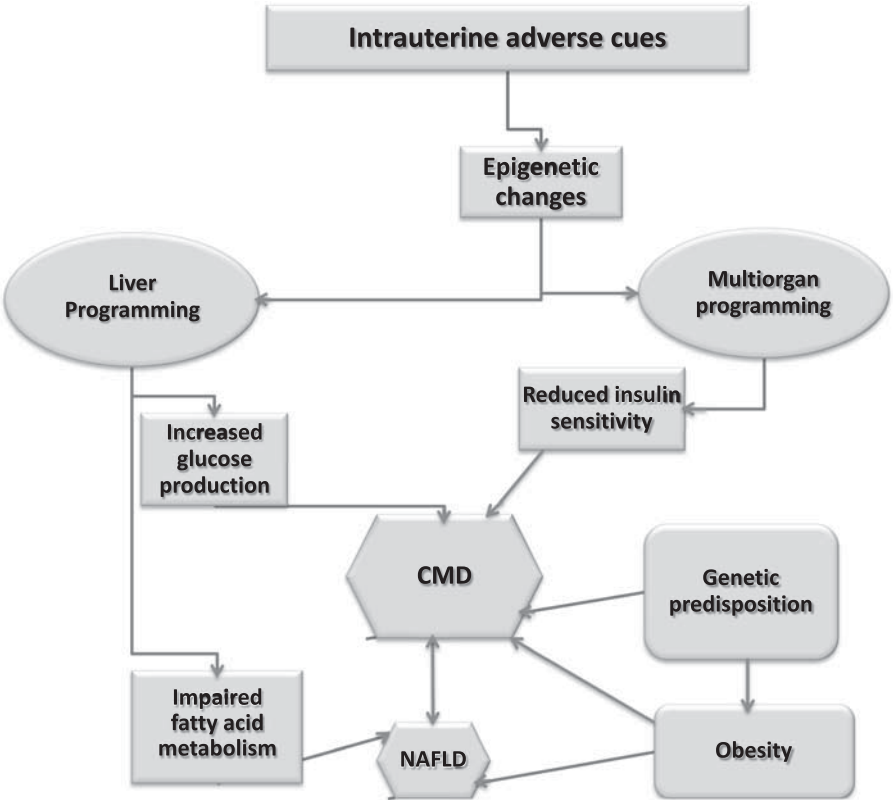


**Figure 1.** Interplay between liver programming and other mechanisms in the development of cardiometabolic disease (CMD), that is, the association between cardiovascular disease (CVD) and type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD).

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 periconceptional 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

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