Early nutrition, epigenetics and cardiovascular disease

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Purpose of review:

Here, we provide a summary of the current knowledge on the impact of early life nutrition on cardiovascular diseases that have emerged from studies in humans and experimental animal models. The involvement of epigenetic mechanisms in the Developmental Origins of Health and Disease (DOHaD) will be discussed in relation to the implications for the heart and the cardiovascular system.

Recent findings:

Environmental cues, such as parental diet and a suboptimal *in utero* environment can shape growth and development, causing long-lasting cardiometabolic perturbations. Increasing evidence suggest that these effects are mediated at the epigenomic level, and can be passed onto future generations. In the last decade, epigenetic mechanisms (DNA methylation, histone modifications) and RNA-based mechanisms (microRNAs [miRNAs], Piwi-interacting RNA [piRNAs] and transfer RNA [tRNA] fragments) have therefore emerged as potential candidates for mediating inheritance of cardiometabolic diseases.

Summary:

The burden of obesity and associated cardiometabolic diseases is believed to arise through interaction between an individual's genetics and the environment. Moreover, the risk of developing poor cardiometabolic health in adulthood is defined by early life exposure to pathological cues and can be inherited by future generations, initiating a vicious cycle of transmission of disease. Elucidating the molecular triggers of such process will help tackle and prevent the uncontrolled rise in obesity and cardiometabolic disease.

Keywords:

Maternal diet, cardiovascular diseases, heart, epigenetics, early nutrition, fetus, obesity, metabolism

KEY POINTS

- The Developmental Origins of Health and Disease (DOHaD) hypothesis suggests that the environment experienced in early in life can influence the long-term cardiovascular health of an individual;
- Studies in humans and animal models suggest that a suboptimal environment during early life increases the risk of cardiovascular disease in the offspring;
- The epigenome is sensitive to dietary challenges applied during critical windows of development (fetal and early post-natal);
- Recently, small non-coding RNAs have been proposed as mediators of intergenerational programming of metabolic diseases through the paternal line;
- The contribution of epigenetic mechanisms to the programming of cardiovascular diseases by early life disturbances is relatively unexplored.

INTRODUCTION

The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that exposure to detrimental stimuli during pre-natal and early post-natal life can shape the long-term health of an individual. The developing fetus is extremely sensitive to the external environment, and adapts its physiology in order to increase its survival post-natally. This idea was first conceptualised as the Thrifty Phenotype Hypothesis in the early 90's by David Barker and colleagues who suggested that the fetal environment is a major determinant of long-term cardiometabolic health [1]. Although initial studies focussed on the detrimental effects of fetal under-nutrition, subsequent studies in humans and animal models have consistently reported that either deficiency (undernutrition) or excess (overnutrition) availability of nutrients, oxygen and hormones influence tissue development, and lead to an increased risk of "non-communicable diseases" in the adult progeny including obesity, type-2 diabetes (T2D) and cardiovascular diseases (CVD) [2]. In this context, nutritional imbalances applied during "critical windows" of development (fetal and post-natal life) can induce permanent changes to the structure and function of organs and systems, predisposing the fetus to an increased risk of diseases later in life.

CARDIOVASCULAR DISEASE RISK: THE IMPACT OF FETAL LIFE

The balance of nutrient supply during gestation and lactation is critical to ensure correct fetal development and growth. Failure of the fetus to meet the nutritional demand may result in *in utero* growth restriction (IUGR) and high risk of delivering a low birth weight (LBW) baby, both of which have been associated with increased risk of cardiovascular diseases [3]. Birth weight is a proxy of fetal health and predicts the risk of T2D in a U-shaped manner, with babies at both ends of the curve being more at risk [4]. LBW is an independent risk factor for cardiovascular diseases (CVD) in adulthood,

inversely correlates with systolic blood pressure (SBP) [5, 6], and associates with increase mortality from coronary heart disease [7] and higher risk of hypertension [8]. IUGR in human pregnancies has been strongly linked to poor cardiovascular health already *in utero*, such as altered parameters of systolic and diastolic function [9, 10], and throughout infancy and adulthood [11]. Consistently, studies conducted on various experimental models of fetal growth restriction (nutritional, hypoxia, glucocorticoid exposure, diabetes) have identified the cardiovascular system as a major target of developmental programming, and have shown that control of cardiomyocyte number and size may play an important role in this process [12]. IUGR babies have a higher risk of being born Small for Gestational Age (SGA), and often undergo a rapid (catch-up) growth early after birth in an attempt to compensate for the slow uterine development. This causes long-lasting changes to the structure and metabolism of an organism.

EARLY POST-NATAL LIFE: NUTRITION AND GROWTH

As well as during fetal life, suboptimal nutrient availability early after birth can impair offspring growth and also program a higher risk of metabolic diseases. In humans, overfeeding and excess post-natal weight gain is causally linked to offspring insulin resistance, obesity and CVD [13-15]. Similarly, a restricted period of overfeeding during lactation results in an increased incidence of obesity in adult baboons [16, 17] and only partially in rodents [18]. More recently, it has been shown in humans that accelerated neonatal growth was associated with the expression of obesity genes [19], confirming once more the strong causal relationship between altered growth trajectory and risk of obesity.

Common ways of promoting post-natal growth in animals are manipulation of litter size or cross-fostering. Litter size reduction induces post-natal overfeeding in rodents, via reducing pup competition for maternal milk and consequently increasing pup energy intake. The cardiovascular system is particularly sensitive to nutritional imbalances during lactation [18]. Studies on overfed pups in rodents have shown that overnutrition during lactation results in left ventricular cardiac hypertrophy and increased susceptibility to ischemia-reperfusion injury [20], impaired insulin signalling [21]* and leptin signalling [22], and leads to dysregulation of gene expression of cardiac structural proteins [23].

Cross-fostering is a husbandry practice where new born pups are assigned to a surrogate control mum in order to promote catch-up growth and development in IUGR pups. Increased cardiac DNA damage and oxidative stress was reported in hearts of IUGR rats born to under-nourished mothers which underwent a rapid catch-up growth post-natally [24, 25], as well as increased risk of T2D [26] and reduced longevity [27] .

THE MATERNAL ENVIRONMENT AND CARDIOVASCULAR DISEASE IN ADULTHOOD

The causal link between suboptimal exposures during fetal life and poor cardiovascular outcomes in later life has been extensively highlighted in human epidemiological studies. Studies on the Dutch Famine Birth Cohort revealed that individuals who were *in utero* during the famine in the Netherlands,

when compared with those not exposed had a lower birth weight and had a higher prevalence of coronary heart disease [28, 29]. Poor cardiovascular outcomes are also common in babies born to type-1 diabetic mothers, and this is thought to be a consequence of the teratogenic effects of maternal hyperglycaemia. Among those, congenital heart defects [30], increased cardiac ventricular output [31], reduced cardiac function, cardiac hypertrophy [32] and increased heart rate [33] are observed in babies born to diabetic mothers. Similarly, maternal hypoxia increases the risk of delivering a growth-restricted baby [34], which will develop high blood pressure and chronic hypertension in the long-term, exposing the offspring to a high risk of heart failure [35]. Similar observations have been reported in offspring born to obese mothers. A positive association between maternal Body Mass Index (BMI) and increased offspring risk of hospital admission and premature death for cardiovascular events has been revealed by the Aberdeen Cohort study [36]; in addition, findings from the Helsinki Birth Cohort Study indicated that higher maternal BMI was associated with an increased risk of CVD and T2D diabetes among the offspring [37]. Excessive gestational weight gain leads to pregnancy complications, such as gestational diabetes and pre-eclampsia, which expose the offspring to increased risk of cardiometabolic diseases [38]. Fetuses from obese mothers were shown to be insulin resistant *in utero* [39], and to develop fetal myocardial dysfunction [40, 41*]. Most importantly, such cardiometabolic perturbations were significantly ameliorated in human offspring born to obese mothers after gastrointestinal bypass compared to age matched siblings born before the surgery [42]. Maternal bariatric surgery resulted in a significant improvement in the metabolic and cardiovascular profile of offspring born after the surgery, reflected by ameliorated insulin sensitivity and reduced systolic blood pressure [43]**.

In parallel, evidence from animal models of dietary manipulation including undernutrition (caloric restriction, low protein diet) and overnutrition (high-fat diet, obesogenic diet) have reinforced the causative link between suboptimal maternal environment and CVD risk in adulthood, and have been important in the characterization of the complex mechanisms underlying the developmental programming of CVD (table 1).

Maternal diet	Species	Timing of exposure	Cardiovascular outcome	Sex studied	Reference
High-fat	mouse	*in utero* and lactation	Hypertension	M and F	[44]
	mouse	*in utero* and lactation	Hyperglycaemia, insulin resistance, obesity, and hypertension	F	[45]
	rat	*in utero* and lactation	Increased lipid peroxidation and evidence of mitochondrial dysfunction	not available	[46]

	rat	*in utero* and lactation	Vascular dysfunction	not available	[47]
	rat	*in utero* and lactation	High systolic and diastolic blood pressure, abnormal vascular function, reduced endothelium-dependent relaxation	M and F	[48], [49] , [50], [51, 52]
	rat	*in utero*	Cardiac vulnerability to ischemic injury in adult male offspring	M and F	[53]*
	rat	*in utero* and lactation	Increased blood pressure, insulin resistance, dyslipidaemia, obesity and mesenteric artery endothelial dysfunction in adult offspring	M and F	[54]
	sheep	*in utero*	Fibrosis and collagen deposition	M and F	[55]
	sheep	*in utero*	Impaired cardiac insulin signalling and impaired left-ventricular-developed pressure in response to high workload stress.	M and F	[56]
	sheep	*in utero*	Myofibril hypertrophy and fascicular disarray	M and F	[57]
	Japanese macaque	*in utero* and lactation	Vascular dysfunction manifested as depressed endothelium-dependent vasodilatation and thickened intima wall	not available	[58]
High fat/high sugar (obesogenic)	mouse	*in utero* and lactation	Hypertension, cardiac hypertrophy and cardiac dysfunction *ex vivo*	M	[59*, 60] [61, 62]
Caloric restriction	mouse	*in utero* and lactation	Increase in systolic blood pressure, perivascular fibrosis of the coronary artery, cardiomegaly and cardiomyocyte hypertrophy	M	[63, 64]
	rat	*in utero* and lactation	Endothelial dysfunction	M	[65]
	rat	*in utero*	Elevated blood pressure	M and F	[66]
	rat	*in utero* and lactation	Persistent hypertension and endothelial dysfunction across F1-F3 offspring	M	[67]
	rat	*in utero*	Reduced heart weight and cardiomyocytes number at birth	F	[68]
	rat	*in utero*	Pathological cardiac remodeling, diastolic dysfunction, altered Ca^{2+}	M and F	[69*, 70]

			handling properties is isolated cardiomyocytes		
	rat	*in utero*	Hypertension and reduced number of glomeruli	M	[71]
	sheep	Gestation and/or lactation	Hypertension and impaired glomerulogenesis	M	[72]
	sheep	*in utero*	Left and right ventricular cardiac hypertrophy (fetus and adult offspring)	F	[73, 74]
Low protein	mouse	*in utero* and lactation	Elevated offspring systolic blood pressure	M and F	[75]
	mouse	*in utero* and lactation	Cardiac hypertrophy	M	[76]
	mouse	*in utero* and lactation	Hypertension and vascular dysfunction	M	[77]
	rat	*in utero* and lactation	Reduced cardiac beta-adrenergic responsiveness	M	[78]
	rat	*in utero* and lactation	Increase in the cardiovascular sympathetic tone	M	[79]
	rat	*in utero*	Higher systolic blood pressure at 4 weeks of age	M and F	[80]
	rat	*in utero* and lactation	Increased oxidative stress	not available	[81]
	rat	*in utero*	Increased systolic blood pressure, impaired recovery of left ventricular developed pressure after ischaemia (Langendorff)	M and F	[82]
	rat	*in utero*	Hypertension and renal dysfunction	M and F	[83]
	goat	Late gestation	Reduced heart and body weight at birth	M	[84]
Low protein and post-natal catch-up growth	Rat	*in utero*	Cardiac DNA damage and oxidative stress	M	[24, 25]

Table 1: Experimental evidence of the effects of maternal under and overnutrition on the offspring cardiovascular system.

DIET AND CARDIOVASCULAR EPIGENOMICS

Current diet is thought to be a potent epigenetic modifier. However, there is also now evidence to suggest that early life exposure to suboptimal nutrition can permanently affect transcriptional regulation through epigenetic alterations, and this is thought to contribute to the long-lasting consequences on

offspring health [85]. Epigenetic regulation of the genome in mammals is mediated by DNA methylation, histone protein modification and epigenetic related RNA-based mechanisms (miRNAs, piRNAs, tRNA fragments and long non-coding RNAs). During embryogenesis the epigenetic information is believed to be globally erased, and subsequently re-established after embryo implantation. The timing and nature of the epigenetic modification will define different cell phenotypes. All the aforementioned epigenetic marks are sensitive and modifiable upon dietary challenges *in utero* [86]. Whether the effects are tissue specific or global will depend on the timing of the challenge. If it occurs early (when only a few cells exist) prior to tissue differentiation, then these changes will be present in all tissues within the body (meta-stable epialleles - see DNA methylation). The impact of the *in utero* environment (F0) on the transmission of certain metabolic traits to the F1 generation is an example of "inter-generational" inheritance. In contrast, "trans-generational" transmission requires the phenotype manifestation to occur in absence of the original stimuli which caused it, nutrition in this case (F2 for inheritance via paternal line; F3 for inheritance via maternal line) [87].

Although epigenetic regulation of gene expression has been extensively implicated in the pathophysiology of CVD, evidence for the existence of a causative link between nutrition, epigenetics and developmental programming of CVD is still limited. More evidence exists for the influence of early life nutrition on epigenetic regulation of gene transcription in adipose tissue, brain, liver and pancreas [85, 86].

DNA methylation

DNA methylation is by far the best characterized epigenetic modification. Changes in DNA methylation patterns have been identified in human patients with heart failure [88] and dilated cardiomyopathy [89]. However in these studies it is impossible to dissect out whether these changes occurred as a consequence of the disease state or are causative in its development. Recent analysis of the DNA methylome of newborn, healthy adult and adult failing cardiomyocytes revealed a prominent role for DNA methylation in regulating various aspect of cardiac biology, from heart development to disease [90].

Human studies have contributed significantly to the evidence for a link between exposure to peri-conceptional cues and permanent changes in DNA methylation. Studies from the Dutch Famine Birth Cohort Study [91] showed low methylation levels at various loci involved in growth and metabolism in individuals exposed to famine peri-conceptionally, and not during gestation [92, 93]. In mammals, the degree of DNA methylation of certain genomic regions called metastable epialleles (MEs) is determined at the early stages of embryonic development, and is susceptible to peri-conceptional disturbances which will ultimately be present in all tissues potentially affecting their functionality [94]. The maternal nutritional status of Gambian women, which are known to undergo seasonal fluctuations in nutrient intake, significantly influenced the DNA methylation status of specific MEs in offspring hair follicles and lymphocytes post-natally [95**, 96], demonstrating that maternal nutrition can permanently shape the fetal epigenome. As mentioned above, maternal bariatric surgery in obese women improves

offspring cardiometabolic health [43]. Two independent studies have reported that babies born to the same obese mother before and after bariatric surgery display alterations in the methylome of genes involved respectively in glucose regulation, inflammation, and vascular disease [43], or in diabetes, obesity and insulin signalling [97]*. Peroxisome proliferator-activated receptor alpha (PPARα) promoter methylation was reduced in adult and neonatal rat hearts exposed to maternal protein restriction, and this was associated with increased mRNA levels of PPARα only in adult hearts [98]. Oxidative stress is known to interfere with DNA methylation. High levels of reactive oxygen species (ROS) and oxidative stress are features of pregnancy complications (such as pre-eclampsia), and can induce permanent changes in transcription [99]. Epigenetic repression by DNA methylation of protein kinase C ε by ROS was reported in rat fetal hearts and increased ischemia susceptibility in adult male offspring, which was restored by treatment with a ROS scavenger [100]. Similarly, exposure to maternal caloric restriction caused impaired endothelium-dependent pulmonary artery vasodilation with alterations in the DNA methylation profile of lung tissue in 12-week old male offspring; both artery dysfunction and DNA methylation profile were restored after maternal anti-oxidant treatment [101].

Histone modifications

Acetylation of histone tails by histone acetyl transferases causes de-compaction of the nucleosomes and promotes transcription. The role of histone deacetylase enzymes (HDAC) in cardiovascular biology has been well characterized [102], as well as their potential as therapeutic targets of heart disease [103].

In vivo HDAC inhibitor treatment attenuated cardiac hypertrophy [104] and fibrosis [105] in hearts exposed to hypertrophic stimuli. HDAC1 and HDAC2 null mice developed dilated cardiomyopathy [106], whereas cardiac-specific deletion of HDAC3 resulted in severe cardiac hypertrophy [107]. Also, mice lacking either HDAC5 or HDAC9 spontaneously developed cardiac hypertrophy [108]. It is well established that dietary challenges *in utero* and post-natally modify the chromatin histones code in a wide variety of tissues [86]; however, not much evidence has been provided so far regarding the impact of *in utero* suboptimal nutrition on the epigenetic control in the heart through changes in histone modification.

Small non-coding RNAs (snRNAs)

Whether poor maternal nutrition can alter and shape offspring metabolic profile and organ function through alterations in snRNAs such as miRNAs, piRNAs and tRNA fragments is a very intense area of research. A few labs have identified miRNAs as potential inter-generational mediators of the fetal response to maternal nutrition in offspring organs of obese or undernourished mothers such as liver [109-111], adipose tissue [112] and skeletal muscle [113]. In the heart, alterations in miRNA profile was shown in the heart of fetal baboons born to high fat/high fructose-diet fed mothers [114]*. Among the dysregulated miRNAs, fifteen were previously associated with CVD in human and animal models and were shown to regulate cellular death, growth, and proliferation. MiR-133a was up-regulated in the

heart of offspring exposed to a maternal obesogenic diet during gestation and lactation [60]. Hearts from those mice presented pathological cardiac hypertrophy [60] and early onset of cardiac dysfunction [59] when compared to age-matched controls. In humans, an obesogenic intrauterine environment perturbs the amniotic miRNA profile, with possible consequences for placental function and fetal growth [115]. Also, a recent study implicates miRNAs as modulators of pre- and post-natal growth of babies born to mothers with gestational diabetes mellitus [116]*. These studies provide evidence that maternal overnutrition can alter the miRNA profile in different offspring tissues, and can potentially influence their long-term health.

Important insights into the role of snRNAs in the programming of metabolic diseases have recently emerged from studies addressing the impact of paternal nutrition. A suboptimal dietary regime in the father, modifies the snRNA profile in mature sperm of humans and mice, making snRNAs excellent candidates for trans-generational epigenetic inheritance of metabolic traits. Mechanisms of inheritance through the paternal line can be studied in absence of the *in utero* confounding effects. Only recently, an exciting series of papers have proposed that in mice alterations in the sperm miRNA [117-119]*, tRNA fragments [120, 121] and piRNA [119] profile can induce metabolic and behavioural perturbations in the progeny. Sperm of mice fed a western diet showed an altered piRNAs profile [119]**. Also, bariatric surgery in obese individuals modified the abundance of miRNAs, piRNAs and tRNA fragments in the sperm [122]**, suggesting that diet and metabolic state can interfere with progeny metabolic health through paternally inherited RNA-based mechanisms, and that these mechanisms are likely to be conserved in humans. MiRNA injection in naïve zygotes was able to initiate in the offspring metabolic alterations similar to a high fat diet-induced phenotype [119], whereas transfer of tRNA fragments isolated from mature sperm into 2-cell embryos induced severe glucose intolerance in 7-week old mice [120]**. Whether maternal diet could modify snRNA expression in the oocyte is unknown. MiRNA function seems to be globally suppressed in the murine female germline [123]; however, components of the piRNA-pathway have been shown to be active in the bovine, macaque, and human ovaries, as well as in the early embryo [124]. The oocyte contribution to the epigenetic transmission of metabolic diseases is still unexplored and perhaps difficult to assess directly in presence of other modalities of inheritance through the female germline (e.g. mitochondria). Oocytes of obese mice display oxidative stress-induced defects in mitochondrial function, as well as altered methylation levels [125], which may contribute to the programming of altered metabolic phenotypes post-natally [126]. Recent work suggests that in mice the insulin resistant phenotype is mainly acquired through the maternal line [127]*, but the underlying epigenetic mechanisms are not known.

CONCLUSIONS

It is an exciting scientific moment for the developmental programming field. Extensive evidence has been provided in the last decades of a direct causative link between a suboptimal intrauterine environment and development of cardiovascular diseases in humans and animal models. Here, we have

summarized the evidence regarding how diet and metabolic disturbances in early life can affect the offspring epigenome rendering the progeny more prone to cardiovascular diseases (figure 1). Despite much effort that has been exerted so far, we are only starting to understand the epigenetic contribution towards this process. Improving our knowledge of the molecular pathways and the modality of programming of cardiometabolic disease by suboptimal early life is demanding, in order to design rational intervention strategies for the mother and the baby.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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Figure 1. Influences of suboptimal pre-natal and post-natal environment on offspring cardiovascular health