Editorial

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Programming, metabolic syndrome, and NAFLD: The challenge of transforming a vicious cycle into a virtuous cycle

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In the present issue, an elegant experiment extending the *programming* hypothesis of pro-steatotic conditions in the offspring of both the intra-uterine and the lactational phases is reported [1].

Programming is defined as the process by which early insults at critical stages of development lead to permanent changes in tissue structure and function [2]. Intra-uterine programming of postnatal physiological functions has been demonstrated experimentally in a number of species, including mammals. Environmental insults can induce programming at different stages of development, including peri-conceptual and pre-implantation periods, up to early postnatal life. During the phases of rapid cell growth, insults, which alter the supply, uptake, and utilization of nutrients, may switch the cell behavior from proliferation to differentiation with adverse consequences for total cell number, and ultimately, organ size and function [2]. As the different fetal organs grow at different rates during development, the timing of the insult is important in determining the specificity of programming.

In mammalian development, environmental information, such as nutrient availability, is transferred from the mother to embryo and fetus through the placenta, and to infant through lactation. Therefore, programming is induced through the adaptive responses that the fetus or infant makes to cues from the mother. Fetal or perinatal responses may include changes in metabolism, hormone production, and tissue sensitivity to hormones that in turn, may affect the development of various organs, leading to persistent alterations in metabolic homeostasis [3]. Maternal hyperglycemia, for example, may lead to fetal hyperinsulinemia and fat deposition, and evidence suggests that the offspring of obese and/or diabetic women are at greater risk for developing metabolic disorders, even during childhood [4]. Furthermore, once the organism has been programmed, the responses to subsequent environmental cues during infancy, childhood, and adulthood may be influenced by the early adaptation and affect the risk of disease.

The ability of an organism to adjust structure and function in response to environmental cues has been named developmental plasticity [5]. There is increasing evidence that epigenetic mechanisms underlie the processes of developmental plasticity, and, ultimately, programming. Environmental cues, transduced by the mother, may result in fine regulation of gene expression through specific changes in methylation, acetylation, and, microRNA expression in fetal tissues [2]. In a rat model, maternal undernutrition affects the expression of PPAR-α in the liver without affecting the related transcription factor PPAR-γ [6]. In a different rat model of intra-uterine growth retardation obtained by the ligation of uterine arteries, the development of type 2 diabetes has been associated with progressive epigenetic silencing of Pdx1, a pancreatic and duodenal homeobox transcription factor that regulates pancreas development and β-cell differentiation [7]. In humans, the peri-conceptional exposure to famine during the Dutch Hunger Winter in 1943–1945, has been associated with persistent epigenetic changes in the IGF2 differentially methylated region [8].

There is increasing evidence that maternal obesity is a risk factor for obesity in the offspring. It has been reported that 24% of children of obese mothers were themselves obese at age 4 years compared to 9% of children born from normal-weight mothers [9]. Furthermore, a close relationship between maternal weight gain during pregnancy and obesity in childhood has been reported [10]. More recently, it has been shown that female mice, fed with high fat diet from before mating through lactation, underwent weight gain and their offspring developed hyperphagia, increased adiposity, reduced locomotor activity, hypertension, and insulin resistance, thus suggesting maternal diet induced developmental programming [11]. The hypothalamus is emerging as a critical site for the integration of nutritional, endocrine, and neural cues signaling the body's metabolic and nutritional status. These signals should normally activate a negative feedback loop between the availability of nutrients and their intake and metabolism [12]. These appetite control circuits are plastic in early life and can be affected ("pro-

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grammed") by nutritional factors. Maternal obesogenic diet is able to alter metabolic function in neonatal offspring eliciting the oversecretion of leptin which in turn leads to central leptin resistance, permanently affecting hypothalamic function. In this rat model both phosphorylation of STAT3 in the hypothalamic arcuate nucleus, and the level of agouti-related peptide in the hypothalamic paraventricular nucleus, are reduced [13]. In the same rat strain, maternal uteroplacental insufficiency reduces the expression of STAT3 and POMC in the hypothalamus of the offspring [14]. Taken together, these data strongly suggest an intra-uterine programming of the hypothalamic control of appetite and energy homeostasis as a response to the nutrient supply from mother to fetus.

Bruce et al. have also experimentally shown that maternal fat intake contributes to the development of NASH in adult offspring, through impaired hepatic mitochondrial metabolism and up-regulated hepatic lipogenesis [15]. These are potentially highly significant findings. The concept of maternal behavior affecting the offspring in adulthood is consistent with the well established association between *in utero* programming and long-term metabolic consequences. Forsen et al. demonstrated that, in a multivariate analysis, maternal obesity in short women is strongly associated with coronary heart disease in adult offspring [16]. The effect is more pronounced in the offspring of women with a history of chronic malnutrition and who subsequently become obese before conception. This finding, as recently reported by Charlton in his Editorial [17], may explain the high prevalence of metabolic syndrome among obese individuals in emerging economies, such as India. A physiological basis for this observation was provided by Mitrani et al., who observed that metabolic programming in the offspring of obese rats induces changes in autonomic activity, including increased parasympathetic and decreased sympathetic activity, in the postnatal maintenance of hyperinsulinemia in response to the high caloric diet *in utero*.

The new information in the paper by Oben et al. demonstrates that the lactational period may exert an imprinting metabolic effect (or, alternatively, unmasking some gene-related functions) on the later development of NALFD and other conditions (from weight increase to biochemical markers) implicated in the development of the metabolic syndrome [1]. Ozanne and Hales nicely demonstrated in an animal model the strict interplay of maternal nutritional conditions *in utero* and then in lactation on the lifespan of the offspring. These influences were clearly stronger than post-weaning dietary habits [18].

Indeed, observations in humans are consistent with the hypothesis that developmental plasticity extends further into early postnatal life. Recently, Nobili et al. have suggested the possibility of an early imprinting effect of breastfeeding and/or human milk that becomes more evident if the infant is later exposed to unfavourable metabolic conditions such as obesity and fatty liver [19]. A similar preventive effect of early breastfeeding has been recently reported for lung compliance in 5- to 17-year-old children with cystic fibrosis, where breastfeeding longer than 4 months resulted in better lung function tests [20]. Some nutritional components of human milk may interact with many regulatory systems, such as peroxisome proliferator activated receptors (PPAR, alpha and gamma) that are implicated in the protection against fibrosis. In particular, long-chain polyunsaturated fatty acids of the $n-3$ series, especially docosahexaenoic acid, delivered by human milk after prolonged lactation, could act as PPAR-agonists either directly or indirectly [21]. PPAR-gamma expression is critical in reducing experimental liver fibrogenesis and a possible therapeutic role of its inhibition on NAFLD has been proposed [22]. However, to what extent the early effects of breastfeeding could translate into later protection is a matter of speculation. Trophic factors present in human milk could interact with hepatocytes within a neurohormonal milieu that is quite different in breastfed as compared to formula-fed infants. In the report by Oben et al., higher leptin levels were identified in the milk of obese mothers, possibly contributing to the negative programming effect.

At present, the programming hypothesis in humans is challenged against the possibility of indentifying key-events leading to a deranged translation of the genetic inheritance, in order to plan decisive preventive interventions – even to an individually tailored basis. The question of the translation of the vicious circle involving laboratory experiments, animal observations, human epidemiology, and clinical trials is a burning one. Most criticisms actually point out that we are still explaining mechanisms, but the fundamental questions regarding treatment and prevention, still rely on speculative standpoints. As a consequence, despite the available experimental data, neonatologists are still focusing their efforts on the stimulation of weight increase and catch-up growth, without any distinction among the various populations of preterm and growth-retarded infants.

Nevertheless, the recent addition of NALFD to the childhood-onset metabolic syndrome is a key event, since the metabolic mis-regulation of the liver may be counteracted by various approaches, and the break-down of the vicious circle involving liver steatosis and decreased insulin sensitivity could respond to relatively simple interventions such as weight control, physical exercise, and targeted use of docosahexaenoic acid [23].

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