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STATE OF THE ART

1 – Developmental origins of health and disease: moving from biological concepts to interventions and policy

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The rising incidence of noncommunicable diseases (NCDs), especially in young adults, presents great humanitarian and economic challenges to high-resource and, increasingly, to low-resource countries. Noncommunicable diseases (NCDs), including cardiovascular disease, diabetes, chronic lung disease, allergy, some forms of cancer, cognitive decline, osteoporosis, sarcopenia, and affective disorders, are the world's biggest killers. Eighty percent of these deaths occur in low- and middle-income countries, especially as these countries undergo socioeconomic improvement after reductions in infectious disease. The World Health Organization predicts a global increase of 17% in NCDs over the next decade. No longer considered to be diseases of affluence, NCDs are exacerbated by urbanization and changes in social and lifestyle factors such as diet and family size. NCDs are preventable, but new initiatives are needed to institute such prevention, especially in early life. We emphasize that all children are affected by their early developmental conditions, not just children exposed to a very de!cient environment, and that this has long-term consequences for their predisposition to NCDs. We highlight the biomedical implications of these developmental origins of health and disease (DOHaD) concept of NCDs and discuss the implications for health policy. New research emphasizes the importance of early life factors in establishing the risk of NCDs through inadequate responses to later challenges, such as an obesogenic environment. A new focus on interventions to promote a good start to life in at-risk populations necessitates revision of public health policy, with implications for the health, education, and empowerment of women and children in particular.

2 – Environmental Stressors in the Developmental Origins of Disease: Evidence and Mechanisms

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A vast body of epidemiological data has suggested that childhood stress is associated with a variety of physical and mental health challenges later in life. The critical question is what is the mechanism? How could either physical- or social- stress early in life be registered in the genome of the offspring and stably affect the phenotype? We have been testing the hypothesis that DNA methylation, a covalent modification of the DNA, mediates the long term effects of early life environmental exposure on genome function.The pattern of distribution of methyl groups in DNA is different from cell-type to cell type and is conferring cell specific identity on DNA during cellular differentiation and organogenesis. This is an innate and highly programmed process. However, recent data suggests that DNA methylation is not only involved in cellular differentiation but that it is also involved in modulation of genome function in response to signals from the physical, biological and social environments. We propose that modulation of DNA methylation in response to environmental cues early in life serves as a mechanism of life-long genome "adaptation" that molecularly embeds the early experiences of a child ("nurture") in the genome ("nature"). Data that supports this hypothesis from rodent, non-human primates, humans and population studies will be discussed. We have established that the state of DNA methylation of a critical gene in physiological stress control, the glucocorticoid receptor is differentially methylated in adult humans hippocampus in association with early life adversity as it is in a rodent model of differential maternal care. We tested the hypothesis that the change in methylation that associates with early life adversity is not limited to several candidate genes but that it involves multiple functional gene networks and that it is not limited to the brain. We show differential DNA methylation landscapes in T-cells from rhesus monkeys that were deprived of a mother early in life as well as changes in DNA methylation in white blood cells from adults who were exposed to high social adversity early in life. Different early life experience are associated with different DNA methylation landscapes. These data support the hypothesis that exposure to stress early in life results in a broad genome-wide and system-wide change in the DNA methylation landscape that is hypothesized to serve as a genome adaptation mechanism.

3 – Developmental Origins of Non-Communicable Diseases and Dysfunctions: Implications for Animal production

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DOHAD mechanisms are shared by livestock. Worldwide, animal breeding activities play a major role in the reduction of poverty and the improvement of food security. We also currently face a growing need for animal products, to provide the necessary protein sources for human populations. Moreover, with the current global climatic changes, the breeding industry is required to adapt to the increasing incidence of biological hazards including

temperature rise, increasing risks and severity of draughts, river flood disasters and decreased crop yields. In terms of animal production, these fluctuations are likely to cause irregularity in the quantity of forage and cereal yield and to induce quantitative and qualitative variations in the diet provided to the animals with short or even long term periods of nutritional restriction. In animals raised for reproduction, these can result in long term physiological effects on animal health and reproductive parameters as well as on the quality and quantity of products. Through a better understanding of the effects and of the epigenetic mechanisms involved in DOHaD, in addition to the careful use of animal genetic selection, both animal production and welfare can be improved and some of the negative effects of feedstuff restriction could be prevented.

KEYNOTE SPEAKER

Neuroendocrine Origins of Obesity and Diabetes

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The incidence of obesity is increasing at an alarming rate and this worldwide epidemic represents an ominous predictor of increases in diseases such as type 2 diabetes and metabolic syndrome. Epidemiological and animals studies suggest that alteration of the metabolic and hormonal environment during critical periods of development is associated with increased risks for obesity, hypertension, and type 2 diabetes in later life. There is general recognition that the developing brain is more susceptible to environmental insults than the adult brain. In particular, there is growing appreciation that developmental programming of neuroendocrine systems by the perinatal environment represents a possible cause for these diseases. This talk will summarize the major stages of hypothalamic development and will discuss potential periods of vulnerability for the development of hypothalamic neurons involved in feeding regulation. It will also provide an overview of recent evidence concerning the action of perinatal hormones (including leptin and ghrelin) and nutrition in programming the development and organization of hypothalamic circuits that regulate energy balance and reproductive function.

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GROWTH PATTERNS AND LONG-TERM CONSEQUENCES

0 – D. Vieau and M.-A. Charles

Growth modelling from data from the French health booklet or use of body silhouettes at targeted age allows an *a posteriori* reconstitution of growth patterns. The studies presented found a link not only between early body mass index rebound and overweight but also between thinness in childhood and type 2 diabetes, height and weight velocities in the 1st year of life and bone structure 3 years. The ongoing French national birth cohort (ELFE) will provide additive data on the socio-economic, familial and individual aspects interacting throughout childhood.

ORAL N°9

Growth trajectories and body composition: influence of nutrition

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Exposure to nutritional factors during early life has been shown to exert a long-lasting influence on health. Anthropometric indicators such as weight, height and body mass index (BMI) predict later risk of obesity. However, recent studies point out the importance of growth patterns to predict later risks. The age at adiposity rebound (AR), which corresponds to the second rise in BMI and occurs by the age of 6 years, is a good predictor of later obesity and metabolic diseases. As a rule, the earlier the AR, the higher the adiposity at the end of growth.[1] Our objective was to investigate the different BMI pathways associated with adult obesity, and to examine whether BMI trajectories correspond to changes in lean or fat body compartments. We conducted a two-decade-long prospective study (ELANCE). Nutrition and body composition were recorded from the age of 10 months to 20 years in 73 subjects. Fat and lean compartments were assessed on the basis of arm fat and muscle areas. Two main trajectories emerge: (i) fat children who remained fat up to adult age and (ii) fat children who became fat after an early AR. As a rule, the early AR is preceded by a low BMI in early life and followed by a high BMI thereafter. The increased BMI after the AR corresponds to an increase in fat rather than in lean body mass. BMI trajectories differed according to nutritional intakes in early life. In conclusion, we report several types of growth trajectories associated with adult overweight. The BMI increase after the AR corresponds to an increase in fat mass, consistent with previous studies.[2] The BMI trajectory associated with an early AR, which displays low BMI level in early life and subsequent high body fatness,[3,4] is particularly associated with metabolic diseases such as diabetes.[3]

Key words: body composition, child growth, critical periods, early life nutrition, obesity

Statement of interest: Authors report no conflict of interest.

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ORAL N°53

The association between body silhouettes before 20 years of age and type 2 diabetes in adulthood

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Several growth trajectories in childhood have been associated with the risk of type 2 diabetes (T2D).[1–4] We evaluated the link between childhood and adolescent BMI and adulthood T2D, considering potential gender and generation differences. Among the national sample of adult respondents to the ObEpi 2009 survey, we included the subjects with available silhouettes at 8 and 18 years reported on a standard questionnaire, T2D diagnosis and maximum body mass index (BMI_{max}) in adulthood: 23,763 subjects (50.1 ± 17.3 years, 52.8% women, 5.6% T2D). We analyzed the relationship between T2D and silhouettes (thinnest: A to largest: G, regrouped in four main categories), as well as growth trajectories by logistic regression. We adjusted on BMI max, age and gender. No significant interaction silhouettes by gender or birth strata were noted in fully adjusted models. Leaner silhouettes (AB and C) at age 8 were positively associated with T2D before and after adjustment: adjusted ORs 1.54 (95% CI 1.30–1.82) and 1.35 (1.14–1.60) *v.* middle silhouette (D). The largest silhouettes at 18 years were significantly associated with T2D but the adjustment for BMI max reversed the association: for a similar BMI max, subjects with the leanest silhouettes at 18 years had a higher risk of diabetes than the D silhouette taken as a reference: OR 1.29 (1.09–1.52). For silhouettes C or larger at the age of 8 years, a change for a leaner silhouette between 8 and 18 years was protective against T2D, whereas a change for a larger silhouette increased the risk (especially in men). After adjustment, the associations remained significant for the changes from the middle silhouette D. These results confirm our previous data[1] showing that leanness in childhood is a risk factor for T2D in the generations now middle-aged. It also shows the detrimental effect of adolescent weight gain independent of the obesity level reached in adulthood.

Key words: child growth, developmental origins of adult disease, diabetes, obesity

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POSTER N°17

The 'CECA' retrospective study: childhood growth and adult nutritional status

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The prevalence of overweight and obesity in the population has increased in recent decades. Anthropometric characteristics in childhood, such as rapid growth or specific type of trajectory, may have a future impact on BMI and should be further studied. We aimed to conduct a retrospective study allowing to evaluate growth characteristics in childhood and assess their association with overweight, obesity and metabolic risks factors in adulthood. Individuals aged 20–60 years were examined in health centres in the central/western part of France. A standardized examination was performed including clinical assessment (anthropometric measurement, blood pressure), blood sampling and self-reported questionnaire (socio-economic characteristics, family and personal history, eating habits and lifestyle). A self-administered growth questionnaire included weight and height data between 0 and 12 years from individual health booklets, and other characteristics such as type of feeding, physical activity or parental weight status. The study ran for 11 months, between September 2008 and August 2009. Of the 24,574 consultants, 2549 of them (42.7% of men) completed the growth questionnaire. Mean age of included subjects was 32.5 ± 8.9 years (21.8% $\geqslant$ 40 years) and mean number of weight/height measures collected from health booklets was 7.8 ± 2.9 (20.8% $\geqslant$ 10 measures). Birth weight (99.7%), birth height (77.3%) and feeding type when leaving the maternity hospital (91.7%) were available for a majority of subjects. A retrospective study using health booklets from subjects examined in adulthood is a feasible and relatively economic approach to obtain childhood growth data on a large number of individuals and to investigate the association between early growth and adult health outcome. Given the increased use of health booklets in France over the last decades, the feasibility of such study is likely to increase in the near future. Association between growth and adult nutritional status will be evaluated.

Key words: child growth, early development and adult disease, epidemiology/public health, infant growth/nutrition, obesity

Statement of interest: Authors report no conflict of interest.

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POSTER N°55

Weight and height growth modeling to study the determinants of growth and later health outcomes and assess BMI trajectories

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Growth follow-up during infancy can alert about major health issues. More subtle changes in weight or height growth are associated with several diseases in later life. Some patterns of body mass index (BMI) changes over time (e.g. early adiposity rebound) have also been associated with an increased risk of obesity. We aimed to model individual weight and height growth curves during infancy to study their determinants and relationships with later health outcomes and to predict BMI trajectories. We collected in the French EDEN mother–child cohort 15 measurements per child of weight and 13 of height between birth and 3 years in 1900 infants, from their health-care booklet and during the clinical study exams. First, we fitted individual weight and height growth trajectories using the Jenss nonlinear model including random effects. Second, we studied whether individual parameters were associated with several determinants and health outcomes. Finally, we modeled BMI change with age (1) with a fractional polynomial method using reported weight and height and (2) with a combined function of Jenss models on weight and height. Postnatal weight growth was negatively associated with breastfeeding, positively associated with some environmental factors (e.g. parabens) and familial determinants (e.g. fathers' anthropometry). Weight and height growth in the first months were associated with bone status at 3 years. BMI trajectories seemed to be better fitted using the combined function than the fractional polynomial method. In conclusion, measuring or collecting weight and height repeatedly during infancy and childhood allows to describe individual trajectories using a reduced number of parameters. We developed a useful tool to study the developmental origins of health and diseases. By modeling observed growth data, we homogenized the data in

terms of number and age of measurements and were able to calculate other specific parameters (e.g. growth velocities, age/level of BMI peak).

Key words: child growth, critical periods, DOHaD, epidemiology/public health

Statement of interest: No conflict of interest statement.

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POSTER N°8

Weight and height growth velocity during the first years of life and infant bone status at 3 years

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Several studies showed a positive association between birth weight, weight at 1 year and bone mass in adulthood,[1] which suggests that growth-related factors of the prenatal and postnatal periods could have a persisting influence on skeletal development; however, the most critical periods for bone development, especially during early infancy, need to be specified in order to direct research on relevant risk factors. Our objective is to study the association between height and weight growth velocity during the first years of life and infant bone status at 3 years. On the basis of data from 1903 infants included in the EDEN cohort, a mathematic model of growth trajectory between birth and 3 years (non-linear mixed effect models) has been realized and weight and height growth velocities have been estimated at 3 months, 6 months, 1 year and 2 years. Bone status has been measured on 1170 infants at 3 years by quantitative ultrasound of phalanxes of the hand (DBM Sonic, IGEA, Italy). Linear regression models have been used to study the association between growth velocities and 3-year bone status [bone transmission time (BTT)]. We showed that birth height, height and weight growth velocities until 1 year are significantly and positively associated with BTT at 3 years both sexes. After adjustment for finger width (bone size indicator), these associations are weakened but remain significant for height growth velocity. Our results suggest that growth-related factors present during the foetal life and the first year of life may influence infant bone growth and structural development.

Key words: critical periods, epidemiology, foetal programming, infant growth, osteoporosis

Statement of interest: Authors report no conflict of interest.

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POSTER N°50

Elfe: The French longitudinal study of children

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Elfe is the first French national birth cohort. Infants and their families were included at birth in 2011 and will be followed to adulthood under a multidisciplinary approach. This cohort will provide a unique source of data in France to analyse the development and health of children in their environment. Interaction between various factors assessed at the socio-economic, familial and individual levels interacting throughout childhood will be the focus of this study. More than 18,000 children were recruited over four periods in more than 300 randomly selected maternity hospitals in metropolitan France. Acceptance rate was 51%. At birth, data have been collected from medical records and self-completed, or face-to-face questionnaires. Although qualified and working women are over-represented, the variety of social and health situations will allow studying the outcomes of expositions during pregnancy. In all, 9.3% of the women have a BMI $> 30\,kg/m^2$ before pregnancy (*v.* 9.9% in the national perinatal study), 16% smoked during pregnancy (*v.* 17.1%) and 7.5% were followed for gestational diabetes (*v.* 7.7%). Biological samples at birth were collected from 2000 to 5000 mothers or infants. The follow-up started with a telephone interview at 2 months fully achieved by 86% of families. Around 9500 families completed a monthly internet or paper questionnaire on infant feeding between 2 and 10 months. Next steps include a phone interview at 1 year (ongoing) and 2 years and a combined phone/home visit at 3 years with psychomotor test, biological sampling and home measures. Linkage with the French Social Security database is planned to collect health-care consumption of mothers during pregnancy and of the children throughout the follow-up. Data from Elfe will be opened to all research teams through a website on a basis of a specified research project and will provide large opportunities for the French DOHaD research.

Key words: children, environment, health, pregnancy, socio-economic status

Statement of interest: Authors report no conflict of interest.

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METABOLIC, VASCULAR AND CANCER PROGRAMMING

1 – D. Vieau and A.-M. Nuyt

Exposition to two or more adversities in childhood should be considered as a potential risk factor for cancer. Using an embryo transfer technology in rats, it has been shown that

maternal diabetes has both short- and long-term consequences on pancreatic β-cell function. White adipose tissue depots and skeletal muscle are sensitive programming targets in intrauterine growth restriction (IUGR). In premature infants, epigenetic modifications of the AMOT gene, involved in angiogenesis, may participate in the development of adult hypertension. Maternal diabetes programs vascular functions of conductance and resistance arteries, which may contribute to hypertension. High-protein feeding in IUGR rat neonates programs long-term metabolic alterations. High-fat regimen has more deleterious consequences in weaned than in adult rats. Children sleep pattern may influence adiposity and overweight.

ORAL N°65

Childhood adversity as a risk for cancer: findings from the 1958 British Birth Cohort Study

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Adverse psychosocial exposures during childhood may result in biological changes and health behaviours potentially involved in the development of cancer in adulthood. The objective of our study was thus to analyse whether Adverse Childhood Experiences (ACE) are associated with an increased risk of cancer. The National Child Development Study (NCDS) is a prospective birth cohort study with data collected over 50 years. The NCDS included all live births during 1 week in March 1958 ($n = 18{,}558$) in Great Britain. Self-reported cancer incidence was based on 533 participants reporting having had cancer at some point and 6080 reporting never having cancer. ACE was measured using reports of: (1) child in care, (2) physical neglect, (3) child's or family's contact with the prison service, (4) parental separation because of divorce, death or other, (5) family experience of mental illness and (6) family experience of substance abuse (0–6), to test for a relationship with cancer. Information on socio-economic characteristics, pregnancy and birth were extracted as potential confounders, and information on behaviours as potential mediators (smoking, alcohol, BMI). Multivariate models were run using multiple-imputed data to account for missing data in the cohort. The prevalence of reporting a cancer before 50 years of age was 14.5% for those with two or more adversities and 6.4% for those with none ($P < 0.0001$). The odds of reporting a cancer increases twofold when an individual has experienced two or more adversities in childhood *v.* individuals who experienced no such adversities (OR: 2.04, 95% CI: 1.42–2.87, $P < 0.0001$), after adjusting for early life confounding factors and adult mediating factors. It was particularly true for women (OR: 2.3, 95% CI: 1.51–3.44, $P < 0.0001$, respectively, for female respondents who had experienced two or more adversities in childhood). An accumulation of ACEs had a positive association with cancer incidence, which could be in part a direct effect involving biological processes. Exposure to adversity in childhood should be considered as a potential risk factor for cancer.

Key words: cancer, early development and adult disease, epidemiology/public health, lifecourse

Statement of interest: Authors report no conflict of interest.

ORAL N°46

Perinatal programming of the endocrine pancreas by maternal diabetes: impact on the development of the β-cell mass and glucose homeostasis in the offspring

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In recent years, epidemiological findings had strongly suggested that *in utero* exposure to maternal diabetes is associated with abnormal insulin secretion and glucose homeostasis in the offspring and may participate in the excess of maternal transmission in type 2 diabetes (T2D). From human studies, isolation of the respective contribution of genetic *v.* perinatal environmental factors is hardly attainable. The Goto-Kakizaki (GK) rat is a spontaneous model of T2D with decreased β-cell mass observed as early as in fetal life, followed by altered β-cell function during postnatal life. This model is a useful tool to investigate whether the deficit of pancreatic β-cell number is determined genetically, environmentally or both. The aim of our work was to determine the contribution of the maternal hyperglycemia on the development of β-cell mass and function in a normal Wistar conceptus (in the absence of diabetes predisposing genes). Using an embryo transfer technology, we implated fertilized Wistar oocytes into pseudo-pregnant diabetic GK females. β-cell mass, cell proliferation and cell neogenesis were measured in the pancreas of E18.5 fetuses. The pups were either suckled by their GK mothers or cross-fostered to non-diabetic Wistar dams to evaluate the proper influence of perinatal nutritional environment. β-cell mass, basal glycemia and glucose tolerance were measured in 8–10 weeks old offspring. We showed that maternal diabetes impairs early development of the β-cell mass in Wistar offspring. This defect is maintained in the pancreas of adult

offspring reared either by Wistar or by diabetic GK dams. In this group, the glucose tolerance was also altered in the adult offspring. Taken together, our data provide evidence for a deleterious impact of maternal hyperglycemia on β-cell development and growth in Wistar offspring at no spontaneous risk of diabetes. These data contribute to the better understanding of the effects of exposure to maternal diabetic environment and could bear important public health implications in the present context of growing diabetes epidemic.

Key words: β-cell mass, developmental programming, gestational diabetes, glucose tolerance

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ORAL N°34

Maternal prenatal undernutrition programs adipose tissue gene expression in adult male rat offspring

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Epidemiological studies have shown that maternal undernutrition during pregnancy leads to intrauterine growth retardation and low birth weight, and may predispose individuals to the development of metabolic syndrome in adulthood. In order to unravel the underlying mechanisms, we have developed a model of prenatal maternal 70% food-restricted diet throughout gestation in pregnant female rats called FR30. Adult male FR30 offspring showed mild hypertension, impaired glucose intolerance and hyperphagia associated with dysregulated light/dark-phase food intake rhythm.[1] Under chow diet, hyperleptinemic and hypercorticosteronemic FR30 rats did not show overt obesity but were predisposed to fat accumulation. Indeed, FR30 rats exhibited a greater adipocyte area with a global increase of white adipose tissue (WAT) lipogenic gene expression profile. Despite no further adipocyte hypertrophy, high-fat (HF)-fed adult FR30 offspring displayed a more important weight gain with a global increase in WAT adipogenesis mRNA transcript profile. In WAT FR30HF, higher leptin sensitivity and enhanced 11b-HSD2 mRNA (that catalyses the interconversion of adipogenic active corticosterone to inactive 11-dehydrocorticosterone) expression levels might be seen as mechanisms designed to limit fat deposition by counteracting adipogenesis.[2] These observations raised questions regarding the role of the glucocorticoid WAT environment on the development of adiposity.[3] In addition, gene expression levels of many peptide precursors and receptors showed marked modifications. It can be seen as either an adipogenesis predisposition or protective mechanisms against further adiposity. Overall, programming occurred in a WAT depot-specific manner in FR30 offspring.[2] In accordance with dysregulated light/dark-phase food intake rhythm, we also found that the daily transcriptional profile of several clock genes was modified within WAT FR30 offspring. Our data indicated that circadian clock underwent long-term nutritional programming that might contribute to the development of adiposity in adulthood.[4]

Key words: animal, fetal programming, metabolic syndrome, obesity

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ORAL N°4

Short- and long-term influences of intrauterine growth restriction on muscle and adipose tissue properties and metabolic flexibility

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Intrauterine growth restriction (IUGR) is associated with several health problems throughout life. Our program aimed at investigating the mechanisms underlying adipose tissue and skeletal muscle development in IUGR pigs, a species of importance for meat industry worldwide and a broadly used biomedical model for adiposity. Pairs of piglets were chosen within litters to have either a medium or a small weight at different stages of gestation or at birth. Because of the hypothesis of a relationship between high-protein (HP) intake in IUGR children and their later adiposity, a subset of IUGR piglets was fed HP formula during suckling, whereas others received a control formula (AP) that mimicked the sow milk composition. In skeletal muscle, the ratio between adult fast and embryonic myosin heavy-chain isoforms was twofold lower in small fetuses than in their medium littermates at 2 days before birth, denoting a lower muscular maturity in IUGR animals. In subcutaneous fat, IUGR counteracted the normal fall of DLK1/Pref-1 expression during gestation, and blunted the temporal increase in expression levels of many differentiating and lipogenic genes; the differences between weight groups were exacerbated around birth. These differences were not associated with modifications in circulating concentrations of energy-producing metabolites between weight groups.

The distribution of HP formula to IUGR piglets resulted in accelerated growth rate and in a temporary reduction in adiposity (until weaning) compared with piglets fed AP formula. This was associated with a decrease in the expression levels of genes related to glucose utilization and lipid anabolism in adipose tissues. In 160-day-old pigs having being fed HP formula, adipocytes were enlarged and their lipogenic rates were reduced. Thus, IUGR affects the temporal development of muscle and adipose tissue. Altogether, dietary strategies at different periods should be further tested to modulate stem cell lineage, tissue development and reinstating optimal growth trajectories.

Key words: body composition, developmental programming, fetal growth, newborn, plasticity

Statement of interest: Authors report no conflict of interest.

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POSTER N°13

Angiogenesis and the programming of hypertension: developmental changes in the methylation profile of the *AMOT* gene in cord blood endothelial progenitor cells

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Premature birth has been associated with increased risk of hypertension at adulthood.[1] Perturbations of angiogenesis may play a key role in the 'early programming' of adult arterial hypertension. Circulating endothelial progenitor cells are involved in angiogenesis and may participate to later hypertension; previous work in our group demonstrated that preterm newborns (with a low birth weight) have a reduced number, decreased self-renewal capacity and decreased angiogenic function of endothelial colony-forming cells (ECFC).[2] Epigenetic changes in the fetus that occur relatively late in human pregnancy can contribute to disease susceptibility and may be molecular mediators of 'early programming'. Furthermore, supplemental evidence reveals an association between gestational age and a differential methylation of genes.[3] The characterization of early epigenetic modifications of genes involved in angiogenesis would increase the chances to identify epigenetic biomarkers of the 'early programming' of adult hypertension in premature infants. The *AMOT* gene is a key regulator of angiogenesis;[4] its expression is reduced in the cord blood ECFC of preterm newborns.[2] However, the epigenetic analysis of the *AMOT* gene in the context of prematurity is unexplored. We therefore performed a comparative analysis of the DNA methylation profile of the *AMOT* promoter CpG island in the cord blood ECFC of 15 preterm (gestational age between 28 and 36 weeks) and 13 term newborns (>37 weeks). Results of cloning–sequencing experiments showed that some CpG dinucleotides are differentially methylated in the two newborn populations: in fact, a methylation at 4.5% of GpG dinucleotides was found in preterm newborns against 2.5% in term newborns ($\chi^2 = 3.842037097$; *P*-value $P = 1.7e{-}02$). By pyrosequencing experiments, we identified five CpG dinucleotides differentially methylated in term and preterm newborns. Furthermore, this CpG-targeted methylation showed a statistically significant decrease with increasing gestational age (*P*-value between $P = 3.5e{-}02$ and $P = 1.3e{-}05$). These results suggest that, given its crucial role in the regulation of angiogenesis, the methylation of the *AMOT* gene could be an epigenetic biomarker of adverse programming of angiogenesis because of preterm birth. Future studies in hypertensive adults born preterm or at term may elucidate the role of epigenetics and of the *AMOT* gene methylation in the programming of hypertension.

Key words: developmental origins of adult disease, epigenetics, fetal programming, hypertension/blood pressure, prematurity/preterm birth

Statement of interest: Authors report no conflict of interest.

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POSTER N°49

Vascular endothelium and early markers of adult cardiovascular disease

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Cardiovascular disease is a major concern of global health worldwide. Perinatal programing, identification of risk factors and early management of adult disease is a widely used search path and should be a priority. Our interest is focused on the vascular endothelium, whose key role has been demonstrated in many chronic diseases.[1–4] The aim of our study is to examine the effects of fetal and neonatal biometries and growth in endothelial integrity. We assume the existence of early markers of endothelial dysfunction. We included 149 volunteers in the main study, healthy and aged from 18 to 30 years, including 39 in an advanced study. Circulating endothelial progenitors, counted by flow cytometry, such as CD34+/CD45−/KDR+, were positively correlated with

weight ($P = 0.0096$) and BMI at 2 years ($P = 0.0011$); other types as CD34+/KDR total were correlated with systolic blood pressure ($P = 0.015$). The study of the 'myeloïd' endothelial progenitors or CFU-EC revealed a significant correlation with low birth weight ($P = 0.0478$). Establishment of a ratio 'endothelial injury/endothelial repair' seems to be interesting by its significant correlation with *Z*-score in adulthood ($P < 0.0001$). Therefore, the count of endothelial microparticles and 'nonmyeloid' endothelial progenitors or endothelial colony forming cells was disappointing in our first analysis. These preliminary results suggest that some endothelial cell markers might be predictive of early cardiovascular risk. However, a wide analysis with other metabolic parameters and vascular, kidney and heart imaging might improve the conclusion of these results. To date, identification of endothelial markers in a healthy population should be considered with caution as preliminary results, considering the clinical consequences in public health and prevention.

Key words: cardiovascular diseases, endothelium progenitors, growth, microparticles, programming

Statement of interest: Authors report no conflict of interest.

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POSTER N°75

Abnormal vascular programming of acid arachidonic metabolism could explain hypertension in rats exposed *in utero* to maternal diabetes

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Epidemiological studies have clearly demonstrated that cardiovascular risk is not only determined by conventional risk factors in adult life, but also by early life events resulting of re-settings of physiological functions. We previously demonstrated that *in utero* exposure to maternal diabetes induces a salt-sensitive hypertension in adult offsprings[1] and identified a specific gene expression profile of the thoracic aorta in favour of vasoconstriction, at a pre-hypertensive stage (3 months). We found an increase of CYP4f2 and a decrease of the prostacyclin receptor (IP) mRNA in aorta of rats exposed *in utero* to maternal diabetes (DMO) compared with rats from control mothers (CMO). We demonstrated a functional implication of these modifications in conscious animals and in isolated aortic rings. Indeed, even before the onset of hypertension (3 months), *in vivo* and *ex vivo* studies in conductance arteries showed a decreased vasodilatory response to a prostacycline analog.[2] In order to evaluate effect on resistance arteries, we conducted vascular reactivity experiments on mesenteric arteries of male DMO and CMO at 3 and 18 months of age. By measuring vascular responses to phenylephrine, acetylcholine, sodium nitroprussiate and a prostacycline analog, we highilghted perturbations in pharmacological reactivity in DMO as soon as 3 months of age, in favour of vasoconstriction. In this study, we clearly demonstrated a foetal programming of vascular functions of conductance and resistance arteries in adult rats exposed *in utero* to maternal diabetes, which could explain a re-setting of physiological functions and the implication in hypertension later in life.

Key words: fetal programming, gestational diabetes, hypertension/blood pressure

Statement of interest: Authors declare to have no conflict of interest.

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POSTER N°21

Does high protein neonatal feeding program metabolic syndrome in adulthood? A study in a rodent model of intrauterine growth restriction

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Infants born with a low birth weight because of intrauterine growth restriction (IUGR) commonly receive enriched, high protein formulas to ensure postnatal catch-up growth. Whether such high protein nutrition administered in early life has deleterious metabolic effects in the long run is

unknown. To determine the long-term metabolic effects of high protein neonatal feeding, pups born with a low birth weight from dams fed a low-protein diet during gestation were separated from their mothers, and equipped with gastrostomy tubes on the 5th day of postnatal life (D5). Between D7 and D21, they received as their sole feeding a milk substitute of either 'adequate' (AP; $n = 14$; 8.7 g/dl), or 'high' (HP; $n = 14$; 13.0 g/dl) protein content administered through the gastrostomy tube in the 'pup in the cup' system, and were then weaned to standard chow until they were killed at adulthood. At D18, HP feeding was associated with higher rates of protein turnover ($P = 0.007$) and synthesis ($P = 0.051$), as assessed using L-[U^{13}C]valine infusion. Rats that had received HP milk in early life gained more weight from puberty through adulthood, had a slightly higher food intake, higher serum insulin (179 ± 58 *v.* 55 ± 7 pmol/l; means $\pm$ S.E.), a higher HOMA-IR index, increased pancreatic β-cell number, plasma triglycerides (95 ± 8 *v.* 73 ± 9 mg/dl), serum leptin (9.7 ± 1.0 *v.* 5.5 ± 1.2 ng/ml), increased mesenteric fat mass and larger adipocytes. In a model of intrauterine growth restriction, high-protein neonatal feeding may 'program' metabolic syndrome in adulthood. Whether such programming effect is relevant for human infants born with IUGR clearly deserves further investigation.

Key words: amino acid metabolism, early development and adult disease, insulin resistance, metabolic syndrome, pup in the cup

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POSTER N°48

Early exposure to a high-fat diet has more drastic consequences on metabolism compared with exposure during adulthood in rats

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The peripubertal period in rats, which is characterized by several alterations, such as increased levels of steroid hormones, is critical for the final maturation of most neuroendocrine circuits, including those that regulate energy expenditure.[1–4] We aimed to determine whether the introduction of a high-fat (HF) diet during the peripubertal phase induces significant changes in body weight control, glucose homeostasis and the parasympathetic tonus than when HF diet is administrated to adult rats. An HF diet was offered at weaning or during adulthood to male Wistar rats. A group of animals received the HF diet for 60 days, from weaning to 81 days old (HF81) or from 60 to 120 days old (HF120), whereas two other groups received a normal-fat diet (NF81; NF120). Adiposity, glucose homeostasis, insulin sensibility and vagal activity were analyzed. HF diet increased the accumulation of adipose tissue in all animals but to a much larger degree in animals fed an HF diet since weaning ($P < 0.001$). The HF rats showed glucose intolerance with high levels of insulin secretion during the glucose tolerance test ($P < 0.01$). Rats that were fed the HF diet presented severe insulin resistance, indicated by a low Kitt ($P < 0.01$). Interestingly, the HF81 rats exhibited greater insulin resistance compared with the HF120 rats ($P < 0.05$). The recordings of vagus nerve activity showed that the HF rats had higher parasympathetic activity than the NF rats irrespective of age ($P < 0.01$). Our results show that an HF diet offered to rats just after the weaning or in adulthood both cause impairment of glycemic homeostasis and imbalance in parasympathetic activity. Importantly, the consumption of HF diet immediately after weaning has more drastic consequences compared with the consumption of the same diet during adulthood.

Key words: glycemic homeostasis, insulin sensibility, nutrition, parasympathetic activity, puberty

Statement of interest: Authors report no conflict of interest.

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POSTER N°51

Effect of perinatal undernutrition in Merinos D'Arles ewes on physiology and reproductive function

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Maternal nutrition can affect postnatal growth and development of offspring. The aim of this study was to evaluate the long-term effects of maternal periconceptional undernutrition

(PCUN) in ewes on postnatal development of male lambs. A total of 52 Merinos d'Arles ewes were fed to requirements (control group, C), whereas 64 restricted (R) ewes received 50% of their dietary needs from −15 days to +30 days post conception. Thereafter, both groups were fed according to needs. Male offspring were weighed at birth and then weekly. They were weaned at 22 kg body weight (BW). Male lambs were raised until about 35 kg BW at commercial slaughter. Plasma leptin and cortisol concentrations were determined monthly. Organ weights were recorded at slaughter, and histological analysis was performed on testicles and adrenals. A total of 22 C and 34 R male lambs were obtained at lambing. Gestation was significantly longer in the nutrient-restricted group ($P < 0.01$).[1] Birth weight and growth rate in all lambs was not significantly different between groups.[1] Plasma leptin concentrations were significantly lower in R male lambs at birth ($P < 0.001$), increased gradually in both groups until 3 months and tended to be higher at 4 months in R group. There was a significant interaction between groups, age and litter size for basal cortisol concentrations: in singletons, cortisol was significantly lower in R at 3 months ($P < 0.05$) and tended to be higher at 4 months, whereas in twins, cortisol was significantly lower at birth ($P < 0.05$) but not thereafter. The ratios of carcass weight/BW and perirenal fat/BW were increased in R lambs. Adrenal on BW ratio tended to be higher in R lambs but adrenal corticomedullary ratio was not different between groups. There was no significant difference for adrenal and testicular tissue analyses. These results obtained in a hardy breed confirm the effects of PCUN on metabolic function but not on male reproductive development.

Key words: cortisol, fetal programming, leptin, male lamb, maternal nutrition

Statement of interest: Authors report no conflict of interest.

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POSTER N°66

Sleep longitudinal study among French children and the impact on adiposity and overweight risks in the EDEN cohort

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Sleep represents one-third of our lifetime and is a vital function allowing physical, psychological and intellectual recuperation. Sleep mechanisms are better understood but their implications in health are still not completely elucidated. In humans, bad sleep has an impact on wake vigilance, body's temperature, energetic stock reconstitution, hormone production, metabolic functions, mood and stress activation regulation, toxin elimination, immune defense system stimulation, learning and memorization mechanisms and physical and intellectual performances. Adult and childhood obesity is associated with diabetes, insulin resistance, sleep obstructive apnea, cardiovascular diseases, hypertension and several cancers. In adults, sleep deprivation (<6 h/night) is associated with overweight, obesity and type 2 diabetes. Similar results have been obtained by cross-sectional studies among children on body mass index (BMI) *z*-score (BMI adjusted on sex and age). Few longitudinal studies have been conducted on healthy children to determine the impact of sleep on overweight and obesity; none in France. EDEN is a mother–child cohort study. Mothers' inclusions during their first trimester of pregnancy were carried out between 2003 and 2006. Mothers and children were followed up by questionnaires and clinical examinations over 5 years. Data, including socio-demographic, economic, nutritional, psychological, physical, intellectual and health variables, are currently available for 1310 mother–child pairs. None of these variables were simultaneously analyzed in the previous longitudinal studies. The project aims at studying children's sleep pattern in the EDEN cohort, taking into account these specific potential modulating factors available (from first trimester of pregnancy to 5 years of age of the children). We will identify factors associated with sleep pattern at each age (2, 3 and 5 years old) through cross-sectional analyses. We will also focus on sleep trajectory and modulating factors over time with longitudinal analyses. Impact of sleep and sleep variations on children's adiposity and overweight in childhood will be studied. Preliminary results will be discussed.

Key words: epidemiology/public health, newborn/children, obesity

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PLACENTA: A PROGRAMMING TISSUE

2 – P. Chavatte-Palmer and R. Levy

The central role of the placenta is emphasized both in humans and in animal models (rats, pigs and rabbits). Differential physiological, transcriptomic and epigenetic responses to adverse maternal conditions were observed in cases of spontaneous intrauterine growth restriction (IUGR), maternal high-fat diet, maternal diabetes and global food restriction. Early embryo adaptations were related to placental observations in the case of high-fat diets in rabbits, the role of placental transporters, circulating angiogenic factors and macrophages were highlighted in several studies and a specific epigenetic signature has been observed in the placenta of IUGR rats. These observations shed light on mechanisms and may provide early markers of subsequent adverse effects in adult offspring.

ORAL N°31

Evaluation of diabetes' effects on placental fetal macrophages

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Fetal placental macrophages defined as Hofbauer cells (HBCs) are localized in the stroma adjacent to trophoblast and capillaries.[1] Several functions have been addressed to HBCs: transportation of ions, stimulation or inhibition of the other mesenchymal cell's proliferation, remodeling of the extracellular matrix, production of angiogenic growth factors[2] and the release of cytokines and chemokines. Several studies focused on the link between HBCs and some pregnancy complications,[3] but nothing was reported about the role of HBCs in the placenta of women affected by maternal diabetes. Macrophages can respond to the hyperglycemic stimulation modifying their phenotypic profile switching from M2 (anti-inflammatory) to M1 (pro-inflammatory).[4] Notably, HBCs in a 'normal' placenta are identified as M2 macrophages. In the light of this background, our goal is to clarify the effect of 'diabetes' on placental fetal macrophages (i.e. HBCs) *in vitro* and *in vivo* (animal models). Our preliminary data demonstrated: (1) an imbalance at transcriptional level between some pro- and anti-inflammatory cytokines and some M1 and M2 markers in diabetic versus control rat placentas; (2) the purified HBCs cultured in high-glucose medium have showed an increase of some of the pro-inflammatory cytokines (Il12b, Tnfα) and a decrease of some anti-inflammatory cytokine (Il10, Il4) at mRNA level. Once we provide evidence that hyperglycemia can switch the fetal placental macrophages from M2 to M1's profile, we will investigate whether maternal hyperglycemia can program, at epigenetic level, the progeny's macrophages toward a pro-inflammatory profile, leading to a predisposition to metabolic diseases in adulthood. The results obtained in our animal model will be used as preliminary data to start the profiling of HBCs in type 1 and type 2 diabetic women's placentas (Cohort DIAMANT).

Key words: diabetes, fetal programming, immune function, placenta

Statement of interest: Authors report no conflict of interest.

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ORAL N°36

Maternal lipid and cholesterol-enriched diet disrupts fetal development and placental function in a rabbit model

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We have shown that maternal administration of a lipid (8%) and cholesterol (0.2%)-enriched diet (HH diet) in a rabbit model leads to intrauterine growth factor (IUGR) and increased offspring susceptibility to excess body fat, overweight and hypertension in adults.[1] To examine the link between the fetal development and metabolic consequences in later life, placental development has been explored. Female rabbits were fed with a control (C) or HH diet from 10 weeks of age and throughout gestation. At 28 days of gestation, dams were anesthetized and a laparotomy was performed to collect placenta and plasma. Fetal weight in HH group was significantly reduced compared with C. Total cholesterol and triglycerides concentrations in HH fetuses were significantly increased by 1.2- and 2.3-fold, respectively, compared with C. The structural analysis of HH placentas revealed an abnormal accumulation of light vesicles, identified as lipid droplets in the trophoblast layer. Total content of cholesterol esters and triglycerides were also significantly increased in HH placentas. The expression of genes involved in placental growth, vascularization and nutrient transfer has been studied. HH placentas were characterized by a significant decrease in *LDL-receptor*, *CD36*, *LXR-α*, *ABC-G1*, *SLC38A1* and *SLC38A2* transcripts. The downregulation of *LXR-α* mRNA was correlated with a decrease in protein expression. These data demonstrate that maternal HH diet reduced cholesterol transport through the placenta as evidenced by placental gene expression and cholesterol ester accumulation. In contrast, fatty acid transport was not regulated, which could explain the excess of body fat in adults.

Key words: body composition, maternal diet, placenta, pregnancy, small animals

Statement of interest: Authors report no conflict of interest.

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ORAL N°54

Hyperlipidic hypercholesterolemic maternal diet affects early embryonic gene expression and trophoblast function

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Maternal diets have been shown to affect fetal development and postnatal health. However, their effects on early preimplantation embryo remain less documented. Feeding rabbit females with hyperlipidic (8%), hypercholesterolemic (0.2%) diet (HH diet) from 10 weeks of age resulted in intrauterine growth retardation of the progeny as soon as day 9 post fertilization.[1] We thus wondered whether early embryo was affected by this maternal diet. Therefore, we compared the transcriptome of embryos developed in HH-fed females with that of their control (C) counterparts at the stage just following the onset of embryonic genome activation (16–20 cell stage). Our transcriptome analyses evidenced the overexpression of ADIPOPHILIN in HH embryos. ADIPOPHILIN encodes for a protein involved in the early steps of lipid droplets formation from the endoplasmic reticulum. Its overexpression at embryonic genome activation stage was confirmed by quantitative RT-PCR analyses. It seems to be transient as transcript quantification at the blastocyst stage did not detect significant differences between HH and C embryos. However, very interestingly, immunocytochemical analysis of ADIPOPHILIN localization at the blastocyst stage showed that ADIPOPHILIN colocalized with Nile Red-stained lipid droplets in the cytoplasm of trophoblast cells in HH embryos. Such lipid droplets accumulation was not found in control embryos. Later on, during gestation, HH conceptuses displayed similar lipid droplets in the labyrinthine zone of their placenta, whereas C conceptuses did not. Thus, our results evidenced that embryo gene expression may be sensitive to maternal diet as early as embryonic genome activation stage, and gene deregulation may be involved in early perturbation of extraembryonic tissues that persists during pregnancy.

Key words: embryo, maternal diet, periods of plasticity, pregnancy, small animals

Statement of interest: Authors report no conflict of interest.

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POSTER N°30

Transcriptomic analysis demonstrated a placental dysfunction during maternal diabetes

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Nowadays, there is increasing evidence for a role of the perinatal environment in the metabolic programming of adult life. A disturbed intrauterine milieu, such as maternal diabetes, can favorthe occurrence of chronic diseases in adulthood. In animal models of streptozotocin (STZ)-induced diabetes, few studies have assessed the potential role of the placenta and particularly the implication of feto-placental genes on fetal programming. In our work, we: (1) evaluate the consequences of maternal hyperglycemia on pups' metabolism; (2) use systems biology approach to analyzedifferentially expressed placental genes involved in intrauterine growth retardation (IUGR). We used three groups of animals: DS ($n = 5$, receiving 65 mg/kg of STZ at G7), D30 ($n = 9$, receiving sequentially STZ and 75 mg/kg of Nicotinamide at G7) and a control group ($n = 9$). We have evaluated metabolic parameters in mothers during gestation and in pups at birth. Placental whole-genome expression was performed to identify genes differentially expressed between experimental groups (Illumina). Diabetes in DS group is more pronounced than in D30 group. We have observed in our treated groups an IUGR with placental hypertrophy. Histological observations showed a clear hypovascularization. These observations have been correlated with our transcriptomic analyses showing a modification of genes implicated in angiogenic pathways. Especially, prolactin gene (Fold change >4) and protein were highly upregulated in the DS group partially explaining the hypovascularization that can be due to prolactin anti-angiogenic effect. We confirmed these observations in human on placental samples collected from patients with type 1 diabetes (DIAMANT Cohort). Maternal diabetes induces a profound modification of placental genes leading to a defect in angiogenesis. IUGR observed is the result of the placental hypovascularization and could profoundly modify the metabolic imprinting of the fetus.

Key words: developmental programming, diabetes, fetal programming, gestational diabetes

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POSTER N°32

Mild gestational hyperglycemia in rat induces fetal overgrowth and modulates placental growth factors and nutrient transporters expression

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Mild gestational hyperglycemia (MGH) constitutes an adverse environment during pregnancy and is often associated with fetal overgrowth.[1] Intrauterine environment is an important determinant for placental development, which is

now recognized as one of the factors contributing to the developmental programming of chronic diseases in later life.[2,3] To date, there is no relevant animal model that displays impaired glucose tolerance without diabetes during gestation to investigate the mechanisms underlying fetal overgrowth in pregnancy complicated with MGH. We have developed a rat model and investigated the effects of maternal dysglycemia on fetal growth and placental gene expression. Female rats were injected with nicotinamide and streptozotocin (N-STZ) 1 week before mating. N-STZ treatment induced impaired glucose tolerance in late gestation, resulting in metabolic disorders and fetal overgrowth in more than 20% of newborns. Placental mass was also increased in N-STZ macrosomic pups compared with normotrophes, and associated with a rise in the labyrinthine zone. Gene and protein expression of lipoprotein lipase was increased in N-STZ placentas from macrosomic pups. We reported that expression of insulin receptor and glucose transporters genes was down-regulated in macrosomic placentas, whereas the expression of amino acid transporters was not modified. For the first time, we showed that insulin-like growth factors and nutrient transporter genes were also differentially expressed in the placentas from normal pregnancies when the number of fetuses within the litter varies. The N-STZ model offers the potential for further studies into the effects of MGH on placental function that will allow better understanding of the mechanisms underlying fetal overgrowth. We propose that the regulation of placental gene expression constitutes a mechanism of physiological adaptation that is taking part during late gestation to optimize fetal growth and assure the viability at birth when the number or the size of fetuses is inappropriate.

Key words: animal, fetal growth, gestational diabetes, placenta

Statement of interest: Authors report no conflict of interest.

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POSTER N°37

Characterization of the placental development in the intrauterine growth-retarded piglet

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In pig production, the selection of hyperprolific animals has lead to an increased rate of intrauterine growth-retarded piglets in litters. Intrauterine growth retardation (IUGR) is an important economic issue inducing a higher mortality rate, a lower growth capacity, a higher muscular lipid content in carcasses and less tender meat. The maternal and fetal environments interact through the placenta to maintain nutrient supply of the fetus. The aim of this work was to explore placental adaptive mechanisms throughout pregnancy in IUGR piglets. Possible changes in placental morphometry and gene expression of the IUGR piglet were prospected, using a natural IUGR pig model. Placental samples from 18 pairs of control (normal birth weight) and IUGR fetuses at gestation days (gd) 45, 71 and 112 were analyzed by stereology. The expression of 10 candidate genes potentially associated with placental development and IUGR was examined by RT-qPCR. The DNA methylation of the insulin-like growth factor 2 (IGF2) imprinting gene was evaluated by pyrosequencing. Glycemia and fructosemia were measured in IUGR and control fetuses at gd 71 and 112. No morphometric abnormality was found in the IUGR placenta. An increased expression of the IGF2 gene, however, was observed in the IUGR chorionic tissue at gd 71 ($\times$1.48, $P < 0.05$). No difference was shown, however, in the DNA methylation levels of the IGF2 gene. Fructosemia was significantly reduced in IUGR fetuses at gd 71, but not at gd 112, whereas glycemia remained normal at both stages. IGF2 affects the placental growth and the placental permeability by modulating the nutrient transport levels. This increase could be a compensatory mechanism from the placenta to meet the fetal nutrient demand and maintain fetal growth at mid-gestation. Indeed, there was a tendency for the expression of glucose transporter GLUT3 to be reduced at gd 71.

Key words: critical periods, fetal growth, large animals, placenta, pregnancy

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POSTER N°45

Maternal food-restriction leads to a drastic downregulation of H4K16 acetylation in IUGR rat placentas

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Undernutrition during gestation is associated with an increased susceptibility to metabolic and cardiovascular diseases. Placenta, as a widely recognized programming agent, contributes to the underlying processes. Alterations in both placental development and activity are well known to constitute programming events for offspring's physiology and metabolism in adulthood. Growing experimental evidences suggest that epigenetic marks may serve as a memory of exposure to inappropriate environments and thus could be implicated in foetal programming.[1] Our aim was to explore whether maternal undernutrition could disturb epigenetic processes in the placenta of intrauterine growth-restricted (IUGR) foetuses. Two experimental IUGR models were used: pregnant Wistar rats were subjected to a 70% food restriction along the gestation (FR30 model);[2] or to a 50% food restriction during the last week of gestation (FR50).[3] We investigated the global level of four epigenetic marks in full-term placentas. DNA methylation was assessed using LUMA and performed western blot assays for H3K9me3, H3K4me3 and H4K16ac, three important histone marks.[4] We did not observe any change in H3K9me3, H3K4me3 and DNA methylation, but a decrease in placental H4K16ac, in both models and in both sexes. High-performance liquid chromatography/high-performance capillary electrophoresis quantified the decrease of H4 monoacetylation: −12% in FR30 males, −18% in FR50 males and −22% in FR30 and FR50 females. As both models were similarly affected, our findings suggest that the last third of gestation may be a critical period for H4K16ac set-up in placenta. This epigenetic mark may constitute a nutritional sensitive target during foetal programming and may be an important link between nutrition and epigenetic programming during foeto-placental development.

Key words: epigenetics, IUGR, placenta, programming

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FOOD PREFERENCES, EATING BEHAVIOUR AND COGNITIVE DEVELOPMENT

3 – A. Chango and C. Delpierre

Studies in the session suggest a prenatal role of maternal PUFA consumption on child psychomotor development. Stress is reported to promote palatable food intake and highlight the critical role of early nutrition in neurodevelopment and behavioral responses later in life. Malnutrition during early life sensitizes the offspring to the development of metabolic and neurological disorders in adulthood. Data indicate that, in addition to induce stable epigenetic modifications in the hippocampus, perinatal malnutrition alters the plastic epigenetic responses underlying learning and memory.

ORAL N°24

Early nutrition: impact on the development of food preferences and eating behavior

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Early nutritional status not only affects health on the long term but it also affects food preferences and eating behavior at different stages in the developing child. Moreover, eating behavior in early childhood tracks until adulthood.[1] Modes of feeding evolve in the first years of life, starting from cord feeding, going through a transitional phase of milk feeding to ultimately end by eating the family diet. These transitions involve a series of adaptations, which will ultimately affect food preferences, eating behavior and, as a result, weight and health status. Understanding the development of eating behavior in the current context of a wide availability of palatable foods is therefore central to address key societal issues such as the epidemics of obesity and to provide parents with science-based feeding recommendations. This presentation aims at showing the impact of breastfeeding and of practices of complementary feeding on eating behavior in the 1st year of life. Maternal milk bears flavors from the foods ingested by the mother and its tastes different from that of formula milk: it will be shown how breastfeeding affects the infant's food preferences around the time of complementary feeding, as shown by experimental studies in human infants and by a longitudinal study.[2,3] At this age, infants display varied reactions toward new foods according to the sensory properties of the foods.[4] Moreover, based on results from varied experimental studies, it will be shown how complementary feeding practices, in particular introduction of a variety of foods, affect further food acceptance.[4]

Key words: early life nutrition, human, infant feeding/ breastfeeding

Statement of Interest: The authors have no conflict of interest to declare.

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POSTER N°3

Rho-kinase inhibition during late-gestation programs hyperphagia and overweight in adult male rats

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Rho-kinase (ROCKs) belongs to the family of serine/threonine kinases and plays important roles in various cellular functions including contraction, motility, proliferation and apoptosis.[1] Recent findings have reported that activation of the Rho to Rho-kinase (Rho–Rho-kinase) pathway may also be implicated in several metabolic dysfunctions such as in glucose intolerance and obesity.[2–4] Physiological functions of Rho-kinase during the prenatal life have been poorly investigated. We investigated in rats the consequences of a prenatal exposure to Fasudil, a synthetic Rho-kinase inhibitor, during late gestation (10 mg/day) on postnatal growth, on food-intake behavior and glucose homeostasis, as well as on organ development and plasma level of several hormones in 9-month-old male rats. Prenatal exposure to Fasudil did not affect birth weight, but increased body weight from postnatal day 7 (P7) to 9 months. At P180, rats exposed to Fasudil showed an increased basal blood glucose associated with mild glucose intolerance at 6 months. In 9-month-old rats, exposure to Fasudil increased the daily food intake and hypothalamic mRNA level of the orexigenic NPY gene without modulation of the anorexigenic POMC gene expression. Altogether, our data demonstrated that prenatal Fasudil exposure programs long-term metabolic disturbances including transient perturbations of glucose metabolism, a persistent increase of body weight gain, hyperphagia and an augmented expression of hypothalamic NPY orexigenic gene. We thus postulated that prenatal Rho-kinase inhibition had altered the development of arcuate nucleus NPY neurons and postnatal leptin surge. Analysis of hypothalamic NPY gene expression level of other orexigenic/anorexigenic factors and postnatal plasma leptin level were not altered during lactation suggesting other etiology to these defects.

Key words: critical periods, fetal programming

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POSTER N°20

Impact of nutritional programming on first meal pattern and gastro-intestinal peptides following energy restriction in adult rat

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Intrauterine growth retardation (IUGR) is associated with a greater incidence of metabolic disorders at adulthood.[1] Nutritional programming is responsive for feeding behavior alteration, which could contribute to the increased metabolic risk.[2,3] Short-term regulation of food intake is initiated by distension of the gastrointestinal (GI) tract and peptides release that may be influenced by nutritional programming. Here, we analyzed the effects of IUGR on feeding behavior following food restriction in adult rats in relation to GI peptides regulating food intake. IUGR male rats (RR) were obtained from protein-restricted mothers (R; 8% protein) and control rats (CC) from unrestricted mothers (C; 20% protein). After weaning, both groups received a standard diet until 150 days. Following 48-h fasting, total food intake (g/kg body weight/24 h) was higher in RR (56.7 ± 3.9) *v.* CC (41.3 ± 4.7). First meal size (g) was higher in RR (10.6 ± 0.99) *v.* CC (7.18 ± 0.34) and the intermeal time preceding the second meal was reduced, leading to a 30% lower satiety ratio in RR. The enhanced appetite in RR could be related to higher fasting plasma desacyl-ghrelin. Plasma acyl-ghrelin was not affected by feeding (2 h) in both groups, but gastric ghrelin *O*-acetyltransferase expression of refed RR was upregulated. Such an alteration of the ghrelin system could contribute to further deficiency in energy metabolism of IUGR rats, especially under high-fat diet.[4] Plasma 2-h postprandial non-sulfated cholecystokinin (CCK)-8 was not different from basal in CC but was higher in RR. This suggests a delayed CCK release in IUGR rats and could explain the lower satiety ratio. No effect of IUGR was observed either on fasting and postprandial plasma concentration of PYY and GIP or on mRNA propeptides in GI tissue. Our study suggests that IUGR may affect postprandial CCK release and ghrelin system, thereby affecting short-term regulation of satiety and energy balance.

Key words: adult, early life nutrition, fetal programming, metabolic syndrome, small animals

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POSTER N°73

Nutritional regulation of ghrelin signaling during neonatal life

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Neonatal overfeeding increases the risk of obesity and metabolic disease in adulthood. Recent data, including from our laboratory, have indicated that developmental malprogramming of hypothalamic appetite-related circuits by the perinatal environment is a possible cause for these diseases.[1] Ghrelin is a hormone secreted by the stomach that promotes feeding by binding to the growth hormone secretagogue receptor (GHS-R) in the arcuate nucleus of the hypothalamus (ARH). Ghrelin has recently been shown to directly modulate the development of neuronal projections from the ARH during early life and affect long-term programming of metabolic function. The objective of this study was to investigate whether neonatal overfeeding disturbs ghrelin signaling and how it may contribute to lifelong metabolic dysregulation. Neonatal overfeeding was induced by raising mice in small litters; postnatally overnourished mice remained moderately overweight in adulthood and had 50% greater fat mass than control mice. Pups were sacrificed at various points between birth and weaning to investigate postnatal ghrelin signaling. As previously reported, normally fed mice displayed a gradual increase in serum ghrelin levels between the 2nd and 3rd postnatal week. In contrast, neonatally overfed pups had reduced total ghrelin levels at P16 and reduced active ghrelin levels at P16 and P22, These changes in circulating ghrelin levels were associated with a decrease in the expression of ghrelin mRNA in the stomach. Neonatal overfeeding also attenuated GSH-R mRNA expression in the ARH at P16 and P22 as compared with control mice. However, ghsr mRNA were not different in the dorsomedial hypothalamic nucleus. In conclusion, neonatal overnutrition attenuates ghrelin secretion and brain ghrelin receptor levels during critical periods of development. As ghrelin is involved in the development of hypothalamic feeding circuits, these results suggest that the attenuated ghrelin signaling caused by neonatal overnutrition may perturb hypothalamic development and result in long-term metabolic dysfunction.

Key words: critical periods, developmental programming, early life nutrition, infant growth, obesity

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ORAL N°1

Polyunsaturated fatty acids intake during pregnancy and child psychomotor development: results from the EDEN mother–child cohort study

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Foetal brain development needs polyunsaturated fatty acids (PUFA) intake, especially in ω-3, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[1] Prenatal exposure to these PUFAs is particularly linked to maternal dietary during pregnancy.[2] The aim of our study was to investigate relationship between maternal PUFA intake during pregnancy and later child psychomotor development. We evaluated psychomotor development at 3 years in 1066 children from the EDEN mother–child cohort study, with the parent having completed Ages and Stages Questionnaire (ASQ).[3] Dietary lipid intake during pregnancy was estimated by crossing a food frequency questionnaire and a food nutrient chart.[4] We studied associations between PUFA intake and psychomotor score at ASQ by linear regressions, adjusted for centre, child age and sex, gestational age, maternal consumption of alcohol and tobacco, parental education, household incomes, sibling number, child caretaker, frequency of maternal stimulations and school attendance. Mean (±S.D.) ASQ score was 270.1 (±29.4) points, maternal ω-6/ω-3 ratio in dietary was 8.4 (±2.3) and total PUFAs intake was 11.1% (±2.5) of total lipid consumption. After adjustments, ASQ score was negatively associated with ω-6/ω-3 ratio ($\beta = -1.1$; S.E. = 0.4; $P = 0.005$), and positively with EPA ($\beta = 3.6$; S.E. = 1.7; $P = 0.029$) and DHA ($\beta = 1.9$; S.E. = 0.8; $P = 0.035$) consumptions. These associations persisted among never breastfed children. Our results suggest a prenatal role of maternal PUFA consumption on child psychomotor development.

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Key words: cognitive function, critical periods, early life nutrition, epidemiology/public health, maternal nutrition/diet

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ORAL N°33

Perinatal high-fat diet attenuates anxiety-like behaviour in adult offspring exposed to maternal separation

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In humans, adverse early life experiences such as childhood traumas increase the vulnerability to mood disorders at adulthood.[1] In rats, chronic neonatal maternal separation of the pups from their mother (>3 h/day) increases anxiety-like behavior and exacerbates stress response in the offspring.[2] Comfort food theory suggests that chronic stressful situations promote nutrient-dense food intake and that pleasurable feeding reduces endocrine and behavioral effects of stress.[3] Recently, it has been demonstrated that high-fat diet ameliorates anxiety- and depressive-like behaviors in mother rats subjected to maternal separation.[4] However, it is unknown whether maternal consumption of a high-fat diet can also protect their progeny against early stress-induced emotional disturbances. The present study aimed to examine the impact of a high-fat diet (20%) during pregnancy and lactation on the effects of maternal separation in adult male rat offspring. Here we report that maternal high-fat diet modulates the behavioral effects of chronic stress separation both in the offspring and in their mothers. Indeed, maternal separation leads to spatial memory impairments and enhances anxiety-like behavior in offspring of mothers fed a normal chow; however, these deficits are counteracted by maternal high-fat diet. Furthermore, we also observed that during stress sessions, dams exhibited a marked increase of the high-fat food intake, associated with a decrease of their anxiety-like behavior. These results reinforce the idea that stress promotes palatable food intake and highlight the critical role of early nutrition in neurodevelopment and behavioral responses later in life.

Key words: animal, early life nutrition, maternal stress, mental health/illness, palatable food

Statement of interest: The authors declare that they have no competing interests.

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POSTER N°80

Grandmothers' transgenerational transmission of the prenatal stress phenotype in rats

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Early life stress may program offspring susceptibility to lifelong health problems, and there is increasing evidence that developmental programming by an altered intrauterine environment can be passed across generations. In rats, we have previously shown that prenatally restraint stress (PRS) induces long-lasting biochemical and behavioral changes, which result in expression of an anxious/depressive phenotype. In mice, PRS increases the expression of type-1 DNA methyl transferase in the frontal cortex and induces epigenetic changes in mGlu2/3 metabotropic glutamate receptors.[1] Here we examined the transgenerational effect of PRS in rats by mating first-generation (F1) PRS female rats with naïve males. Remarkably, most of the behavioral and neurobiological alterations associated with PRS persisted in the second-generation (F2) rats, despite the fact that these males were reared normally (i.e not directly exposed to stress *in utero*). We observed enhanced anxiety-like behavior, prolonged corticosterone response to stress and increased brain-derived neurotrophic factor and reduced mGlu2/3 receptor expression in the hippocampus in both F1 and F2 rats. In addition, we identified several genes stably regulated by PRS that were transmitted to F2 generation by a microarray analysis of the hippocampal transcriptome. As vulnerability to stress-related disorders can be epigenetically programmed by maternal behavior, we scored dams exposed to the repeated restraint stress during gestation (F0, grandmothers) as well as in their female offspring (F1, mothers). Gestational stress in grandmothers markedly reduced the amount of nursing and licking/grooming behavior, and enhanced anxiety during

and after lactation. Interestingly, PRS stress affected more mildly maternal behavior in F1 dams and had no effect on their anxiety-like profile. Our results show that the pathological programming induced by PRS in rats can be transmitted across generations and that transmission involves mechanisms independent of maternal behavior.

Key words: anxiety, epigenetics, maternal

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ORAL N°43

Effects of early protein restriction on the plastic epigenetic responses associated with learning and memory

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Malnutrition during early life sensitizes the offspring to the development of metabolic and neurological disorders in adulthood. This enhanced disease susceptibility would be the result of epigenetic modifications (DNA methylation and posttranslational modifications of histones) that fix the effects of early and transient nutritional events imposing a memory effect that can modulate an individual's phenotype over his whole life. However, epigenetic processes are plastic and can change in response to a wide range on environmental stimuli beyond the prenatal and postnatal periods of development. Nevertheless, we ignore whether, and how, an early nutritional insult also alters these plastic epigenetic responses. Here we have addressed this question by examining the effects of prenatal protein restriction of the epigenetic responses associated with learning and memory. Pregnant Wistar rats were fed *ad libitum* either a control (20% protein) or a low-protein (8% protein) diet throughout pregnancy and lactation. At weaning, pups received a standard diet, and at 3 months of age their memory capacities were evaluated using the fear condition paradigm. In addition, we analysed, in the hippocampus, several posttranslational modifications of histones 3 (H3) and 2B (H2B), which have been identified as epigenetic marks of learning and memory. Naive protein-restricted pups exhibited increased acetylation of H2B and of H3 on lysine 9 (H3AcK9) in relation to their control counterparts along with decreased dimethylation of H3 on lysine 9 (H3diMeK9); however, there were no significant differences between the two groups in the acetylation of H3 on lysine 14 (H3AcK14). Malnourished pups showed less freezing behaviour during fear conditioning and this learning deficit was associated with an impaired posttranslational profile of H3 and H2B in response to the memory test. These data indicate that, in addition to induce stable epigenetic modifications in the hippocampus, perinatal malnutrition alters the plastic epigenetic responses underlying learning and memory.

Key words: developmental programming, epigenetics, learning and memory, molecular/cellular

Statement of interest: The authors have nothing to declare.

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ORAL N°47

Early postnatal leptin blockade predisposes rats to overweight and modifies hypothalamic microRNA expression pattern

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Perinatal leptin impairment has long-term consequences on energy homeostasis leading to body weight gain.[1,2] The underlying mechanisms are still not clearly established. The present study aimed to analyze the long-term effects of early leptin blockade, using a pegylated rat leptin antagonist (pRLA), on body weight gain, insulin/leptin sensitivity and expression profile of miRNAs at the hypothalamic level. Daily injection of pRLA from postnatal d2 to d13 predisposes rats to overweight under chow or high-fat diet as compared with control rats and promotes the onset of leptin and insulin resistance in both hypothalamus and liver at adulthood. Furthermore, pRLA treatment affects genes involved in energy homeostasis, such as UCPs and AdipoRs. In pRLA rat muscle, UCP2/3 and AdipoR1/R2 were upregulated at d90, whereas, in liver, pRLA treatment upregulated AdipoR1/R2 following high-fat diet challenge. These genes are known to be involved in insulin resistance and type 2 diabetes.[3,4] Finally, using a large-scale analysis, we showed that pRLA modifies hypothalamic miRNA expression profile at d28 as mirrored by 34 miRNAs upregulated and 4 miRNAs downregulated as compared with controls. For quantitative reverse transcription polymerase chain reaction confirmation, we have focused on miRNAs that have been linked to metabolic disorders. In conclusion, the present paper highlights the consequence of leptin blockade early in life on leptin/insulin resistance and hypothalamic miRNA expression modulation in the adulthood, suggesting a potential

link between hypothalamic miRNA expression pattern and the predisposition to impaired insulin/leptin responsiveness.

Key words: high-fat diet, insulin resistance, microRNA, overweight, postnatal leptin

Statement of interest: Authors report no conflict of interest.

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ENDOCRINE DISRUPTORS AND OTHER TOXIC SUBSTANCES

4 – R. Levy and J. Lepeule

Regarding endocrine disruptors, French studies have shown an association between maternal exposure to BPA, parabens and chlorpyrifos during gestation and increased postnatal growth and impaired locomotion in male offspring.

ORAL N°57

Effects of pregnancy exposure to non-persistent endocrine disruptors on foetal development and postnatal growth: overview of recent results from mother–child cohorts and perspectives

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Wildlife observations and toxicological experiments indicated that early-life exposure to endocrine disruptors may affect development and postnatal growth. Human biomonitoring studies have indicated that exposure to many endocrine disruptors is widespread in the general population. For these reasons, characterizing the impact of early-life exposure to such endocrine disruptors in human populations is warranted. We present here results of the impact of pregnancy exposure to select phenols and phthalates on the basis of Eden (Nancy, Poitiers) and Pélagie (Brittany) mother–child cohorts. We focused on male births only. Pregnant women were recruited and followed up with their offspring to monitor foetal growth (ultrasound measures), postnatal growth in the first 3 years of life (520 children) and anomalies of male genital organs (undescended testis at birth and hypospadias, 71 cases). The levels of phenols and phthalate exposure biomarkers in maternal spot urine samples collected during pregnancy were used to assess exposure during intrauterine life. For most women, urinary levels were above the detection limits for most compounds. There was no clear evidence of increased risk of malformation of male genitalia in association with the compounds considered. Bisphenol A pregnancy level was associated with increased abdominal circumference during pregnancy and weight growth velocity at 1 year of age. Parabens tended to be associated with increased birth weight and weight at 30 months not adjusted for height. Our results need to be interpreted in the light of the toxicological literature and considering multiple associations tested and potential exposure misclassification because of the reliance on a single urine sample. Continuing efforts, many of which are under way, are required to further incorporate results from the DOHaD and endocrine disruptors field based on animal models in human cohorts; these include progress in exposure assessment, consideration of exposure mixtures, assessment of epigenetic marks or gene expression levels in epidemiological studies.

Key words: endocrine disrupters, epidemiology/public health, exposures, fetal growth, pregnancy

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ORAL N°69

Low-dose effects of endocrine dirsruptors: towards metabolomics-based phenotypic biomarkers?

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Human beings are exposed to numerous chemicals through environment and food. Many of these compounds, known as 'endocrine disruptors' (ED), have been shown to interfere with development and/or homeostasis. These include the model xeno-estrogen bisphenol A, for which extensive literature documenting low-dose effects is available for the perinatal period. ED effects span far beyond the classical reproductive system target, including metabolism (obesity,

metabolic syndrome), cognition, etc. Because classical tools of toxicology cannot always address low-dose exposure issues, it is necessary to develop new approaches adapted to the detection of subtle changes in the global metabolism. Innovative approaches in the field of metabolomics can highlight metabolic changes in organisms exposed to nutritional, pharmacological or toxic stimuli. Their use for the investigation of metabolic shifts induced *in vivo* can contribute to a better understanding of disrupted metabolic pathways with possible consequences in adult life. To achieve this goal, studies should first rely on the use of model ED for which observed metabolic shifts can ultimately be connected with the occurrence of well-documented biological effects. *In vivo* characterization of biomarkers of exposure can then be extended to more complex situations, involving mixtures and individual susceptibility factors to address current challenges in human health. On the basis of human *in vitro* models, global approaches (non-targeted metabolomics) should also allow to increase our understanding of the mechanisms by which low doses of contaminants trigger biological effects. We present *in vivo/in vitro* proofs of concept based on studies using low doses of bisphenol A. ^{1}H-NMR fingerprinting is a powerful tool enabling to discriminate between exposed/non-exposed animals. Further characterization of the metabolic network of *in vivo/in vitro* systems, and its shift under ED exposure, is expected to largely contribute in deciphering the mechanisms of action of low doses of chemicals.

Key words: bisphenol A, endocrine disrupter, metabolomics, metabolic fingerprints, metabolic networks

Statement of interest: Authors report no conflict of interest.

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POSTER N°18

Behavioural modifications in rat pups following early exposure to organophosphorus chlorpyrifos

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Chlorpyrifos is an organophosphorus insecticide largely used in agriculture worldwide. Behavioural and neurological modifications were reported in both humans and animals exposed during foetal life.[1,2] Chlorpyrifos exposure during critical phases of development in rodents led to impaired learning abilities,[1] decreased or increased locomotor activity and anxiety, depending on the dose and period of exposure,[2–4] with sex-selective effects.[1,2] Both in humans and rodents, chlorpyrifos passes the placental barrier, and after birth, is found in maternal milk. To reproduce what happens in humans, we exposed female rats to chlorpyrifos by oral treatment from the 1st day of gestation until pup's weaning at a subtoxic dose (1 mg/kg/day). Female Wistar rats were force-fed with chlorpyrifos diluted in corn oil or with vehicle (corn oil). The study aimed at looking for cognitive, sensory and motor function modifications in pups born to chlorpyrifos-exposed dams (CPF) compared with those exposed to vehicle (V). Pups (21 males, 21 females) underwent behavioural tests from neonatal period to adulthood, such as startle reflex and open field. We observed behavioural modifications in males only. Open-field tests revealed that CPF pups were and tended to be less active than V pups at 16 days and 18 days ($P = 0.01$ and 0.06, respectively), but this difference disappeared at 20 days ($P = 0.6$). Startle reflex tests were conducted on 31 and 60-day-old rats: no difference between CPF and V rats were found for the startle after basal pulse, but CPF rats exhibited a larger and longer startle when there was the prepulse before the pulse ($P < 0.05$). As a consequence, prepulse inhibition area tended to be smaller in CPF rats compared with V rats ($P = 0.06$). These results suggest impairment in locomotion and in signal processing between auditory and motor system in male rats born to CPF-exposed dams.

Key words: cognitive function, developmental programming, small animals

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POSTER N°56

Pregnancy urinary phenol concentrations in relation to postnatal growth of male offspring

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Phenols are endocrine disruptors, and animal studies suggest a possible role in the development of obesity. We aimed to study the association between maternal pregnancy exposure to phenols and postnatal weight and height growth of male offspring. In 520 boys from EDEN mother–child cohort, a median of 17 weight and 15 height/length measures per child were assessed as part of the follow-up or collected from the child health booklet. Individual weight and height growth were modeled using a nonlinear growth model, including random effects. From the individual equations, growth velocities were calculated at specific ages. Concentrations of nine phenols were measured in maternal urine sampled at 22–29 gestational weeks. We used multiple regression models to study associations between ln-transformed phenol concentrations and birthweight, weight and height at 30 months, weight and height growth velocities at 3, 12 and 24 months adjusting for maternal anthropometry, socioeconomic status and other potential confounders. Interactions with maternal overweight body mass index (BMI $>25\,kg/m^2$) and any breastfeeding up to 3 months were also assessed. The sum of parabens concentrations PB was positively associated with weight and height growth velocity at 2 years and weight and height at 30 months. An increase in one interquartile range of PB was associated with a 198 g difference in weight (95% CI, 22, 374) and a 0.39 cm difference in height (95% CI, 0.00; 0.77) at 30 months. These associations with weight parameters were no longer significant when adjusted for height parameter ($\beta_{30\,mo} = 76\,g$; 95% CI, −51, 203). Bisphenol A (BPA) was significantly associated with weight growth velocity at 1 year when adjusted for height growth velocity at the same age (adjusted $\beta = 8.1$ g/month; 95% CI, 2.2, 14.0; non-adjusted $\beta = 5.9$ g/month; 95% CI, −1.0, 12.8). Associations were not modified by maternal BMI or breastfeeding status. To conclude, exposure to PB during pregnancy might be associated with height growth and BPA with weight growth.

Key words: child growth, DOHaD, endocrine disrupters, epidemiology/public health

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MECHANISMS OF PROGRAMMING

5 – A. Vambergue and L. Storme

Mechanisms of perinatal programming are the subject of intensive research. Novel pathways have now been identified, and some interesting hypothesis could be confirmed. In that way, anti-angiogenic factor-induced endothelial progenitor cells dysfunction were found in cord blood plasma from low birth weight infants. This mechanism may link intrauterine growth restriction and hypertension at adulthood. Folate deficiency-mediated epigenetic dysregulations may impair long-term neurodevelopment through decrease in hippocampal progenitor differentiation and synaptic plasticity. Early postnatal nutritional environment (overnutrition and undernutrition) was found to cause lasting and deleterious effects on the organization of hypothalamic neural circuits. Growing evidence highlighted the key role of microRNA as a mechanism of long-lasting epigenetic regulation by the environment in the early life. In several experimental models, small RNAs have also been identified as vectors of epigenetic inheritance. Indeed, transgenerational transfer of obesity and type 2 diabetes by injection in naïve embryos of testis RNA prepared from obese and diabetic males rises on high-fat diet. Therefore, sperm RNAs may act as a vector of parental inheritance. Finally, bacterial pathogens are able to reprogram host cell transcription. In particular, listeria is able to modulate chromatin-silencing complex, suggesting a possible role of bacteria in long-lasting imprints on host chromatin.

ORAL N°19

Folate deficiency impairs hippocampal progenitor differentiation and synaptic plasticity

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Folate plays an important role in brain development and neuronal homeostasis. It acts as a cofactor in the one-carbon metabolism, and folate shortage leads to decreased levels of S-adenosylmethionine (SAM), the universal substrate for transmethylation reactions, and to elevated levels of homocysteine, a risk factor for various brain pathologies. During development, DNA methylation participates to correct gene expression, and it is likely that the action of folate goes through this process to influence the expression of genes involved in neuroprogenitor proliferation and differentiation. Thus, we studied the consequences of folate deficiency on hippocampal neuronal progenitors issued from the H19-7 cell line. A transcriptional study of 84 genes related with neurogenesis and differentiation identified an altered neuronal interacting network of various gene products involved in the neurogenic program. Decreased production of SAM and increased expression of histone deacetylases (HDACs) led to epigenetic dysregulations of key proneural basic helix–loop–helix transcription factors that impair the differentiation process. Moreover, in folate-deficient progenitors, induction

of differentiation was associated with a lack of cell polarization, disruption of cytoskeleton components and impaired synaptic plasticity. It is known that the ability of neurons to polarize is influenced by cytoskeletal proteins and is crucial for the process of neurite growth, emergence of the axon and for synaptic plasticity. Immunohistochemical and Western blot analyses showed significant abnormalities in the expression and localization of cytoskeleton components, including actin, tubulins and motor proteins, and the loss of polarization was partially restored by a treatment with an HDAC inhibitor. Therefore, folate deficiency may affect neurodevelopment through epigenetic dysregulations and altered cytoskeleton components.

Key words: early life nutrition, epigenetics, maternal nutrition/diet/body composition, molecular/cellular, plasticity

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ORAL N°42

Evidence for the involvement of the nutrient sensors mTOR and AMPK as a common tissue system underlying the nutritional programming of metabolic disorders

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The fact that diverse nutritional interventions in rodent pregnancy establish common phenotypic outcomes in the resulting offspring (i.e. hypertension, obesity and insulin resistance), has led to suggest that the metabolic alterations because of early nutritional stress (over nutrition or under-nutrition) might result from the programming of only a small number of genes, which act as gatekeepers of a key gene program.[1] A reasonable extension of this proposition is that the gatekeeper genes or signaling pathways affected by nutritional programming are the same across diverse cell types, but their common nature is masked by the cell-specific processes they initiate and because of the complex signaling network between the different organs that maintain energy homeostasis. To get insight into the shared cellular responses that may ultimately constitute a common nutritional programming mechanism, in this study we examined the effects of perinatal protein restriction on the activity of the nutrient sensors mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) in hypothalamus, liver and skeletal muscle. mTOR is activated under conditions of high nutrient supply, favors lipogenesis and protein synthesis and inhibits food intake. Conversely, AMPK is activated in response to ATP depletion, inhibits feeding and protein and lipid biosynthesis and stimulates β-oxidation, glucose uptake and glycolysis. We observed that the adult offspring born to protein-restricted (PR) dams exhibit enhanced hypothalamic mTOR activity in the fed state and impaired mTOR responses to fasting and refeeding that differed from one hypothalamic nucleus to another. At the peripheral level, *ad libitum* fed PR rats displayed reduced mTOR activity along with increased AMPK phosphorylation. mTOR activity decreased with fast and returned to pre-fasting levels after refeeding in control but not in PR rats. The widespread tissue alterations of mTOR and AMPK activity in PR animals suggest that these two kinases constitute a major gatekeeper system underlying the developmental programming of metabolic disorders.

Key words: AMPK, developmental programming, mTOR, molecular/cellular, nutrient sensing

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ORAL N°29

Endothelial progenitor cells dysfunction in low birth weight infants: involvement of circulating inhibitors

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Low birth weight (LBW) is a risk factor for hypertension at adulthood. Endothelial progenitor cell (EPC) dysfunction has been characterized in LBW neonates. We hypothesized that changes in soluble, plasma pro- or anti-angiogenic factors are associated with EPC dysfunction and impaired angiogenesis in LBW neonates. Venous umbilical cord blood was collected from 42 normal, term neonates and 75 LBW neonates. Cord blood endothelial colony forming cells (ECFC) from control patients were cultured in the presence of 10% of serum obtained from both groups. The proliferation and the migration of ECFC were significantly reduced when cultured with 10% of serum of LBW neonates compared with serum of control neonates. Matrigel invasion assay was not significantly altered. Umbilical vein plasma vascular endothelial growth factor (VEGF) concentration was significantly reduced in LBW neonates while that of sVEGFR and PF4 were significantly higher. Addition of VEGF corrected the inhibitory effect of LBW serum on normal ECFC proliferation.

We conclude that serum obtained from LBW babies contains factors that exhibit an anti-angiogenic effect on ECFC proliferation and migration. VEGF/sVEGF/PF4 pathway seems to be involved in the EPC dysfunction in LBW neonates.

Key words: cardio-vascular disease, DOHaD, neonate, prematurity

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ORAL N°6

Genome-wide analysis of epigenetic signatures induced by exposure to high glucose concentrations in endothelial cells supports the implication of epigenetic phenomena in diabetic endothelial dysfunction

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Recent studies show that poor glycemic control in diabetes induces persistent epigenetic modifications that may underlie the phenomenon of glycemic memory observed in the prospective DCCT/EDIC and UKPSD clinical trials. These studies, analyzing a type 1 and type 2 diabetic patients' cohort, respectively, have demonstrated the beneficial effects of early tight glycemic control on microvascular and macrovascular complications caused by diabetes. In this study, we have submitted endothelial cells to high glucose concentrations (25 mM) and we have assessed the consequences of such exposure to a 'hyperglycemic' milieu on the epigenome by chromatin immunoprecipitation of acetylated histone H3 and methylated DNA linked to next-generation sequencing. In parallel, gene expression profiles have been determined by microarray analysis. The resulting transcriptional and epigenomic profiles have been analyzed with respect to Gene Ontologies and by Ingenuity Pathway Analysis (IPA) to determine biologically relevant responses elicited by exposure to high glucose. We sequenced $>10^9$ base pairs for each experimental condition. Such sequencing depth allowed to identify genome-wide glucose-dependent H3 hyperacetylation and CpG methylation signatures, which were associated to specific gene expression patterns induced by glucose. IPA and gene ontology analyses indicated that high glucose significantly affected the human vascular chromatin, with the transcriptional upregulation of genes involved in metabolic and cardiovascular disease. HMOX1, CCL2 and ICAM2 were among the most upregulated genes following exposure to 25 mM glucose. These results have elucidated the epigenetic changes taking place following exposure to a high glucose concentration on a genome-wide scale. Histone H3 acetylation and DNA methylation were found to be inversely correlated, and genes that participate to diabetic-induced endothelial dysfunction were found to be affected by exposure to excessive glucose concentrations.

Key words: diabetes, epigenetics

Statement of interest: Authors report no conflict of interest.

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POSTER N°16

Effect of citrulline supplementation on fetal growth, and protein synthesis in a rodent model of intrauterine growth restriction (IUGR)

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Intrauterine growth restriction (IUGR), defined by a birth weight <3rd percentile for gestational age, is associated with increased neonatal mortality, and a higher risk of chronic disease in adulthood. We hypothesized that oral administration of L-citrulline may enhance fetal growth as: (1) citrulline is a precursor of arginine,[1] the sole endogenous source of nitric oxide (NO); (2) NO increases placental blood flow;[2] (3) moreover, citrulline has a protein anabolic effect in other models of malnutrition (e.g. aged[3] or enterectomized[4] rats). To assess the putative role of citrulline, gestating rats were fed either a control (C; 20% protein) or a low-protein (4% protein, LP group) diet. In addition, LP dams were randomized to receive tap water either as such, or supplemented with citrulline (CIT; 2 g/kg/day), arginine (ARG) or an isonitrogenous mix of non-essential amino acids (NEAA). On the 20th day of gestation, dams received a 2-h intravenous infusion of L-[1-^{13}C]valine and L-[1-^{13}C]alanine until fetuses were extracted by C-section. Isotope enrichments were measured in free amino acids and fetal muscle protein by gas chromatography–mass spectrometry. Maternal protein restriction reduced fetal weight (3.81 ± 0.03 and 5.37 ± 0.05 g in LP and C, respectively; $P < 0.001$). CIT, ARG or NEAA increased fetal weight to 4.12 ± 0.04, 4.00 ± 0.03 and 4.11 ± 0.04 g, respectively ($P < 0.05$). Plasma fetal/maternal ^{13}C-alanine enrichment ratio, an index of placental alanine transfer, was 0.65 ± 0.43 and 0.36 ± 0.04 in C and LP, respectively (NS). None of the supplements altered this ratio. Fetal muscle protein fractional synthesis rate (FSR) was lower in LP than control fetuses

(41 ± 11 *v.* 61 ± 13%/day, $P < 0.001$). Fetal muscle protein FSR was enhanced by CIT (56 ± 4%/day), not with ARG or NEAA (45 ± 7 and 50 ± 19%/day, NS). We conclude that: (1) citrulline enhances fetal growth in a model of IUGR; and (2) such effect may be mediated by enhanced fetal muscle protein synthesis.

Key words: fetal growth, maternal diet, metabolism, placenta, stable isotopes

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POSTER N°35

The apelinergic system is implicated in fetoplacental development and is a target in developmental nutritional programming

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Apelin (APL) and its receptor APJ are expressed in numerous tissues in mammals.[1] APL is also present in the blood where its level is related to the nutritional status and is correlated to insulin plasma level.[2] Recent studies pointed out an emerging role of APL in cardiovascular regulations, energy metabolism and glucose homeostasis.[1,3] We investigated the gene expression profile of this system and circulating APL levels during the gestation in rat. Plasma APL level is reduced in mothers at day 7 of pregnancy (E7) and then augmented until E21. In fetuses, APL concentration decreases from E17 to E21 and is comparable, at term, to maternal level. High levels of APJ and APL mRNAs were found at the fetoplacental interface (i.e. the mesometrial triangle and the placenta) and exogenous maternal administration of APL increases both placental glucose uptake and transplacental transport of glucose. In addition, high APJ/APL mRNAs were detected in numerous fetal tissues at term. Maternal food-restriction (FR) modulates drastically APJ/APL mRNAs levels at the fetoplacental interface and reduces circulating APL levels in both mothers and in growth-restricted fetuses. In adult male rat offspring from FR mothers, an increase basal plasma APL level was found suggesting a prenatal nutritional programming of this new endocrine system. Altogether, our findings propose that the apelinergic system is implicated in the fetoplacental unit development and may be targeted by prenatal nutritional disturbances resulting to the programming of metabolic diseases.

Key words: developmental programming, fetal growth, pregnancy

Statement of interest: The authors declare no conflicts of interest.

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POSTER N°39

Nutritional epigenetics: considering maternal dietary and fetal programming

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Growing evidence suggests that epigenetic mechanisms of gene regulation, such as DNA methylation and chromatin modification, are influenced by the nutritional factors and play an important role in the fetal basis of adult disease susceptibility. Epigenetics refer to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence. The epigenetic marks get added to genes or chromosomal proteins during fetal life. These epigenetic marks become fixed after early life and may become a problem when environment or diet changes later in life. This is thought to be one of the underlying mechanisms of fetal programming and its consequences on disease risks in the adult are now well accepted by the scientific community. It is also probable that the first few years after birth are more likely to be influenced by dietary components nutrition. To address these essential issues, we investigate in the laboratory the influence of dietary components on epigenetic processes, including DNA methylation, histone modification and miRNA gene expression. In our studies, the potential *in vitro* effect of some dietary components, dietary quality (nutrients deficiency and the presence of contaminants) on DNA methylation and chromatin accessibility were carried out by using different epigenetic analysis methods and approaches.[1–4] Results of studies have shown that folate deficiency and fumonisin B1 decreased global genomic DNA

methylation and gene-specific methylation in growing HepG2 cell line. Interestingly the presence of polyphenolic compounds extracted from seeds, tea and grapes and changed chromatin accessibility in Caco2 cell line. These preliminary results suggest that the presence of such components in the maternal diet may modulate epigenomes and gene expression of the fetus. The studies may open a novel field for investigation pertaining to the identification of the possible role of some components of epigenetic modulators in fetal programming.

Key words: contaminant, dietary, epigenetic, gene expression

Statement of interest: The authors declare no conflict of interest.

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POSTER N°44

MicroRNA expression profiling of hypothalamic arcuate and paraventricular nuclei from single rats using Illumina sequencing technology

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MicroRNAs (miRNAs) finely tune messenger RNA (mRNA) expression. As the brain is a highly heterogeneous tissue, physiologically relevant miRNA expression profiling greatly benefits from sampling brain regions or nuclei. miRNA expression profiling from individual samples is also important for investigating potential differences between animals according to their physiological and pathophysiological status. We have punched the arcuate (ARC) and paraventricular (PVN) nuclei from the hypothalamus of seven male Wistar rats and used them to establish a novel method for the characterization of the miRNA expression profile of individual rat brain nuclei. The identity of the ARC and PVN samples was checked for proopiomelanocortin and arginine vasopressin mRNA expression, respectively. Individual cDNA libraries were constructed from purified RNAs between 16 and 26 bases, using barcoded adapters. Libraries were multiplexed and sequenced using Illumina technology to a read depth $>10^5$. The ARC and PVN profiles displayed similar expression from a set of more than 210 miRNA genes. Expression was high or moderate for about 20 miRNAs that may be used to define a common ARC/PVN prototype profile of male Wistar rats. These miRNAs included seven of the eight genes of the let-7 family, the two miR-7 genes, miR-9 gene and 5′ copy of the three miR-30 loci. Our method shows that the ARC and PVN from a single rat are accessible for miRNA digital characterization. This method will allow miRNA transcriptome characterization for any rat brain substructure or nuclei that can be microdissected.

Key words: arcuate nucleus (ARC), hypothalamus, microRNA (miRNA), paraventricular nucleus (PVN), rat brain, RNA-seq

Statement of interest: Authors report no conflict of interest.

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POSTER N°77

RNA-mediated inheritance of acquired metabolic disorders

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Recently several studies have led to the remarkable conclusion that not only does over-nutrition promote metabolic syndrome but that the unhealthy parental diets (lipid-rich and low-protein regimens) contribute to its development. Understanding the mode of inheritance of these severe pathologies is clearly relevant to the societal problems raised by the current obesity epidemic. However, inheritance of acquired phenotypes is a new concept of heredity; little is known about the molecular basis of this process. Several questions are still awaiting experimental answers – the first being that concerning the transgenerational carrier identity. In the last few years, growing evidence suggests the involvement of epigenetic (non-genetic) modifications in this process. Until now, DNA methylation, histone modifications and nuclear proteins involved in the regulation of chromatin structure were proposed as players. However, several experimental models systems from *Caenorhabditis elegans*, Drosophila, plants and mice have led to the identification of small RNAs as vectors of epigenetic inheritance. Indeed, we

previously reported three instances of RNA-mediated heredity of an epigenetic state, a quantitative change in the transcriptional activity of a locus induced by sequence-related non-coding RNAs. We then investigated whether the same mode of transgenerational determination may apply to diet-induced epigenetic variations. As in our previous studies, the main analytical tool used was microinjection of RNA molecules into mouse one-cell embryos, an efficient and well-tolerated procedure. We observed the efficient transgenerational transfer of obesity and type 2 diabetes by injection in naive embryos of testis RNA prepared from obese and diabetic males raised on high-fat diet. We conclude that sperm RNA acts as a vector in parental inheritance.

Key words: adaptative response, developmental biology, epigenetics, molecular/cellular, obesity

Statement of interest: Authors report no conflict of interest.

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POSTER N°79

Consequences of protein malnutrition during prenatal and early postnatal stages

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The main factors leading to metabolic syndrome-associated pathologies (obesity, type 2 diabetes, hypertension, etc.) include genetic predisposition and environmental factors such as nutrition. Epidemiologic studies show that the intrauterine environment plays an important role. In mammals, as essential amino acids cannot be synthesized by the body, the proper development of an individual requires an optimum protein nutrition during the perinatal period. If missing, complex adaptive processes, set up at both the mother and fetus levels, will leave an imprint in the offspring that will last throughout his future life. One of the goals of our team is to identify the molecular mechanisms responsible for the nutritional imprinting acquired perinatally to understand the origin of predispositions to certain diseases during adult life and for future generations. We chose a model of protein undernutrition during pregnancy and lactation. The resulting offsprings, called F1-CD (Control diet) and F1-LPD (low-protein diet), are fed from weaning with CD. We have shown that adult F1-LPD mice had a lower body weight and a higher food intake. The phenotype of these animals prompted us to study the regulation of leptin expression, one of the main hormones involved in the control of energy metabolism. We have shown that, in F1-LPD animals, the promoter of the leptin gene showed an epigenetic modification correlated with a change in the regulation of its expression in response to a meal.[1] The knowledge of the molecular mechanisms involved in metabolic imprinting is an important step in understanding the predisposition to diseases associated with metabolic syndrome in adult life.

Key words: adaptive responses, developmental programming, epigenetics, fetal programming, metabolic syndrome

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POSTER N°78

Transposable element regulation under stress condition

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Eukaryotic genomes are prone to the accumulation of repetitive sequences, including transposable elements that represent a very large proportion of the genome. In the past few years, it has become clear from many transcriptomic studies that most of the eukaryotic genomes are transcribed. This complex network of transcripts includes several types of small RNAs classified as non-coding RNAs. The vast majority of small RNAs act as transcriptional, postranscriptionnal and translational regulators, controlling specific target genes involved in various cellular functions. They are classified on the basis of their biogenesis and mode of action. A subclass of small non-coding RNA, the Piwi-interacting RNAs (piRNAs), ensures genomic stability by silencing endogenous transposable elements in both germline and somatic gonadal tissues. piRNAs are produced through two mechanisms: (1) The primary processing pathway from long single-stranded precursors produced by some specific loci in the genome, the piRNA clusters and (2) The secondary pathway by the amplification loop called the ping-pong. piRNA clusters are composed of fragment of active mobile elements and are located in heterochromatic region.[1–4] Barbara McClintock was the first to suggest that stress can reactivate transposable element mobilization. The activity of transposable element can be induced by environmental stresses in various organisms. Despite the numerous examples of stress-induced activation of transposons, little is known about how the

signals induced by stress are transmitted to the transposons. We have investigated this question using *Drosophila* as a *model organism*.

Key words: epigenetic, stress, transposable element

Statement of interest: The authors declare that they have no competing interests.

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POSTER N°60

Search for prediabetes markers: comparison of muscle methylome of lean and obese women with or without type 2 diabetes

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There is convincing experimental evidence to suggest that stable changes in epigenetic marks act as a memory of exposure to unbalanced nutrition or metabolic disturbances during crucial developmental periods and throughout life.[1] These marks induce long-term changes in gene expression, thereby influencing the susceptibility to mental and physical health, including obesity and type 2 diabetes (T2D).[2] Until now, only one large-scale epigenome-wide study investigated how the epigenome changes were mediated by hyperglycemia in primary vascular cell[3]. So far, there has been no such study comparing T2D and non-T2D obese patients. Our aim was to identify genome-wide differentially methylated DNA sites in abdominal muscle from operated patients and to test their involvement in obesity and T2D. We selected from the ABOS (Atlas Biologique de l'Obésité Sévère) collection of biological samples obtained during bariatric surgery[4] muscle samples (80 mg) from obese women without T2D [25, body mass index (BMI) = 48.1 ± 6.9], and obese women with T2D (25, BMI = 48.6 ± 6.0), and lean control (15, BMI = 22.7 ± 2.4). Using Illumina Infinium 450K Methylation Beadchip, which allows the simultaneous quantitative monitoring of more than 480,000 cytosines across the genome, we identified sequences with methylation differences. Data normalization and analyses with bioinformatics tools including IMA and SWAN were performed. Some differentially methylated CpG sites were validated by pyrosequencing. Furthermore, the expression of genes close to these sites was investigated by RT-qPCR. For example, hypomethylation of some CpG within the promoter region of SIM1 was found in obese groups but especially in the obese and diabetic group. A differentially methylated region was found in chromosome 17q21, in which HOXB gene family and two HOX-related miRNA-encoding genes map. Interestingly, some CpGs within this region were hypomethylated, whereas some others were hypermethylated in obese groups. Data will be presented to show the differentially methylated regions, the genes and the potential networks concerned. The author(s) declare that they have no competing interests.

Key words: CpG, DNA methylation, gene expression, methylome, obesity, type 2 diabetes

Statement of interest: Authors report no conflict of interest.

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POSTER N° 76

Fetal programming of β-cell dysfunction in type 2 diabetes: implication of the glucocorticoids and their signalling pathway

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Several studies have shown that many adult diseases originate from adverse foetal environment that alters organ development and programs their dysfunction later in adult life. The risk to develop type 2 diabetes, a metabolic disease characterized by peripheral insulin resistance and insufficient insulin secretion by pancreatic β cells, is, for example, increased in individuals with low birth weight.[1] To explain this association, we and others have proposed that perturbations of the foetal environment alters the development of pancreatic β cells that will not be able to secrete insulin properly in adult life. We developed murine models of altered foetal environment through caloric restriction of pregnant females. Offspring submitted to this abnormal foetal environment develop glucose intolerance as adults with decreased β-cell mass and function. In those models, we observed that caloric restriction in pregnant females led to

increased plasma levels of corticosterone, both in the mothers and foetuses, and that increased glucocorticoids (GC) levels were responsible for decrease β-cell mass.[2] We next conducted several studies that led to the conclusion that GC inhibited β-cell development[3] and demonstrated that, in humans, such inhibition was possible as the glucocorticoid receptor (GR) was expressed early in the foetal human pancreas.[4] To decipher the molecular mechanisms involved, we focused on the GC signalling pathway and particularly PGC-1α, a GR transcriptional coregulator activated both by GC and caloric restriction. Using both *in vitro* and *in vivo* strategies of PGC-1α overexpression, we showed that excess of PGC-1α led to reduced β-cell mass and function. More importantly, we showed that PGC-1α overexpression was sufficient only during foetal life to program adult β-cell dysfunction. Thus, our results place the GC pathway as a major actor in the foetal programming of β-cell dysfunction and this role is actually being tested in clinical studies.

Key words: foetal environment, glucocorticoids, pancreatic β cells, PGC-1α, type 2 diabetes

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POSTER N°70

Alterations of postnatal nutrition permanently disrupt the neural pathways controlling reproductive function in female mice

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It is increasingly accepted that alterations of the early life environment may have lasting impacts on physiological functions. In particular, epidemiological and animal studies have indicated that changes in growth and nutrition during childhood and adolescence can impair reproductive function.[1,2] However, the precise biological mechanisms that underlie these programming effects of neonatal nutrition on reproduction are still poorly understood. Here, we used a mouse model of divergent litter size[3] to investigate the effects of early postnatal overnutrition and undernutrition on the maturation of hypothalamic circuits involved in reproductive function. Neonatally undernourished females display attenuated postnatal growth associated with delayed puberty and defective development of axonal projections from the arcuate nucleus to the preoptic region. These alterations persist into adulthood and specifically affect the organization of neural projections containing kisspeptin, a key neuropeptide involved in pubertal activation and fertility. Neonatal overfeeding also perturbs the development of neural projections from the arcuate nucleus to the preoptic region, but it does not result in alterations in kisspeptin projections. These studies indicate that alterations in the early nutritional environment cause lasting and deleterious effects on the organization of neural circuits involved in the control of reproduction, and that these changes are associated with lifelong functional perturbations.

Key words: critical period, developmental origins of adult disease, early life nutrition, reproductive function

Statement of interest: The authors have nothing to disclose.

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POSTER N°71

Epigenetic regulation by DNA methylation in fetal membranes: the actors of the phenomenon and the consequences of its deregulation

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Epigenetic changes are defined as 'heritable changes in gene expression associated with modifications of DNA or chromatin proteins that are not due to any modification in the DNA sequence'.[1] Epigenetic modifications can influence gene transcription by acting at three different major levels: histone modifications, noncoding RNA and DNA methylation. As transitory but essential 'organs', the placenta and the

fetal membranes play vital roles in pregnancy. The fetal membranes enclose the developing embryo and form the amniotic cavity protecting the developing fetus. Precise control of gene regulations is essential for their healthy functions. Gene deregulations can impair that function and is directly conducive to preterm birth or chorioamnionitis. Today, the epigenetic components of pregnancy pathologies are now increasingly researched and have been independently linked to the placenta (preeclampsia, intrauterine growth restriction) but only recently to the fetal membranes (chorioamniotis, preterm premature rupture of membranes: PPROM).[2] For this last point, it appears more evident that particular environmental factors called 'epigenators' (chemicals, diet, vitamins, stress, etc.) may influence the epigenetic marks and cause fetal membrane pathologies. To better understand how these factors could lead to pathologies, the aim of our work was to detail the presence/expression level and the geographical zone in fetal membrane (intact-ZIM or altered-ZAM zone) where the DNA methyltransferase (DNMT)[3] and DNA demethylases (GADD45/TET)[4] are expressed. Furthermore, as PPROM concerns 3% of French annual pregnancies, a cohort was constituted to characterize the DNA methylation state between ZIM and ZAM zones. At the end, this analysis had to permit the arising of new hypothesis concerning some changes in the methylation state that could conduct to a weakening of an amnion/chorion zone and predispose a future mother to a PPROM.

Key words: epigenetics, human, molecular/cellular, pregnancy, prematurity/pre-term birth

Statement of interest: None.

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POSTER N°74

Epigenetics and bacterial infectious diseases

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During infection, bacterial pathogens subvert a variety of host cell functions to survive in body tissues and control host defenses. There is growing evidence that bacteria modify chromatin in order to reprogram host cell transcription to their benefit. This is particularly well exemplified by *Listeria monocytogenes*, an intracellular bacterium that can control gene expression at the chromatin level in two ways, either indirectly by activating signaling pathways in the cytosol of host cells or directly by manipulating the chromatin-remodeling machinery in the nucleus. In particular, *L. monocytogenes* tightly control the secretion of a nucleomodulin,[1] LntA, to manipulate the function of BAHD1, the core component of a novel chromatin-silencing complex.[2] Upon signaling induced by *L. monocytogenes* infection, the BAHD1 complex represses a set of immunity genes induced by type III interferons in epithelial cells. When produced, LntA translocates to the nucleus and counteracts this repression. This positive and negative control of a chromatin repressor allows Listeria to tune interferon responses.[3,4] We have gathered novel data used by this bacterium to alter the host epigenome in another cell type, the hepatocytes. In addition, in-depth characterization of the BAHD1 complex suggests a possible role of this epigenetic regulator in other human diseases. These studies highlight that bacteria can be potential epimutagens that might generate specific, long-lasting imprints on host chromatin. Conversely, deregulation of epigenetic regulators could affect host responses to microorganisms.

Key words: epigenetics, immune functions, maternal infection, molecular/cellular

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GUT AND MICROBIOTA

6 – D. Darmaun and L. Najar

Intrauterine growth restriction results in long-term alterations in gut permeability, energy utilization and epigenetic marks in rat colon, suggesting that a low birth weight may increase the long-term risk of intestinal inflammation or cancer (abstract no. 15). Feeding healthy pups specific prebiotic oligosaccharides in neonatal period (abstract no. 22) results in long-term alterations of intestinal microbiota, and perinatal antibiotic treatment alters intestinal permeability (abstract no. 12) and the effect of a high-fat diet on gut mucosa later in life in piglets (abstract no. 38). Maternal separation at 10 days of age alters gene expression in the colon of rat pups (abstract no. 26). Taken together, such data suggest that perinatal nutrition may program gut health disease in adulthood.

ORAL N°22

Can intestinal microbiota be involved in nutritional programming?

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Increasing evidence indicates that nutrition in early life has sustained effects on adult health. Identifying mechanisms underlying this nutritional imprinting may enable new disease prevention strategies. Intestinal microbiota could be a key player in this, as (i) it affects the physiology of its host;[1] (ii) its postnatal implement can be modulated by nutrition;[2] and (iii) its initial set-up is thought to have a sustained impact on microbiota composition throughout life.[3,4] Thus, this study was aimed to determine whether early modification of intestinal microbiota could have long-lasting effects in rats. Suckling rat pups were supplemented by oral gavage with 3.2 g/kg BW fructo-oligosaccharides (FOS), galacto-oligosaccharides/long-chain fructan mix (GOS/lcFructan, 9/1), acidic oligosaccharides (AOS) or control solution from postnatal days 5 to 15, and then were weaned to standard chow until day 130. We characterized caecocolonic microbiota at days 15 and 130 using RT-qPCR and pyrosequencing analyses. At day 15, the different oligosaccharides did affect gut microbiota. Firmicutes were decreased in all rats fed oligosaccharides, whereas bifidobacteria were specifically increased in FOS and GOS/lcFructan rats. At day 130, the sole GOS/lcFructan preweaning treatment exerted a sustained effect as reflected by an increased OTU richness ($P = 0.03$) and decreases of Roseburia intestinalis and Erysipelotrichi family subgroups ($P = 0.01$ and $P < 0.05$). GOS/lcFructan provided before weaning had long-lasting effects on microbiota in rats. These sustained effects were not observed with other oligosaccharides and did not reflect the initial direct impact. Demonstrating the physiological relevancy of these sustained effects is crucial to gain more insight into impacts of oligosaccharides in early life. Moreover, our findings suggest that intestinal microbiota may, under specific circumstances, serve as a relay of neonatal nutrition and potentially contribute to nutritional programming.

Key words: animal, developmental programming, early life nutrition, intestinal microbiota, oligosaccharides

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ORAL N°38

Early antibiotic-induced alteration of gut microbiota colonization affects gut adaptation in adult offspring

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Maternal environment during pregnancy and lactation influences health of the offspring and its microbiota.[1] Antibiotics used in pediatric practices are recognized to deeply affect gut barrier function and disrupt the normal process of antigen presentation and handling.[2] However, less is known about the long-term consequences of early alteration of gut colonization. Our aim was to investigate gut barrier function and galactosyltransferase (GALT) response to lipopolysaccharide in piglets born from sows whose microbiota had been manipulated by antibiotics. Long-term consequences on gut adaptation to a high-fat (HF) diet were examined in adult offspring. Sows were given amoxicillin per os (ATBQ, $n = 11$ *v.* CTRL, $n = 12$) from 10 days before to 21 days after parturition. One piglet per litter was killed at postnatal day (PND) 14, 21 and 28. The remaining piglets were given either a low-fat (LF) or a HF diet from PND140 to 170. At PND14, increased ileal permeability in ATBQ piglets was associated with altered cholinergic regulation, and decreased mucosal acetylcholine concentration in ATBQ *v.* CTRL. At PND21, TNFα secretion of LPMCs in response to LPS was observed in ATBQ piglets, but not in CRTL. HF diet did not affect glucose tolerance at PND170. However, although ileal permeability was increased in HF-fed CTRL pigs compared with LF-fed ones, no such increase was observed in ATBQ animals, and carbachol-induced permeability was only effective in LF-fed CTRL pigs. Secretion of TNFα by ileal explants stimulated by pokeweed mitogen was blunted in ATBQ pigs compared with CTRL ones, irrespective of the diet. Moreover, TNFα secretion by ileal explants was not increased in response to LPS in HF-fed ATBQ pigs as opposed to LF-fed ATBQ pigs or LF- and HF-fed CTRL pigs. Manipulating the maternal microbiota modified both ileal barrier function and GALT response to LPS during the neonatal period. It also deeply influenced the intestinal adaptive response to an HF diet in adult offspring, suggesting a microbiota imprinting in gut response to an HF diet.

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Key words: adaptative responses, developmental origins of adult health, immune function, large animals, microbiota

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ORAL N°7

Effects of early postnatal undernutrition on intestinal maturation in mouse

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It is well known that the perinatal nutritional environment can affect both structure and physiology of a range of organs and tissues, thereby increasing susceptibility to metabolic disorders later in life.[1] During the perinatal period, the gastrointestinal tract undergoes profound structural and functional maturation.[2] Experimental studies suggest that alteration of the perinatal nutritional environment may lead to abnormal intestinal development in foetus and neonates.[3,4] We hypothesized that growth retardation may impair intestinal maturation. Growth retardation was induced by undernutrition during the suckling period in FVB/j mice, by adjusting the litter size to 15 pups per mother on day 4 of life (eight pups per mother in control group). Effects of postnatal undernutrition on the structure and functions of the ileal and colonic epithelium were studied at postnatal day 21 (P21) (weaning). Pups from large litters had a lower body weight (median 5.4 *v.* 8.8 g at P20; $P = 0.0002$) and a lower growth velocity until P21 (median 0.17 *v.* 0.35 arbitrary unit (AU); $P = 0.0005$). They showed catch-up growth after the weaning period with a more important body weight gain until adulthood (median +280.7% *v.* +88.2%; $P = 0.0045$). Postnatal undernutrition induced a delay in the maturation of the ileal epithelium characterized by the presence of vacuolated villus enterocytes that normally disappeared at P21, abnormal expression of the tight junction proteins, associated with an increase of the paracellular permeability, and intracellular expression of brush border enzyme dipeptidyl-peptidase IV. Colonic structure in underfed mice was impaired with a thinner muscularis externa (median 30.2 *v.* 44.5 μm; $P = 0.0029$), submucosal detachment, hyperplasia in mucosa and fragile surface epithelium. We conclude that postnatal growth retardation induced by early undernutrition alters maturation of the intestinal epithelium. Long-term consequences of such anomalies remain to be assessed.

Key words: critical periods, developmental mismatch, early life nutrition, small animals

Statement of interest: None.

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POSTER N°12

Short- and long-term alterations in colonic permeability, absorptive-secretory physiology and heat shock proteins in pigs born to antibiotic-treated mothers

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The widespread use of broad-spectrum antibiotics around parturition and in neonates has raised the question of their delayed health consequences in infants, but long-term studies are scarce.[1,2] Impact of antibiotics on gut function is poorly documented, despite their known effects on the microbiota.[2] Neonatal amoxicillin altered gut developmental and barrier gene expression in mice.[3] Effects were stronger on colonic (*v.* small intestine) transcriptome, particularly affecting ion transport and oxidative stress response genes.[3] However, kinetic studies are often short and functional data are scarce. We hypothesized that perinatal administration of antibiotic durably affects colonic permeability and absorptive-secretory physiology. This was tested in a swine model of neonatal disturbance of the gut induced by oral amoxicillin (AMOX) administration to mothers around parturition (controls = no antibiotic). Pig offspring were studied between 14 and 170 days. Colonic permeability and physiology were analysed basally or under oxidative stress (monochloramine) in Ussing chambers. Heat-shock proteins (HSP27 and HSP70), as protective components against oxidative stress,[4] were analysed by western blot analysis. Between d14 and d42, $Na+$ glucose absorption capacity (but not carbachol-induced $Cl-$ secretion) and HSP70 (but not HSP27) were higher in AMOX pigs. At day 170, paracellular permeability was lower in basal state in AMOX pigs. However, it was higher, and transcellular permeability was lower under oxidative stress. Basal colonic physiology was unaffected, but $Na+$ glucose absorption capacity was higher under oxidative stress in AMOX pigs. Neither carbachol-induced $Cl-$secretion nor HSPs were affected by AMOX treatment. In conclusion, our data demonstrate that perinatal antibiotic administration does have selective short- and long-term consequences on colonic

permeability and Na+ absorption capacity. By contrast, changes in HSP protein levels were only transient, whereas Cl− secretory capacity was not affected in this model. Work is in progress to elucidate the underlying molecular mechanisms and the involvement of the gut microbiota on colonic function.

Key words: adult, animal, DOHaD, maternal stress, newborn/neonate

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POSTER N°15

Intrauterine growth retardation induces alterations of colonic epithelial barrier and increases the risk of colonic diseases in adult rats

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Infants born with intrauterine growth retardation (IUGR) are at an increased risk for developing metabolic diseases in adulthood, such as type 2 diabetes. It has been proposed that an adverse intrauterine environment could induce stable epigenetic modulation of gene expression, which in turn alters the function of metabolic organs later in life.[1] Epigenetic and metabolic modifications are also involved in the pathogenesis of inflammatory bowel disease and colorectal cancer but their origins are not completely understood.[2,3] The objective of this study was to determine the impact of IUGR upon colonic epithelial barrier and colonic diseases. The rat model of IUGR was obtained by restricting protein intake in pregnant rats. Birth weights of IUGR pups are 15–20% lower than controls. By the age of 5–8 months, colons were collected and colonocytes were isolated. Proliferation of epithelial cells was decreased in colonic crypts from IUGR rats without modification of apoptosis, suggesting a lower self-renewal of colonic epithelium. IUGR increased intestinal permeability as assessed by mounting colonic tissues in Ussing chambers. This effect was associated with destabilization of tight junction proteins. The expressions of the transporter (MCT1) and β-oxidation enzyme (scACAD) of butyrate were downregulated in IUGR colonocytes, suggesting impairment of butyrate utilization, the main energy source for colonocytes.[3] Among the post-traductional modifications of H3 and H4 histones, IUGR induced a drastic loss of H4K16 acetylation, an epigenetic mark of colorectal cancer.[4] The severity of DSS-induced colitis was higher (histological scores and inflammatory cytokines) in IUGR rats than in controls. Moreover, the number of AOM-induced preneoplastic lesions (mucin-depleted foci) was higher in IUGR rats. Our study suggests that IUGR induces epigenetic and metabolic modifications in colonic epithelium, which could affect the intestinal barrier and predispose to gastrointestinal diseases.

Key words: cancer, DOHaD, early life nutrition, epigenetics

Statement of interest: Authors report no conflict of interest.

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POSTER N°26

Short-time maternal separation in early neonate rats markedly increases intestinal permeability, induces bacterial translocation and affects gene expression in the liver

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Intestinal permeability (IP) is high at birth[1] for maturation of gut barrier[2] and liver. In neonate rats, chronic maternal deprivation induces defect of IP in adulthood.[3,4] In human newborns, transient maternal separation (MS) is common practice, mainly when medical care is required; however, whether a short episode of MS affects gut–liver axis has not been explored. We investigate whether a short-time single MS during postnatal development affects IP and liver gene expression. Randomized female rat pups were either separated from their dams for 4 h or not separated (controls) at postnatal day (PND) 10 or PND20. First, total IP was determined by administration of fluorescein isothiocyanate (FITC)-dextran immediately after MS at PND10 or PND20, and at PND10 after ML7 pretreatment before MS. Second, colonic paracellular permeability (CPP) to FITC-dextran and transcellular permeability to horseradish peroxidase were measured in Ussing chambers at PND10. Bacterial translocation (BT) was assessed in liver and spleen at PND10 and PND20. Hepatic transcriptome was obtained using Agilent microarrays. Single short-time MS significantly increased total

IP (+90%, $P < 0.001$) at PND10 compared with controls but not at PND20. In PND10 pups, ML7 pretreatment blocked MS-induced raise of IP (−47% compared with vehicle-MS controls, $P < 0.05$). MS markedly increased CPP, compared with controls (1.7 ± 0.3 *v.* 0.6 ± 0.1 nmol/cm^2/h, $P < 0.05$), and transcellular permeability (2.4 ± 0.3 *v.* 0.8 ± 0.5 pmol/cm^2/h). At PND10, control pups showed no BT to extraintestinal sites. MS induced BT in the liver and spleen of 60% and 80% of MS rats, respectively, whereas nothing was detected in PND20. A significant impact of MS at PND10 was observed on liver with a downregulation of genes involved in cell cycle. Our study shows that a single episode of short-time MS in PND10, but not at PND20, exacerbated total IP through a myosin light chain kinase-dependent pathway, induced bacterial translocation to the liver and spleen and altered hepatic transcriptome expression.

Key words: bacterial translocation, gut barrier maturation, intestinal permeability, liver development, maternal separation

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CHRONOBIOLOGY

7 – Chroniobiologie

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Deciphering the critical periods will improve our understanding on underlying mechanisms of programming. Prevalence of biliary atresia, based on a follow-up of French Polynesia population, indicated a significant seasonality. Maternal undernutrition induced epigenetic changes on proteins involved in circadian clock in rodents.

ORAL N°14

Long-lasting effect of perinatal exposure to L-tryptophan on circadian clock of primary cell lines established from male offspring born from mothers fed on dietary protein restriction.

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Maternal undernutrition programs metabolic adaptations that are ultimately detrimental to adult. We recently demonstrated an effect on circadian clock of young rats recovering from perinatal denutrition.[1] Here, L-tryptophan supplementation was given to manipulate the long-term sequelae of early-life programming effects and explore whether cells sampled by non-invasive means retain circadian clock dysregulation. Male rat pups from mothers fed a low-protein (8%, LP) or control (20%, CP) diet were given an oral bolus of L-tryptophan 1 h before light off (125 mg/kg) between days 12 and 21 of age. Body weight, daily food intake and blood glucose circadian profiles were measured during the young (45–55 days) and adult (110–130 days) phases. Morphology, adhesion capacity and tryptophan-hydroxylase expression of primary cell cultures from rat tail were characterized. Circadian clock oscillations were reinduced by a serum shock over 30 h on near-confluent cell monolayers to follow PERIOD1 and CLOCK proteins by Fluorescent Linked ImmunoSorbent Assay (FLISA) and of period1 and bmal1 mRNA by RT-PCR. Cell monolayer survival in amino acid-free conditions was used to measure circadian expression of MAP-LC3B, MAP-LC3B-FP and Survivin by FLISA. By three-way ANOVA of blood glucose, a significant interaction between daily bolus (tryptophan, saline) and diets (LP, CP) were found during young and adult phases. In adult phase, the capacity of colonization at seeding of primary cells from low-protein rats was twice lower than controls. By three-way ANOVA of PERIOD1 perinuclear/nuclear immunoreactivity during young phase, we found a significant effect of diets, daily bolus and synchronizer hours. MAP-LC3B, MAP-LC3B-FP and Survivin were only altered according to diets in young phase. The availability of 50 primary cell lines retaining nutritional stress-related alterations in PERIOD1 expression open the way to design functional assays on living cells on the dynamics of the circadian epigenome.[2]

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ORAL N°23

Environmental influence on biliary atresia assessed by a 30-year whole-population-based study

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Biliary atresia is the leading cause of liver transplantation in children. Despite initial description of biliary atresia over a century ago, very little is known about its onset. Recently, a possible effect of environment was raised through the assessment of decreased DNA methylation in the disrupted development of bile ducts.[1] To check the hypothesis of the influence of environment on biliary atresia, we took the opportunity of a 30-year cohort of all biliary atresia cases in French Polynesia that displays the highest incidence worldwide. On the basis of a whole population-based study that combines both simplified two-season climatic condition and a population cluster, we collected birth months of the cohort and of the total population over 30 years. Radar plotting of the data clearly evidenced an unexpected shift of patient's births towards the dry season. Comparison of birth distribution between dry and wet season in patients with biliary atresia *v.* the total population indicates a highly significant difference ($P = 0.007$, χ^2-test). Our observation reveals for the first time a significant seasonality of biliary atresia in a geographic isolate with monocentric recruitment. Furthermore, we believe that the seasonal dynamics we encounter fit nicely with a model resulting from an infection, likely influenced by genetic background, for which the mouse models of biliary atresia may be regarded as paradigms. Finally, we also discuss why seasonality still remaines debated at the time of study,[2,3] and how our methodology should help solving old controversial debate and cast lights on the most recent results.[4]

Key words: critical periods, developmental programming, exposures, fetal programming, newborn/neonate

Statement of interest: The authors declare no conflict of interest.

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SEXUAL DIMORPHISM

A. Gabory and U. Simeoni

Communications in this session showed striking sex differences in the programming effects of metabolic disorders in the offspring of various animal species. Males and females show a clear differential sensitivity to both maternal obesogenic diet and exposure to endocrine dysrupting pollutants, expressed as both metabolic and epigenetic markers.

ORAL N°62

Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta

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There is mounting evidence that placenta can be considered as a programming agent of adult health and diseases.[1] Placental weight and shape at term are correlated with the development of metabolic diseases in adulthood in humans. Maternal obesity and malnutrition predispose the offspring to develop metabolic syndrome, a vicious cycle leading to transmission to subsequent generation(s), with differences in response and susceptibility according to the sex of the individual. Adaptations in placental phenotype in response to maternal diet and body composition alter fetal nutrient provision. This implies important epigenetic changes.[2] However, the epigenetics of placental development in DOHaD studies is still poorly documented, particularly concerning overnutrition. We used histology, microarray analysis and epigenetic techniques to investigate the effects of a high-fat diet (HFD) on mouse development. We showed for the first time that not only the gene sets but also their biological functions affected by the HFD differed markedly between the two sexes. Remarkably, genes of the epigenetic machinery and global DNA methylation levels showed sexual dimorphism. Imprinted gene expression was altered, with locus-specific changes in DNA methylation. Thus, these findings[3,4] demonstrate a striking sexual dimorphism of programming trajectories in response to the same environmental challenge. Explaining the sex-specific causal variables and how males *v.* females respond and adapt to environmental perturbations should help physicians and patients anticipate disease susceptibility.

Key words: epigenetics, nutrition, placenta, sexual dimorphism

Statement of interest: Authors report no conflict of interest.

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ORAL N°5

Gender differences in the aggravation of metabolic disorders induced by food contaminants in offspring of obese mice

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Several data indicate that endocrine-disrupting compounds are involved in the epidemic incidence of obesity and type 2 diabetes. In this study, we aimed at defining whether a mixture of low-dosed pollutants may aggravate metabolic disorders induced by obesity in mice lifelong fed a high-fat high-sucrose diet (HFSD). The mixture consisted in two persistent (Dioxin, PCB153) and two non-persistent (DHEP, BPA) pollutants, to which humans are largely exposed through diet on a daily basis. In brief, female mice were fed HFSD with or without pollutants, each added at their tolerable daily intake (TDI) in the mixture, and the progeny was given the same diet than its dam from weaning. Metabolic parameters were monitored in 12-week-old F1 mice that were either exposed (HFS-TDI group) or not exposed (HFS-0 group) to the pollutant mixture. F1 mice were obese with no difference in body weight and food intake between groups of the same sex. Glucose tolerance tests demonstrated that upon HFSD, female mice remained less glucose intolerant than male mice. However, pollutants aggravated this metabolic disorder in females but not in males, thus leading HFS-TDI females to the same glucose intolerance than HFS-0 and HFS-TDI males. To better understand the basis of these gender differences, we studied by RT-qPCR the expression of candidate genes related to lipogenesis in liver, and to inflammation in sub-cutaneous adipose tissue (scAT). Interestingly, effects were pollutant- and gender-dependent. Particularly, the hepatic expression of PPAR-encoding gene was enhanced in HFS-TDI males, and that of SREBP1c-encoding gene was decreased in HFS-TDI females, as compared with the respective HFS-0 group. In scAT, pollutants increased IL6 gene expression in females and MCP1 in males, as compared with the respective HFS-0 group. In conclusion, a mixture of low-dosed pollutants altered the metabolic profile of obese mice. Effects were gender specific with males being more sensitive to the diet and females more sensitive to pollutants.

Key words: endocrine disrupter, food contaminants, maternal exposure, metabolic disorders, nutrition, obesity

Statement of interest: Authors report no conflict of interest.

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ORAL N°25

Maternal methionine-restricted diet, epigenetics and offspring development: the case of force-fed ducks for 'foie gras' production

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The current studies on DOHaD in human and animal models[1,2] have stimulated studies at INRA in the field of fatty liver (foie gras) production, focusing on early nutrition and epigenetics. Foie gras production results from the storage of lipids (mainly triglycerides) synthesized from the starch of the feed during a 2-week force-feeding. It exploits the hybrid mule duck[3] (common duck female × Muscovy drake). The early nutritional modulation studied was methionine restriction in order to target DNA-methylation, as experimented in the sheep.[4] It was applied to the mule duck's dam. Three levels of methionine contents were designed: 4.2 g/kg (control, C), 2.6 g/kg (maximum restriction, R_m) and an intermediate level (R_i). The dams were fed the experimental diets from the age of 10 weeks until the conception of mule ducks offspring of both sexes, at 31–32 weeks of age. The mule ducks were force-fed from the age of 12 weeks and slaughtered. The traits studied were body weight at 4, 8, 12 and 14 weeks of age, carcass weight at slaughter, fatty liver weight, pectoral muscle (magret) weight and subcutaneous fatness of the magret, an indicator of the overall subcutaneous fatness. They were analysed by analysis of variance with the fixed effects of the sex, of the maternal diet and their interaction. The effect of the diet was significant for 12-week body weight ($R_m = R_i > C$, $P = 0.06$) and for magret fatness ($R_m > R_i > C$, $P = 0.09$). The most striking results concerned fatty liver, exhibiting a significant sex by diet interaction ($P < 0.01$): the ranking of the diets was $R_m > R_i = C$ in males and $C > R_i = R_m$ in females. The 20% increase in fatty liver weight in male mule ducks in the

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R_m diet could allow reducing the duration of force-feeding. These results are currently under confirmation by replicating the experiment.

Key words: DNA methylation, duck, foie gras, hepatic steatosis, methionine restriction

Statement of interest: Authors report no conflict of interest.

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ORAL N°10

Lessons in epigenetics from the human parasite *Schistosoma mansoni*: sexual differentiation under environmental stress

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Schistosomes are parasitic plathyhelminthes that are responsible for schistosomiasis (bilharziosis), an important parasitic human disease.[1] The life cycle of the parasite is characterized by passage through two obligatory hosts: a fresh-water snail for the asexual larval stage; and humans or rodents as hosts for the sexual adult stage. Male individuals are homogametic (ZZ), whereas female inviduals are heterogametic (ZW). We used massively parallel DNA sequencing to identify unambiguously Z-specific, W-specific and pseudoautosomal regions of the *Schistosoma mansoni* sex chromosomes. We showed that more than 90% of *S. mansoni* W and Z are pseudoautosomal. The W-specific region is composed almost entirely of 36 satellite DNA families. Transcription and chromatin status of female-specific repeats are correlated to life stage; for example, if repeats were transcribed, transcription would be restricted to the larval stages lacking sexual dimorphism. In addition, levels of histone modifications typically associated with transcriptionally active euchromatin decreased around the W-specific repeats, as assayed by ChIP and ChIP-Seq. Our study provides evidence for the hypothesis that repeat-induced chromatin changes may have been an initial event in sex chromosome emergence. These chromatin structure changes have probably an effect in *cis* and/or *trans* and control (pseudo)autosomal genes that are responsible for sexual dimorphism.[2,3] To investigate how the epigenome of the parasite reacts to environmental stress, we conducted experimental evolution experiments in which the larvae were exposed to two different snail host strains. We measured life history traits, and used RNA-Seq and ChIP-Seq to follow changes in gene expression and chromatin structure through three generations. We show that stress-induced bias towards females in sex ratio occurs, and that transcriptional changes are correlated with modifications in chromatin structure. We hypothesize that chromatin structure provides a link between the environment and the development of females.

Key words: developmental programming, epigenetics

Statement of interest: The authors declare not to have competing interests.

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POSTER N°58

Sexual dimorphism of hepatic epigenetic marks and machinery in offspring of obese and diabetic mothers fed a control diet during periconceptional/gestation/lactation period

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Early nutritional events may have an influence on later life health mainly through epigenetic processes.[1] In our two-generation mice model, providing obese and diabetic mice with a control diet during the periconceptional/gestation/lactation period led to a pronounced sex-specific shift from susceptibility to resistance to a high-fat diet (HFD) in the female offspring only.[2,3] The aim of this study was to detect sex-specific differences in the expression of candidate genes and epigenetic marks and machinery in the liver of both sexes and both generations. As a key organ for lipid processing and detoxification, liver plays a major role in conditions of chronic lipid oversupply. According to the sex, female (F) or male (M), the generation, first (F1) or second (F2), and diet types, CD or HFD, mice were divided into eight groups (F-F1-CD, F-F1-HFD, M-F1-CD, M-F1-HFD, F-F2-CD, F-F2-HFD, M-F2-CD and M-F2-HFD). Body weight, blood glucose level and blood cholesterol levels were measured. Liver morphology was identified by hematoxylin–eosin staining and oil red O staining. Hepatosteatosis was found to be more common in all HFD groups with adaptation of

the liver phenotype in F2 females but not in males, in parallel with obesity and cholesterol levels.[4] Global DNA methylation and histone modifications were investigated by LUMA and Western blot analysis, respectively. Interestingly, although no significant difference was found within groups, global DNA methylation level was significantly negatively correlated to steatosis percentage. Using RT-qPCR, sexual dimorphism was observed for the gene expression of 12 genes encoding enzymes of the epigenetic machinery. These marks may help us to understand the sex-specific epigenetic mechanisms of the underlying sex-specific responses to HFD and improve the early life nutritional environment in a sex-specific manner. The author(s) declare that they have no competing interests.

Key words: epigenetics, gene expression, hepatocellular carcinoma, hepatosteatosis, high-fat diet, histone modification, methylation, obesity, sexual dimorphism, type 2 diabetes

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POSTER N°68

Exposure to the diabetic intrauterine environment, growth in infancy and childhood and development of adiposity at 7 years

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Recent meta-analyses pointed out inconsistent evidence of an association of gestational diabetes mellitus (GDM) with offspring overweight and obesity.[1,2] Our objective was to examine the associations of maternal gestational glucose tolerance with child growth trajectory from birth, and child body composition at 7 years. Among 914 women in the prebirth cohort Project Viva, maternal glucose tolerance was assessed in the second trimester by non-fasting 1-h glucose challenge test (GCT), followed, if needed, by fasting 3-h glucose tolerance test (OGTT). We categorized women as normoglycemic (Norm: 83.3%) if GCT was normal, isolated hyperglycemia (9.1%) if GCT was abnormal but OGTT was normal, impaired glucose tolerance (IGT: 3.3%) if one abnormal value on OGTT and gestational diabetes (GDM: 4.5%) if women had two or more abnormal OGTT values. We obtained child weight and height from medical records and research examinations, and body composition at 7 years using DXA ($n = 760$). We modeled growth trajectories. We adjusted the multivariate linear regressions for parental body mass index, gestational weight gain, child age, race/ethnicity and socio-demographic characteristics. Boys born to GDM mothers were heavier throughout infancy and childhood. At the age of 7, they had a higher overall adiposity (1.89 kg; 95% CI: 0.33, 3.45) compared with boys of Norm. Girls born to GDM mothers did not show a different growth trajectory compared with Norm. Yet, girls of IGT mothers showed a slower growth in infancy, followed by a steep acceleration in early childhood that resulted in a higher overall adiposity at 7 years (+2.23 kg; 95% CI: 0.12, 4.34), compared with girls of Norm. The exposure to maternal glycemia of fetuses of IGT and GDM mothers may differ in timing and intensity. This, combined with a different sensitivity of male and female fetuses *in utero*, may result in different programming and metabolic consequences in the long run.

Key words: body composition, child growth, developmental programming, gestational diabetes, human

Statement of interest: The authors declare that they have no competing interests.

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ALLEVIATION OF PROGRAMMING DYSFUNCTIONS

9 – A. Vambergue and L. Storme

Evidence indicates that the consequences of programming dysfunctions can be partly corrected. In an experimental model of metabolic syndrome obtained in the offspring of undernourished rats, lactation by adoptive mothers is able to prevent occurrence of metabolic syndrome later in life. In the same way, extracted polyphenolic compounds from seeds, teal and grapes in the maternal diet may modulate DNA methylation and chromatin accessibility, suggesting potential perspectives for modulating programming.

ORAL N°40

Role of postnatal leptin on organ maturation and development

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Human neonates born with intrauterine growth retardation (IUGR) often experience adverse perinatal outcomes. Their general developmental delay affects the growth and functional properties of various organs, leading to immediate defects in key biological functions. This situation renders difficult their adaptation to extrauterine life and contributes to the development of diseases, affecting their survival and long-term adult health. A new role for leptin in developmental processes has recently emerged from several studies, mostly in rodents. During this period, leptin acts as a neurotrophic factor to coordinate the establishment of the hypothalamic neuronal network responsible for food intake regulation.[1] Moreover, our recent work demonstrated that leptin may constitute a key hormone for the postnatal maturation of numerous peripheral organs.[2] However, in rodents, the temporal windows of development for many organs differ from those of humans, making it difficult to extrapolate these results in humans. Pigs are an advantageous model for many human physiological aspects and for the timing of development and maturation of human organs. Interestingly, IUGR occurs naturally and IUGR results in similar long-term pathological consequences as in humans, including increased adiposity, cardiovascular risk and glucose intolerance.[3] We have previously shown that treatment of IUGR piglets during the first 10 days of life enhances their weight index and linear growth, and improves the growth of several organs.[4] In a further study, we aimed to underline some of the physiological processes that occur at the cellular level of the immune, gastrointestinal and reproductive systems after leptin neonatal supply. Notable structural and functional changes in the pancreas, secondary lymphoid organs and ovaries were observed following leptin administration. The effects of leptin were particularly investigated on ovarian development. In IUGR piglets, leptin was found to enhance germ cell maturation and follicular growth activation. An important role of leptin in developmental processes is highly suggested.

Key words: early development and adult disease, early life nutrition, fetal programming, immune function, oocyte

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ORAL N°64

Feeding patterns over the 1st year of life: relations with child growth and late fruit and vegetable intake

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Early eating patterns can determine later eating habits and food preferences and they have been related to the development of childhood overweight and obesity. We aimed to identify feeding patterns over the 1st year of life and examined their associations with weight, height and body mass index (BMI) at 1 and 3 years of age, and then with fruit and vegetable intake at 3 years.

Our analysis included 715 children from the EDEN mother–child cohort. Principal component analysis was applied to derive patterns from breastfeeding duration, age of introduction of several food groups and type of food used at 1 year. Associations between these patterns and weight, height and BMI at 1 and 3 years were tested by linear regressions adjusted for weight, length and ponderal index at birth, respectively. Relations with fruit and vegetable intake (>1 serving/day) at 3 years were analyzed by logistic regressions.

Feeding patterns over the 1st year of life can be summarized by three independent components: pattern 1: 'Late complementary food (CF) introduction and use of baby foods'; pattern 2: 'Longer breastfeeding, late CF introduction and use of home-made foods'; and pattern 3: 'Use of adults' foods'. Weight and height change between birth and 1 year were significantly related to the second pattern (for a change of 1 S.D. of the pattern: β(S.E.) = −0.11 (0.04), $P = 0.004$ and −0.27(0.08), $P = 0.001$, respectively). Relations were weaker at 3 years. No significant association was found with BMI at both ages. High fruit and vegetable intake at 3 years were related to the second pattern [OR = 1.4 (1.2–1.6), $P = 0.0003$ and OR = 1.5(1.3–1.8), $P < 0.0001$, respectively] but not to the other patterns.

Pattern characterized by longer breastfeeding, late CF introduction and frequent use of home-made food was associated with growth parameters at 1 year but not at 3 years of age and appeared to be strongly related to both fruit and vegetable intake at 3 years.

Key words: fruit and vegetables intake, infant growth/nutrition, newborn/children, obesity

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ORAL N°41

Adoption prevents metabolic alterations in male rats with intrauterine growth restriction resulting from maternal undernutrition

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Epidemiological and experimental data show that intrauterine growth restriction (IUGR) sensitizes the development of adult chronic diseases such as obesity, diabetes and/or hypertension.[1–3] It was also reported that growth alterations during the early postnatal period (i.e. lactation) can exert long-term effects on programming/deprogramming of metabolic diseases.[4] Using an experimental model of maternal perinatal undernutrition of 50% (FR50 model) in rats that induces intrauterine and extrauterine growth retardation in the offspring, we have shown that FR50 male animals exhibit reduced body weight, alterations in carbohydrate metabolism and mild hypertension at the age of 6 months. In neonates, adoption of IUGR newborns from mothers fed *ad libitum* allows normalizing their body weight as soon as postnatal day (PND) 4, and restoring PND10 and PND21 plasma leptin concentration to levels comparable to those observed in control newborns, suggesting that mother regimen during lactation dictates leptin plasma levels in pups. Interestingly, adoption also prevents the development of hypertension and alterations in glycaemic control observed in response to an oral glucose tolerance test. Finally, undernutrition during lactation decreases milk leptin concentration. In our experimental model, adoption allows an early catch-up growth and restoration of plasma leptin levels during lactation, and is associated with long-term prevention of metabolic alterations programmed perinatally in adult male rats. This work opens new perspectives for identifying compounds that may enter in the composition of infant formula, in an attempt to precociously 'deprogram' diseases in adults.

Key words: Barker hypothesis, breastfeeding, developmental programming, early development and adult desease

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ORAL N°59

Locus-specific epigenetic changes associated with peripheral leptin resistance in increased resistance to a high-fat diet in mice born to obese mothers fed a control diet during gestation

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Epigenetic mechanisms are evoked to explain interindividual and across-generation variations in the proneness or resistance to develop diet-induced obesity (DIO) of inbred C57BL/6J mice even under the same high-fat diet.[1,2] Feeding obese and diabetic mothers a control diet during the periconceptional/gestation/lactation period led to a pronounced sex-specific shift from proneness to resistance to DIO in the offspring.[3] In this study, Affymetrix microarrays highlighted the prominent role of leptin–leptin receptor cytokine pathways and a cross-talk between muscle and liver. The top-ranking genes were the leptin (Lep) gene upregulated in muscle, and the leptin receptor (Lepr) and suppressor of cytokine signaling 2 (Socs2) genes drastically downregulated in liver only. Weight-gain proneness as opposed to resistance was associated in muscle with an inflammatory process related to altered levels of adipokines, and in liver with an altered lipid processing and increased lipid droplets formation, suggesting a strong connection of Lepr and Socs2 to fat metabolism. Locus-specific epigenetic analyses by bisulfite-pyrosequencing and ChIP PCR of Lep, Lepr and Socs2 revealed a role for specific CpG methylation and histone acetylation and methylation marks in the tissue-specific regulation of these genes with specific profiles associated with the obesity-prone phenotype, whereas resistant mice epigenetic landscapes evoked a strong tendency toward 'normality'. Emphasizing the hitherto unsuspected role of Lepr gene expression in peripheral leptin resistance[4] and its liver specificity, this work is the first to describe epigenetic changes in Lepr associated with its downregulation as potentially useful markers in the follow-up of leptin resistance treatment in obese patients.

Key words: DNA methylation, epigenetics, histone post-ranslational modifications, leptin resistance, leptin signaling, metabolic adaptation

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POSTER N°61

Transcriptional and epigenetic signatures of adaptive increased resistance to diet-induced obesity by dietary alleviation of malprogramming by maternal obesity during gestation

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Maternal obesity and type 2 diabetes (T2D) at conception and during gestation promote the development of obesity and diabetes in adulthood.[1] However, very few studies have considered whether and how appropriate nutrition could alleviate this malprogramming. An important proportion of inbred animals develop resistance to the obesogenic effects of a high-fat diet (HFD), regardless of the species, the window and mechanisms at stake.[2] In a previous study, we showed that despite maternal obesity and T2D, a control diet (CD) during the periconceptional/gestation/lactation period led to a pronounced sex-specific shift from susceptibility to resistance to a HFD in the female offspring.[3] The aim of this study was to determine the molecular mechanisms of resistance and susceptibility, and how a CD could alleviate the effects of maternal obesity and T2D on the fetus and increase resistance. Despite being similarly lean (resistant) or obese (sensitive), F2 and F1 mice clearly differed in several aspects of their metabolism, with F2 mice presenting obvious features of 'adaptation' on the HFD. Expression data using a custom-built mouse microchip for the liver and quantitative RT-PCR for muscle and adipose tissue highlighted that adaptative processes in F2 mice were associated in the liver with an enhancement of pathways protecting against steatosis, the recruitment of unexpected neurotransmission-related genes and the modulation of a vast imprinted gene network. In the adipose tissue, adipogenesis and lipid storage were also modified in F2 mice. Global DNA methylation and several histone marks assessed using LUMA technique and western blot analysis, as well as the expression of 15 genes encoding chromatin-modifying enzymes, supported the response and adaptation to HFD, in a generation- and tissue-specific manner.[4] Thus, improvements in the nutrition of obese and diabetic women during pregnancy would be an efficient management strategy, with lower risks than current strategies on the basis of weight loss or nutrient supplementation, which may have a negative impact on fetal programming.

Key words: developmental programming, DOHaD, epigenetics, fatty liver, hepatocellular carcinoma, high-fat diet, metabolic syndrome, nutrition, obesity, sexual dimorphism, type 2 diabetes

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TOWARD DIETARY AND THERAPEUTIC RECOMMENDATIONS?

10 – L. Najar and D. Luton

Long-term consequences of prematurity, oxidative stress, noxious neonatal environmental and diet-induced obesity on metabolic and cardiovascular disorders are examined. Regarding the programing of appetite, an assessment of neonatal weight loss with child weight at 1 and 3 years was presented. In view of the importance of dietary supplementation for optimal development, authors provide insight into the impact of prenatal vitamin D on cord blood level. Intake of bioactive molecules (docosahexaenoic acid and arachidonic acid) from milk during development of premature newborn was examined.

ORAL N°52

Long-term consequences of neonatal pain and analgesia on growth, behavior and corticotroph axis in rat

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Previous data reported that a noxious neonatal environment alters brain development and is responsible for diseases in adulthood (metabolic syndrome, obesity).[1] With advances in medical care over the last two decades, the number of newborn infants exposed to chronic pain has considerably increased,[2,3] and this has led to the augmentation of the use of opioid to induce analgesia.[4] However, the long-term effects of perinatal

chronic pain are still to be elucidated. In this study, we examined whether a chronic pain in early life can affect growth, corticotroph axis activity and behavior (locomotion and anxiety) in adulthood. Neonatal pain was induced by the injection of complete Freund's adjuvant into the hind paw of newborn rat pups on postnatal day (P) 3. Similar experiments were conducted by inducing analgesia by fentanyl (P2 to P20) only, or by associating pain and analgesia. Neonatal chronic pain has long-term consequences on the weight, anxiety and ACTH plasma levels. Analgesia with fentanyl, without noxious stimulation, has no drastic consequence, whereas it decreases side effects both on weight gain and anxiety. Together, our data suggest that early neonatal chronic pain affects hormonal function and behavior in adulthood, these alterations being partly reversed by analgesia using fentanyl.

Key words: adult, developmental programming, DOHaD, exposures, small animals

The present protocol was examined and approbated by the 'Nord-Pas-de-Calais Ethical Committee for Animal Experimentation', agreement CEEA 06/2009.

Statement of interest: Authors report no conflict of interest.

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ORAL N°11

Long-term alterations of intestinal key enzymes – alkaline phosphatase and peptidases – after perinatal disturbances in two animal models

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The role of the gut in metabolic diseases and obesity is still obscure, especially from a programming perspective. Gut pro-inflammatory bacterial components (lipopolysaccharides) may be causative factors through a defect in gut permeability.[1] Among enzymes, intestinal alkaline phosphatase (IAP) detoxifies LPS and controls bacterial translocation and inflammation.[2] Peptidases also play important metabolic roles. A high intestinal dipeptidyl peptidase IV (DPP-IV) activity specifically deteriorates glucose tolerance and reduces plasma insulin in mice. IAP limits, but aminopeptidase N (APN) favours intestinal absorption of fatty acids and cholesterol, respectively. Gut peptidase inhibitors reduce chronic inflammation. Therefore, in two independent animal models, we tested the hypothesis that these key intestinal enzymes are early programmed. Intrauterine growth-retarded (IUGR) rats were generated through protein restriction (8% *v.* 20% in controls) of their dams during pregnancy and lactation, and 8-month-old rat offspring received a normal (NF) or a high (HF)-fat diet. IUGR is a risk factor for metabolic syndrome and obesity. A swine model of neonatal disturbance of gut bacterial colonization was developed by antibiotic (amoxicillin) administration to mothers around parturition. Pig offspring were slaughtered at 6 months of age. Alteration in bacterial colonization has lasting effects on gut function in rats.[3] Enzyme activities were determined in jejunal mucosa. Adult IUGR rats fed HF displayed lower IAP ($P < 0.05$) but higher APN ($P < 0.01$) activities. Similar observations (lower IAP, higher DPP-IV) were observed in pig offspring born to antibiotic-treated sows. In conclusion, our data show that similar intestinal key enzymes are imprinted in an unfavourable way in rats and pigs, regardless of the neonatal stimulus. Our findings fit with the 'gatekeeper' hypothesis,[4] suggesting that early independent stimuli could programme the expression of a common set of genes involved in a common pathological phenotype appearing in later life. The studied enzymes probably belong to this set of genes in the intestine.

Key words: adaptive responses, adult, animal, DOHaD, early life nutrition

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ORAL N°27

Consequences of a diet-induced obesity during infancy on the peripheral intrinsic nervous system control of gut and pancreatic functions

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Many children under the age of 5 are obese or overweight. Yet, infancy is a period of still intense maturation of the peripheral nervous systems, namely, the enteric nervous system (ENS) and the pancreatic intrinsic nervous system (PINS) controlling gut and pancreatic functions. An inadequate nutrient supply during this period may interfere with the physiological development of ENS and PINS

phenotypes with functional consequences on gut and pancreas. The aim of our study was to determine the impact of obesity upon the maturation of ENS and PINS and its functional consequences in young mice. C57BL/6J mice aged 4 weeks received either a normal diet (ND) or a western (hyperlipidic hyperenergetic) diet (WD) for 12 weeks. Four-week-old mice were also used as initial controls (iC). Gastric emptying (GE) was measured *in vivo* after saline or N^{ω}-nitro-L-arginine methyl ester (L-NAME, inhibitor of NO synthase) IP injection. After killing, pancreas was removed and placed in short-term organ culture for 1 h. The impact of PINS on endocrine function was studied by adding to the culture the nicotinic receptors agonist dimethylphenylpiperazinium (DMPP) in the presence or absence of sodium nitroprusside (SNP, NO donor) or L-NAME. Supernatant was collected for analysis of insulin by ELISA. Immunohistological analyses were performed to determine ENS and PINS density and to phenotype cholinergic and nitrergic neurons. GE was increased in WD as compared with ND mice, and this was prevented by IP injection of L-NAME. Stimulation by DMPP induced a time-dependent increase in insulin secretion (IS), which was significantly larger in ND as compared with WD mice. Interestingly, the IS profile was identical in WD and iC mice. The addition of L-Name or SNP significantly inhibited insulin release in ND mice, whereas neither L-Name nor SNP altered IS in WD mice. ENS and PINS density was less in ND as compared with iC mice, whereas there was no difference between WD and iC mice. Nitrergic innervation was increased in WD mice as compared with ND mice in ENS, whereas it was decreased in WD mice as compared with ND mice in PINS. Altogether, our study suggests that WD interferes with age-associated loss of neurons in both ENS and PINS but with differential nitrergic neuroplasticity in ENS and PINS. This defective maturation could be involved in gut and pancreatic dysfunctions observed during obesity.

Key words: early life nutrition, obesity, periods of plasticity, small animal

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ORAL N°67

Are the first few days of life a critical window for programming? A study on neonatal weight loss

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Animal studies suggest that the first few postnatal days may be critical for the programming of appetite and energy metabolism, setting the lifetime regulation of weight control.[1] For most babies, this period is characterized by an initial neonatal weight loss (NWL). We aimed to assess the associations of: (1) potential maternal, obstetrical and fetal determinants with NWL ($n = 1557$); (2) NWL with child weight at 1 and 3 years of life ($n = 926$) in the French EDEN mother–child cohort. Neonates were weighed every day until discharge that occurred on average 4.5 days after birth. Neonatal weight loss at day 3 (NWL) was expressed as a percentage of birth weight lost in the first 3 days of life. Breastfed neonates lose more weight in the neonatal period than their formula-fed counterparts as previously shown.[2–4] Other factors associated with greater NWL, regardless of the feeding mode, were: higher birth weight, lower gestational age, gestational diabetes and caesarean section. The association between maternal pre-pregnancy BMI and NWL differed by feeding mode. In breastfed babies, NWL was the lowest (4.9%) in neonates of underweight mothers and highest (5.8%) for neonates of obese mothers. In formula-fed babies, NWL was highest for neonates of underweight mothers (4.1%) and lowest for those of obese mothers (2.6%). In these early days, formula-fed neonates may be relatively 'overfed' compared with the breastfed neonates. Offspring of obese mothers may be particularly responsive to this 'overfeeding'. There was no association of NWL with child weight at 1 year ($P = 0.74$). However, at 3 years, the children with NWL $\geqslant 10\%$ weighed 475 ± 220 g less than those with NWL in the normal range ($P = 0.03$), whereas those with NWL $\leqslant 0\%$ tended to be heavier. Future analyses will examine the associations with child growth and metabolism up to 5 years.

Key words: child growth, critical periods, infant feeding/breastfeeding, maternal nutrition/diet/body composition, newborn/children

Statement of interest: Authors report no conflict of interest.

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ORAL N°63

Increased blood pressure and susceptibility to type 2 diabetes in adulthood can be linked during postnatal life: an implication of oxidative stress

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Cardiovascular diseases are increased in industrialized countries and represent an important cause of mortality.[1] Many studies showed that prematurity is associated with later indices of vascular dysfunction, increased blood pressure and type 2 diabetes in adulthood.[2] Premature babies are susceptible to early oxidative injury. However, long-term consequences of neonatal oxidative insults on cardiovascular system and metabolism are still largely unknown. Sprague–Dawley pups were kept with their mothers in 80% O_2 (O_2) or room air (RA) from D3 to D10 of life. At weeks 4–15, tail blood pressure was measured as the cardiovascular parameter. During adulthood, vascular reactivity (*ex vivo*, carotid) to Angiotensin II (AngII) and carbachol ±tempol was studied; nitric oxide (NO) production ±L-arginine and ±L-sepiapterine (immunohistochemistry, aorta) and endothelial NO synthase expression (eNOS; western blot analysis, aorta) were analyzed; aorta superoxide anion production ±apocynin and ±L-NAME, (chemiluminescence) and plasma levels malondialdehyde (MDA, HPLC) were measured; and nephrons were counted (hydrochloric acid digestion). Increases in body weight were followed as the metabolic parameter. During adulthood, body composition and glucose tolerance were evaluated. In a comparison between adult O_2 and RA rats, the following were observed: blood pressure was seen to have increased; vascular reactivity to Ang II was seen to have increased but decreased on carbachol stimulation, and tempol abolished these dysfunctions; NO production had decreased in basal condition and after carbachol stimulation but was restored after preincubation with L-arginine and L-sepiapterine; eNOS expression had increased; aorta superoxide anion levels had increased after AngII stimulation and were mediated by NADPH oxidase and eNOS uncoupling; the plasma levels of MDA had increased; nephron counts had decreased; body composition was similar; and glucose tolerance had decreased. The increase in body weight was less in the O_2 group but a recovery was observed in adulthood. These results support the hypothesis of a developmental programming of vascular and metabolic diseases in adulthood after neonatal oxygen exposure.

Key words: glucose intolerance, hypertension, insulin resistance, oxidative stress, vascular reactivity

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ORAL N°28

Intake of bioactive molecules from milk during an early postnatal critical window and development of premature newborn

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Long-chain polyunsaturated fatty acids [docosahexaenoic (DHA) and arachidonic (ARA) acids] are crucial for organ development and short/long-term health outcomes.[1] Phospholipids exert cardio, hepato, brain protective or anti-oxidant effects,[2] or favor intestinal maturity (sphingomyelin, SM).[3] Human milk should program an optimal development by providing these bioactive molecules. We aimed to quantitate their real intakes in very-low-birth-weight premature neonates in an early postnatal critical window of development. Forty neonates (1224 ± 242 g; 29.6 ± 0.8 weeks) fed native milk ($n = 13$) or pasteurized donors milk ($n = 27$) were followed-up from the start of minimal enteral feeding (MEF) to 4 weeks. Samples of feeding were analysed by GC for fatty acid composition[2], and by ^{31}P NMR for phospholipids. The needs for DHA (40–70 mg/day, i.e. 280–490 mg/week)[4] are not met (40–220 mg/week) because of MEF, moderate-to-low levels in milk (0.34 ± 0.08% tfa) and no parenteral DHA supply. SM intakes were lower (17–166 mg/week) than amounts beneficial for intestinal development (60–150 mg/day)[3]. Early postnatal nutrition should be improved by (1) systematic use of DHA supplements during lactation, (2) selecting donor milk high in DHA and SM levels and (3) developing milk fortifiers, formulas and parenteral emulsions enriched in DHA and ARA.

Key words: breastfeeding, cardiovascular disease, DOHaD, early life nutrition, prematurity

Statement of interest: Authors report no conflict of interest.

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ORAL N°2

Impact of current prenatal vitamin D-recommended supplementation on cord blood level: a French cohort

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Despite recommendation for vitamin D supplementation, vitamin D deficiency rates are still high in mother and infants of both industrial and developing countries.[1,2] Data from North America and Europe reported hypovitaminosis D in general pregnant population, with alarming rates among immigrant and especially in dark skin mothers.[3] The vitamin D pool of the fetus and the newborn depends entirely on their mother's status, and circulating 25-hydroxyvitamin D3 (25(OH)D) is a good index of vitamin D status in general. This observational prospective study conducted in a teaching hospital from July 2008 to March 2009 determines the impact of current recommended vitamin D prenatal supplementation on cord blood 25(OH) D level in a French cohort and determines population at risk of higher needs. There were two large groups of newborns, one born after summer and the other after winter period. A total of 399 mother/newborns pairs were enrolled, and cord blood results were available for 225 newborns in the post-summer group and 174 newborns in the post-winter group. Maternal supplementation during pregnancy was recorded from medical notes and questionnaires. The levels of 25(OH)D were generally low with mean at 50.9 ± 24.7 nM. Vitamin D supplementation was prescribed in only 37.6% over all the study period. Studying general population, 25(OH)D level was significantly higher in the supplemented group; however, current recommended supplementation failed to cover needs for large subgroups of newborns. After winter, 25(OH)D cord blood level was in the deficiency range for 40.7% of the general population, and in pigmented mothers group deficiency rates even rose up to 61.9%. Despite national guidelines on vitamin D supplementation, the rates are currently low. In addition, although the recommended 100,000 IU single dose helps to limit deficiency in newborns, it fails to cover infants' needs for optimal status. Some high-risk groups need more intensive protocols.

Key words: deficiency, 25(OH) vitamin D, newborn, pregnancy, supplementation

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