EFFECT OF MATERNAL OBESITY AND PRECONCEPTIONAL WEIGHT LOSS ON FOETO-PLACENTAL GROWTH AND OFFSPRING HEALTH IN MICE: expression of epigenetic modifiers at the interface with metabolism

P.E. Panchenko[1], M. Jouin[1], S. Voisin[1], S. Safi-Stibler[1], M.C. Lacroix[2], L. Jouneau[1], K. Badonnel[2], N. Meunier[2], H. Jammes[1], C. Junien[1], C. Baly[2] and A. Gabory[1]

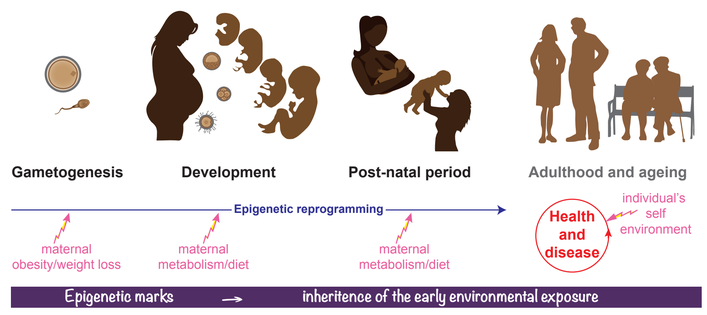
Introduction: intergenerational transmission of obesity ... what about preconception weight loss?

Maternal obesity is associated to a wide range of fertility troubles, obstetrical and gestational metabolic complications [1]. The babies are also at risk for stillbirth, growth phenotype (small or large birthweight), congenital malformations [2-4]. Moreover, according to the 'developmental origins of health and disease' concept, maternal obesity predisposes the offspring to adult onset non-communicable diseases [5].

A preconceptional weight loss is widely recommended to obese women. However, its long-term outcomes on the offspring have been poorly assessed.
In human cohorts, it may have positive or negative effects on fetal growth [6-11]. Interestingly, none of these studies followed up the metabolic development of these children and adolescents and additional studies are therefore needed to reveal the long-term health profiles of offspring born to obese mothers who lost weight prior to conception [12-13].

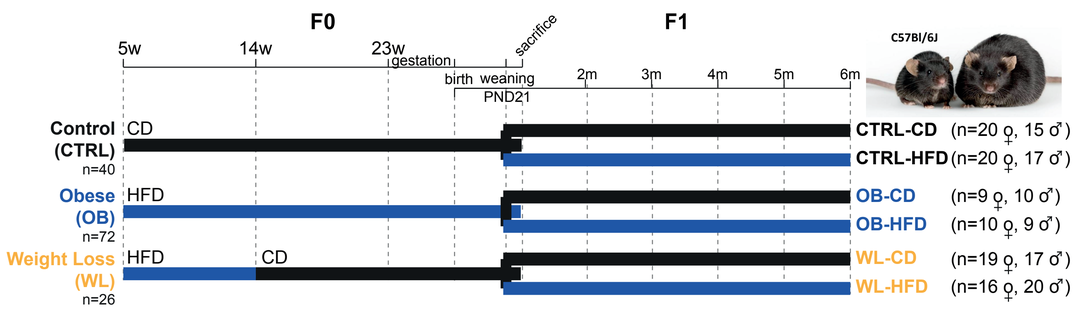
In animal, preconceptional weight loss has been assessed in rodents, sheep and non human primates (macaque). In two rat models, a nutritional intervention in obese dams was beneficial to the offspring, even if all parameters were not normalized [14-15]. In sheep, a strict nutritional intervention before mating had long-term benefits on offspring weight gain but deleterious effects on offspring stress response and glucose metabolism [16-17].

The epigenome is reprogrammed during gametogenesis and development, untill final differentiation of tissue and continue to be modified with ageing. This plasticity makes the epigenome a good candidate to participate in DOHaD.
Alteration of the epigenome early during ontogenesis, could be a mechanism of "memorization" of the *in utero* environment, contributing to particular gene expression patterns and thus to adult phenotype establishment.

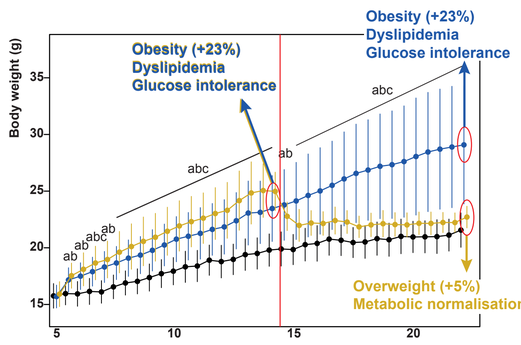


We recorded E18.5 mouse development and measured the mRNA expression of epigenetic modifiers and metabolic genes in foetal liver and placenta. Offspring born to 'control', 'obese' or 'weight-loss after diet-induced obesity' mothers were put on a control or high-fat diet, and we tracked their metabolic parameters and olfactory behaviour.

Results: Obesity induced foetal growth restriction, transcriptional changes and worsen diet-induced obesity in adulthood. Maternal weight loss is beneficial with some possible adverse outcomes.

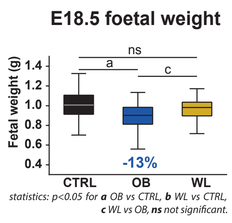


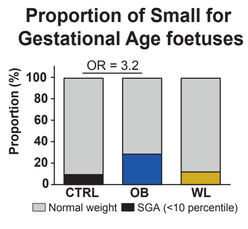
1 Maternal phenotype



After 4 months of HFD, OB mothers presented the characteristics of the **metabolic syndrome**. Diet change induced **weight loss, normalisation of lipidic and glucidic metabolims**, although WL mother kept a slight 5% overweight.
*statistics: p<0.05 for **a** OB vs CTRL, **b** WL vs CTRL, **c** WL vs OB.*

2 Offspring foetal phenotype



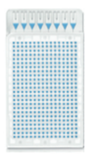


*statistics: p<0.05 for **a** OB vs CTRL, **b** WL vs CTRL, **c** WL vs OB, **ns** not significant.*

Maternal obesity is associated with **fetal growth restriction** (-13%) and increased proportion of **small for gestational age foetuses** (odd ratio =3.2).
Maternal preconceptional weight loss lead to a **complete normalisation** of the foetal growth phénotype.

3 Foetal gene expression

The expression of 60 epigenetic machinery genes and 32 metabolic genes was measured in the fetal liver, placental labyrinth, and junctional zone by RT-qPCR using TaqMan Low Density Assay.



Epigenetic machinery (60 genes):

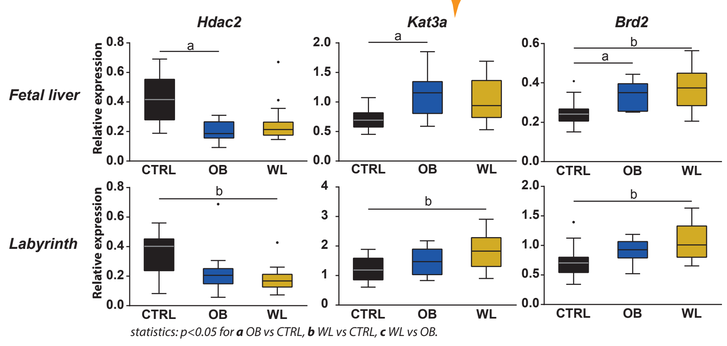
DNA Methylation (13):
✓ DNMTs
✓ Tet methylcytosine dioxygenases
✓ methylated DNA binding proteins
Histone Methylation (18):
✓ Lysine demethylases KDMs
✓ Lysine methyltransferases KMTs
✓ Arginine methyltransferases PRMTs
Histone Acetylation (29):
✓ Histone deacetylases HDACs
✓ Lysine acetyltransferases HAT
✓ Bromodomaine proteins Brd

Candidate for metabolism/development (32 genes):

Glucidic and lipidic metabolism:
✓ *Ppara, Ppary, Pparδ, Pepck, Pgc-1a, C/Ebp-a, C/Ebp-β, Lpl, Gck, Rev-erba, Nocturnin, Oxtr, GcGr, Irs-1, Insr*
Appetite regulation:
✓ *Leptin, Lepr, Pomc, Npy, Bdnf*
Glucocorticoïds regulation and response:
✓ *11βHsd-1, 11βHsd-2, Gr*
Serotonine pathway:
✓ *Tph1, Slc6a4, Maoa, 5-HT-r2a, 5-HT-r2c*
Feto-placental development:
✓ *Gcm1, Gcgr, Igf2, Igf2r, Slc16a10, Irs-1, Insr*

23 genes were affected by maternal weight trajectories in at least one of three tissues. The fetal liver and placental labyrinth were more responsive to maternal obesity than junctional zone.
One third (18/60) of the epigenetic machinery genes were differentially expressed between at least two maternal groups.
Genes involved in the **histone acetylation pathway** were particularly altered (13/18).

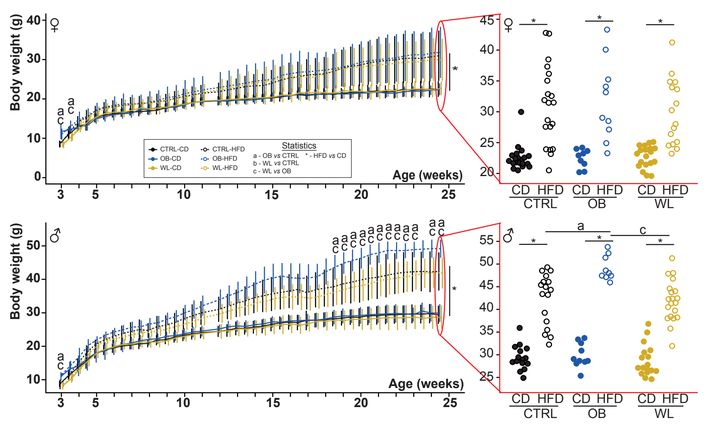
In OB group, while most *Hdacs* were downregulated, *Kats* and *Brd2* were upregulated. In WL group, the expression of only a subset of these genes was normalized.



*statistics: p<0.05 for **a** OB vs CTRL, **b** WL vs CTRL, **c** WL vs OB.*

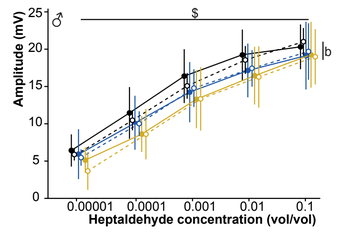
4 Offspring long-term outcomes

We tracked metabolic and olfactory behavioural trajectories of offspring born to CTRL, OBor WL mothers. After weaning, the offspring were either put on a CD or a HFD.
The offspring's **own diet explained most of the variability** in metabolic and olfactory phenotypes. After only few weeks of HFD, the offspring developed obesity, metabolic alterations and olfactory impairments, independently of maternal context.

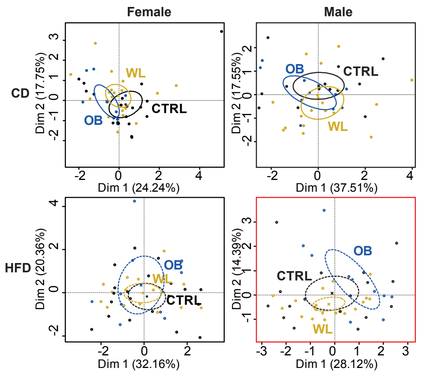


However, maternal obesity had a sex-specific *conditioning* effect:
Male offspring born to obese mother gained even more weight under HFD than their counterparts born to lean mothers.
Preconceptional WL normalized the offspring metabolic phenotypes but had an unexpected *programming* effect on olfactory performance:
a reduction in olfactory sensitivity, along with a lack of fasting-induced, olfactory-based motivation.

Electroolfactogram (EOG) recordings demonstrated a decreased sensitivity of the olfactory epithelium of male offspring born to WL mothers, whatever their post-weaning diet.



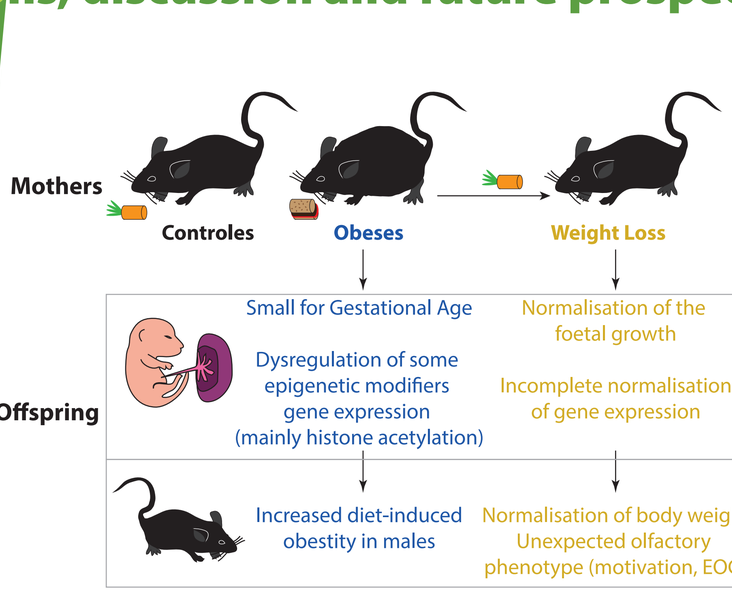
*statistics: p<0.05 for **a** OB vs CTRL, **b** WL vs CTRL, **c** WL vs OB, **$** dose response.*



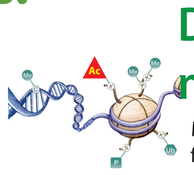
Multiple factor analysis (MFA) confirmed that **post weaning diet** has a major effect on F1 phenotype and **HFD-fed males are additionally influenced by maternal group.**

MFA analysed a set of 18 variables regrouped in 3 variable categories:
"Biochemistry" grouped the last measure of fasting glycaemia, insulinaemia, cholesterolemia and leptinemia, and glycemic AUC in OGTT.
"Biometry" grouped the organ to body weight ratio for BAT, Pr WAT, Pg-WAT, Sc-WAT, Total-WAT, Liver, Heart and Kidney, and week-24.5 body weight.
"Behaviour" grouped the week-23 relative caloric intake, day-1 hidden-cookie test retrieving time, HFD filled hole preference ratio and EOG at dose 0.001.

Conclusions, discussion and future prospects:

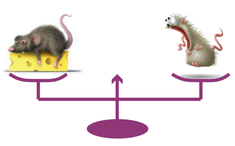


Deciphering epigenetics mechanisms in DOHaD.



Most of the research projects studying the effect of maternal obesity focuses on DNA methylation. However our results, as well as other publications, point out that the **histone acetylation** pathway can be a key component [18-21].
This highlights the importance of investigating the mechanisms of regulation of **histone marks** in response to environmental insults. The link between histone modifiers, histone acetylation levels, and placental and hepatic function should be established.

Memory of weight loss or stress?



In our model, it is unclear whether the decrease in olfactory sensitivity in WL males and the resistance of WL-CD to fasting stem from:
- the preconceptional **caloric depletion** associated with the transition to CD in mothers
- to a **maternal stress linked to the transition** per se, as stress may lead to intergenerational phenotypes [22-23].

Further studies are needed to discriminate the effect of energy depletion from the effect of stress on the maternal effects reported herein.

References

Expression of epigenetic machinery genes is sensitive to maternal obesity and weight loss in relation to fetal growth in mice.
Panchenko PE, Voisin S, Jouin M, Jouneau L, Prézelin A, Lecoutre S, Breton C, Jammes H, Junien C, Gabory A.
Clin Epigenetics. 2016

Effect of Maternal Obesity and Preconceptional Weight Loss on Male and Female Offspring Metabolism and Olfactory Performance in Mice.
Panchenko PE, Lacroix MC, Jouin M, Voisin S, Badonnel K, Lemaire M, Meunier N, Safi-Stibler S, Persuy MA, Jouneau L, Durieux D, Lecoutre S, Jammes H, Rousseau-Ralliard D, Breton C, Junien C, Baly C, Gabory A.
Nutrients. 2019

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Bibliography

1-Lee and Koren, J Obstet Gynaecol. 2010; **2**-Rajasingam et al., Am J Obstetrics and Gynecology 2009; **3**-Gaudet et al., BioMed Res Int 2014; **4**-Acosta et al., 2015; **5**-Hanson and Gluckman, Physiol Rev 2014; **6**-Villamor and Cnattingius, Lancet 2006; **7**-Kjaer and Nilas, Acta Obstet. Gynecol. Scand. 2013; **8**-Smith et al., J. Clin. Endocrinol. Metab. 2009; **9**-Jain et al., Am. J. Obstet. Gynecol. 2013; **10**-McBain et al., Eur. J. Obstet. Gynecol. Reprod. Biol. 2016; **11**-Diouf et al., Eur. J. Epidemiol. 2011; **12**-Forsum et al., Food Nutr. Res. 2013; **13**-Matusiak et al., J. Obes. 2014; **14**-Nathanielsz et al., Nutr. Rev. 2013; **15**-Dennison et al., Front. Endocrinol. 2017; **16**-Zhang et al., Exp. Diabetes Res. 2011; **17**-Nicholas et al., PLoS ONE 2013; **18**-Aagaard-Tillery etal., J Mol Endocrinol. 2008; **19**-Strakovsky etal., J Physiol. 2011; **20**-Suter et al., FASEB J. 2012; **21**-Suter etal., Am J Obstet Gynecol. 2014; **22**-Taouk and Schulkin, J Dev Orig Health Dis 2016; **23**-Zhang et al., Endocrinology 2013

1-UMR INRA, ENVA «Developmental Biology and Reproduction»
2-UR INRA «NeuroBiology of Olfaction»
Université Paris-Saclay
Centre INRA de Jouy-en-Josas, Domaine de Vilvert
78350 Jouy-en-Josas

Anne Gabory
anne.gabory@inra.fr