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REPRODUCTIVE ENDOCRINOLOGY

You are what your grandmother ate—inherited effects of *in utero* undernourishment

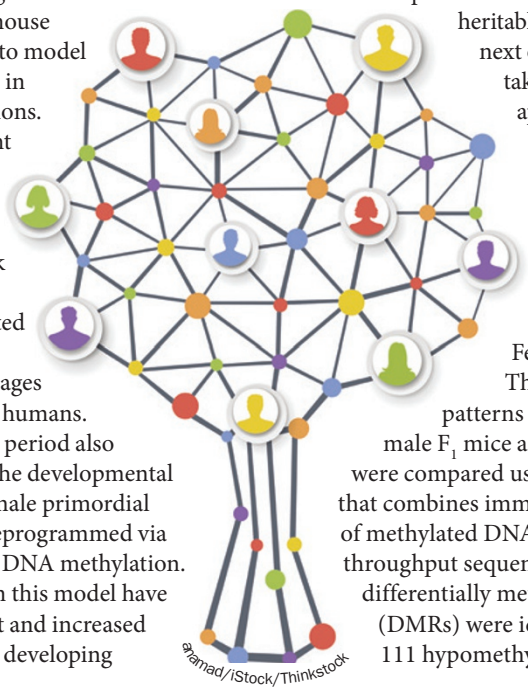
Nutritional perturbations during *in utero* development have long-lasting effects that are passed through multiple generations. Alterations of methylation patterns in male germline DNA are markers of compromised sperm in this process, reveals new research.

The nutritional status of a pregnant mother influences *in utero* development and can have effects on a child's health later in life. Moreover, emerging evidence suggests that exposure of an individual to environmental perturbations can have effects up to the third generation; however, the mechanisms that underlie intergenerational transmission of these effects remain largely unknown.

The collaborative study was led by Mary-Elizabeth Patti at the Joslin Diabetes Center, USA, and Anne Ferguson-Smith at the University of Cambridge, UK. The research teams used a mouse model of low birth weight to investigate the effects of undernourishment and an altered *in utero* environment on the DNA methylation profile of adult germ cells.

An outbred mouse strain was used to model potential effects in human populations. Low birth weight was induced by maternal undernutrition in the final week of pregnancy, which is estimated to parallel the middle to late stages of pregnancy in humans. This gestational period also coincides with the developmental stage in which male primordial germ cells are reprogrammed via reacquisition of DNA methylation.

F_1 offspring in this model have low birth weight and increased susceptibility to developing phenotypes such as increased fat mass and insulin resistance and decreased glucose tolerance, which are associated with diabetes mellitus later in life.



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In the new study, male mice from the F_1 generation were bred with healthy control mice (without any further environmental or nutritional insults) and the metabolic phenotypes of the subsequent F_2 generation pups analysed. Similar to their fathers, these mice had low birth weight and at 8 months of age exhibited reduced muscle mass with a concomitant increase in adiposity and reduced glucose tolerance. Analysis of markers of lipid metabolism in F_2 mice at embryonic day (E)16.5, such as levels of saturated fatty-acid-conjugated triglycerides and expression of genes involved in fatty acid oxidation, revealed that even *in utero* these mice had abnormal metabolism.

In earlier work, the authors had excluded the possibility that methylation at imprinting control regions was primarily responsible for the observed heritable phenotypes. "The next obvious step was to take a genome-wide approach to try to determine whether other parts of the genome might be epigenetically susceptible to this *in utero* insult," explains Ferguson-Smith.

The DNA methylation patterns in sperm from male F_1 mice and control mice were compared using a technique that combines immunoprecipitation of methylated DNA with high-throughput sequencing. In all, 166 differentially methylated regions (DMRs) were identified, including 111 hypomethylated regions and 55 hypermethylated regions. Loss of methylation in hypomethylated DMRs was confirmed in 90% of the unique regions that were analysed with bisulfite pyrosequencing in an independent set of sperm samples; however, of the eight hypermethylated DMRs selected for validation none were confirmed.

Examination of the genomic distribution of the hypomethylated DMRs in primordial germ cells of affected male F_1 mice revealed a unique temporal pattern of reprogramming, which suggested that *in utero* undernutrition interferes with the DNA remethylation process that normally occurs between E13.5 and E16.5. Hypomethylated DMRs in these mice also retained nucleosomes, which suggested that these effects might also be transmitted via altered chromatin structures.

Importantly, the differential methylation patterns did not persist in the brain and liver tissues of F_2 mice at E16.5, demonstrating that the metabolic phenotypes seen in this generation do not result from long-term heritable memory of a compromised germline.

"A father's previous [environmental] exposures can affect the health of his offspring," says Patti. "However, key questions that remain unanswered are if these effects are reversible, and if improvements in the health of the father [can] improve the health of his offspring."

The researchers plan to continue investigating the mechanisms that drive intergenerational effects of maternal undernourishment on the metabolic health of future generations, including the mechanisms that contribute to transmission of these effects through the maternal F_1 germline.

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