Lactation undernutrition leads to intergenerational alterations in reproductive parameters of male progeny.

Alicja Żochowska, Sylwia Judycka, Monika M. Kaczmarek

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Nutritional alterations including both over- and undernutrition experienced during a critical period of fetal or postnatal development may have a long-term impact on the health, which can persist over the generations. Early-life nutritional status may affect maturation of hypothalamus-pituitary-gonadal (HPG) axis, which is a key regulator of the development and functioning of reproductive system. However, it is still not well documented how postnatal malnutrition affects reproductive performance in male progeny across the generations. Here, we investigated the effects of maternal undernutrition during lactation on body weight and composition, puberty attainment, testicular gene expression, and sperm motility and kinematics of first (F1) and second (F2) generation male progeny. C57BL/6 mice were assigned to 1) control group (CON), where dams were fed a standard diet *at libitum* during lactation and 2) lactation undernutrition group (LUN), where dams were fed 50% of standard diet. In both groups, litter size was fixed at 8 pups. Next, to acquire F2 male offspring, three different combinations of breeding pairs from F1 progeny were used: CON female x CON male (CC), LUN female x CON male (LC) and LUN female x LUN male (LL). F2 offspring were fed standard diet *at libitum.* Decreased body weight and body composition alterations were indicated in LUN male offspring (F1) at postnatal day 21 (PND21; $p<0.0001$) and postpubertal period ($p<0.05$), contrary to F2 male offspring where any significant changes in body parameters were observed. However, malnutrition of ancestors during lactation contributed to delayed puberty in F2 male offspring; the most significantly affected in LL males ($p<0.0001$). Altered expression of testicular genes involved in steroidogenesis and sexual maturity (*e.g., Hsd3b1, Lhcgr, Cyp19a1, Cd2ap, Nsd1)* was observed in both F1 and F2 males at PND21 ($p<0.05$), while expression of *Cd2ap* was maintained altered until adulthood only in LL males. Sperm kinematics showed no significant changes in F1 male offspring, whereas meaningful alterations were visible for VAP (Average Path Velocity), VCL (Curvilinear Path Velocity) and VSL (Straight Line Velocity) parameters in LL males ($p<0.05$). Sperm motility was not affected by malnutrition in both generations. Summarizing, early postnatal exposure to malnutrition induce long-term reproductive consequences in the subsequent generations of males. Alternations in timing of puberty, changed testicular gene expression and impaired sperm parameters persisting over generations hint at the necessity of further studies on the mechanism governing this phenomenon.

Research supported by Polish National Science Centre grant OPUS 2018/31/B/NZ4/03527