Non-caloric sweeteners are associated with decreased sperm viability and morphology and altered testicular gene expression in male mice

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Infertility is global issue affecting approximately 15% of couples worldwide, with male infertility contributing up to 50% of cases. There is growing evidence that obesity can alter sperm parameters and negatively affect sperm function and induce epigenetic changes to spermatozoa. Non-caloric sweeteners (NCS) are widely consumed owing to their intense sweetness while providing minimal or no energy, however their detectability in different body tissues, including the reproductive system is unclear. Limited rodent studies have shown high NCS intakes can reduce sperm function, however most studies to date have looked at excessive NCS consumption.

The present study was carried out to evaluate the impact of modest NCS intakes, in combination with High fat diet (HFD) on sperm parameters and glucose tolerance in male mice.

32 adult male mice were randomly assigned to a control diet (CD) (10% kcal fat) or HFD (45% kcal fat) and assigned to 8 groups of 4 mice each and received either water (control), fructose (20% solution in water) or NCS (Ace-k at a concentration of 12.5mM solution in water or Reb A at a concentration of 1mM solution in water). Mice were weighed weekly, and food and solution intakes were monitored. Following 8 weeks, mice were humanely killed. Testes were weighed and sperm isolated from the cauda epididymis and parameters, including sperm viability and morphology were evaluated. Sperm abnormal morphology and live viability were evaluated for at least 200 spermatozoa of each animal. The percentage of morphologically abnormal spermatozoa and viable sperm were assessed by Eosin and Eosin-Nigrosin staining respectively and imaged using light microscopy. RNA was extracted from testes. The expression of 84 genes relevant to male infertility was analysed using the Mouse Male Infertility RT^2 Profiler PCR Array (SABioscience).

Following 8 weeks on the diet, HFD mice gained significantly higher body weight than CD mice ($p<0.001$) with a mean body weight of 30.1g ±1.0 for CD and 33.7±1.4 for HFD mice. Following NCS consumption, mice had increased abnormal sperm morphology (Ace-k 68.0± 9.2, Reb-a 78.3±10.3) compared to CD fed mice (CD control 59.6±6.8, HF control 64.2±9.0). Sperm viability decreased in CD NCS groups (Ace-k 27.9±1.1, Reb-a 39.9±11.2) compared to the CD Con group (43.2±8.5), however sperm viability increased in both HFD NCS mice (Ace-k 38.1±0.6, Reb-a 46.6±4.0) compared to CD NCS fed mice. Among the CDRebA groups there was downregulated expression of Boll gene (0.24±0.01, $p<0.05$) and HFCon downregulated expression of Akap14 gene (0.5±0.04, $p<0.05$).

This study has shown that NCS consumption reduced sperm parameters and testicular gene expression in male mice**.** Furthermore, consumption of a HFD had a protective effect on sperm viability in mice consuming artificial sweeteners compared with control and fructose

groups. HFCon mice had downregulated expression of Akap14, thought to play a role in sperm motility. Our results indicate that modest consumption of artificial sweeteners may induce negative effects on sperm parameters in mice, however these negative effects on sperm viability may be reduced with the addition of a high fat diet.

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