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TREADMILL EXERCISE PREVENTS LEPTIN-INDUCED DECLINE IN TESTICULAR AND SPERM PARAMETERS IN MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Elevated leptin levels have detrimental effects on sperm parameters and testicular tissue in Sprague-Dawley rats. Although some forms of low-to-moderate physical activity has been shown to improve health, whether regular physical exercise could prevent the adverse effects of exogenous leptin administration on sperm and testicular parameters, remains unknown. This study, therefore, examined the effects of treadmill running on leptin-induced adverse effects on sperm and testicular parameters in male rats. A total of 32 male Sprague-Dawley rats, aged 12 weeks, were divided into 4 equal groups. The rats received intraperitoneal injections as follows: Group 1 (Control) received 0.1 ml of normal saline; Group 2 (Leptin) received 60 μg/kg of leptin; Group 3 (Leptin+Exercise) received 60 μg/kg of leptin combined with exercise; and Group 4 (Exercise) received exercise alone. The exercise regimen consisted of treadmill running 4 times per week for 42 days (6 weeks). Each exercise session consisted of running at a speed of 0.5 m/s at 5° inclination on the treadmill for 30 minutes. Food and water intake, and body weight were measured weekly. Following the completion of treatment on day 43, the rats were euthanised and reproductive organs were collected. The weight of the organs (testes and epididymis) was determined. Total sperm count and fraction of sperm with abnormal morphology, seminiferous tubular height and diameter, concentration of 8-OHdG, sperm DNA damage, catalase and glutathione peroxidase activities, and expression of connexin-43 and occludin were determined. Data were analysed using ANOVA and Repeated Measures (RM) ANOVA followed by post-hoc analysis, and are expressed as mean ± SEM.

Sperm count was lower, and fraction of sperm with abnormal morphology was significantly higher in rats in Group 2 when compared to those in Group 1. Sperm count was significantly higher, and fraction of sperm with abnormal morphology was significantly lower in rats in Groups 3 and 4 when compared to those in rats in Group 2. Seminiferous tubular epithelial height and diameter were significantly lower in Group 2 rats when compared to those rats in Groups 1, 3 and 4. No significant differences were evident in the weight of the reproductive organs, sperm 8-OHdG concentration, and activities of catalase and glutathione peroxidase between the four groups. Sperm DNA fragmentation was significantly lower in rats in Groups 3 and 4 when compared to that in Group 2 rats. There was no significant difference in gene expression of occludin between the 4 groups. In contrast, the gene expression of connexin-43 showed a significant upregulation in Group 2 compared to that in the other groups, suggesting an increase in cell proliferative activity. In conclusion, leptin's adverse effects on sperm count, fraction of abnormal morphology, seminiferous tubular epithelial height and diameter and sperm DNA fragmentation were prevented by the exercise intervention. Exercise could, therefore, be recommended to those with leptin-induced decreases in sperm count and overall sperm function. This might be particularly relevant to males who are overweight or obese where serum leptin levels are persistently elevated.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Leptin is a non-glycosylated peptide hormone, predominantly secreted from white adipose tissue. Its presence in the circulation is directly proportional to body fat mass (Wiesner et al., 1999; Perakakis et al., 2021; Picó et al., 2022). Leptin was initially shown to play an important role in energy homeostasis and regulation of body weight (Considine et al., 1996; Friedman, 2011; Hussain & Khan, 2017; Liu et al., 2023). However, research over the last couple of decades has found that leptin has roles in numerous physiological functions in the body, including reproduction (Clarke & Henry, 1999; Almabhouh et al., 2019; Childs et al., 2021), metabolism (Overton et al., 2001; Pereira et al., 2021), immune responses (Wu et al., 2003; De Heredia et al., 2012; Kiernan & MacIver, 2021), inflammation (Wahba & Mak, 2007; Pérez-Pérez et al., 2020) and stress (Ahima et al., 1997; Bouillon-Minois et al., 2021; Hosseini et al., 2024), to the extent that it is now considered a pleiotropic factor having both direct and permissive functions.

Although leptin is necessary for sexual maturation and normal reproductive function, raised serum leptin levels, as seen in overweight and obese individuals, seem to adversely affect male reproductive function. Leptin has a role in obesity-induced derangement of the hypothalamic-pituitary-gonadal (HPG) axis leading to lower testosterone levels in human males (Craig et al., 2017). In addition, leptin has also been shown to have direct effects on the testes and spermatogenesis (Esmaili-Nejad et al., 2015; Arora et al., 2022). Leptin's effects on the testes are positive at low levels and negative at high levels. Leptin receptors are widely distributed on germ and somatic cells of the testes (Ishikawa et al., 2007; Gao et al., 2022). Leptin levels in human seminal plasma were found to have a significant negative correlation with sperm progressive motility and serum total testosterone (Guo et al., 2014).

Studies *in vivo* on laboratory animals have demonstrated the negative impact of leptin on rat spermatozoa. Sprague-Dawley rats given single-daily intraperitoneal injection of leptin, in doses ranging from 5 to 30 μg/kg body weight for 6 weeks, had significantly lower sperm count, and higher fraction of sperm with abnormal