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Ameliorative Effect of SR48692 on the Testicular and Epididymal Impairments of High-Fat Diet-Induced Swiss Albino Mice

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Abstract

This study investigated the role of Neurotensin (NT) receptor-1 antagonist SR48692 on High-Fat Diet (HFD)-induced considerable morphological alterations in the testis and epididymis in mice. Excessive intake of fat and a sedentary lifestyle are major causes of metabolic comorbidities in the current obesogenic environment. Consumption of HFD induces NT release in the gut, which promotes lipid absorption. Elevated NT is associated with metabolic alterations and imbalanced energy status which could be responsible for a negative impact on reproductive outcomes. Male mice (n = 24) were randomly assigned, based on diet into Control Diet (CD) and HFD for 4 weeks; they were further divided into 2 sub-groups and administered saline and SR48692 (100 µg/kg b.w.) intraperitoneally for the next 4 weeks. The present findings revealed that HFD-induced obesity caused significant alterations in circulating lipid and reproductive hormone profiles leading to deterioration of testicular and epididymal histoarchitechture. However, the co-treatment of SR48692 with HFD has significantly restored the HFD-induced detrimental effect on lipid and reproductive hormone profiles, justified by the improvements in histoarchitechture of the testis and epididymis. However, only SR48692 administered group showed adverse effects on the studied parameters. The results reported herein showed that SR48692 have the efficacy to actively overcome the HFD-induced detrimental effects. Therefore, SR48692 might be considered for the therapeutic role in HFD-induced reproductive impairments.

Keywords: High-Fat Diet, Histopathology, Neurotensin, Reproduction, SR48692

1. Introduction

Globally obesity, one of the major health issues of the current scenario considered a chronic health disease that affects various physiological processes, it grows rapidly due to excessive dietary lipids intake and leads to obesity-mediated numerous health disturbances such as cardiovascular disease, insulin resistance different types of cancer and infertility[1-3]. Epidemiological investigations have revealed that HFD-intake has been proven to trigger the progression of obesity by increased insulin resistance, metabolic inflammation, and imbalance in lipid profiles[1,4,5]. The appropriate integration of lipid metabolic regulation is governed by various neuropeptides such as ghrelin, kisspeptin, and neuropeptide-Y, including NT[6,7]. NT is a brain and gut 13 amino acid peptide released by the enteroendocrine N-cells after fat ingestion, and promotes lipid absorption by three specific receptors (NTR1, NTR2

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and NTR3) in the gut; however, in the brain, it regulates energy balance and appetite[7,8].

Normal reproductive function is governed by the proper availability of metabolic fuels to secure balanced energy status and endocrine factors which are regulated by central and peripheral energy sensors[9,10]. Earlier studies well documented that under HFD conditions, NT-mediated lipid absorption cooperated with other regulators like histamine, and reflected in elevated levels of lipids[7]. HFD-induced NT-mediated histamine secreted by the mast cells which promotes bile acid absorption and vasodilation resulted in enhanced lipid level[11]. As per the studies, elevated plasma Total Cholesterol (TC), Triglycerides (TG), and Low-Density Lipoprotein-c (LDL-c), and decreases in High-Density Lipoprotein-c (HDL-c) have been associated with the prevalence and severity of metabolic dysregulations[12,13].

There is a multifactorial relationship between metabolic dysregulation and reproduction has been revealed by impaired regulation of the Hypothalamic-Pituitary-Gonadal (HPG) axis[14]. The hypothalamus situated at the top of the axis synthesizes and secretes Gonadotropin-Releasing Hormone (GnRH), and participates in the regulation of reproduction by activating the HPG axis[15,16]. In the hypothalamus, GnRH neurons are the key neurons of the complex neural circuits that coordinate numerous internal and external factors, crucial for the regulation of reproductive function[17]. GnRH controls the gonadotropin; Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) secretion from the pituitary which consequently regulates testicular performance[18,19]. Cooperatively LH and FSH stimulate the testis to synthesize and release sex hormones such as testosterone. Testosterone produced by the Leydig cells through steroidogenesis is governed by the HPG axis[20]. Under HFD condition the testosterone level is declined which is directly associated with negative impacts on the HPG axis and impairments of fertility[2,21]. Oestrogen levels are elevated in men with obesity due to the conversion of testosterone to oestrogen by aromatase[3], which is associated with a reduced testicular size and sperm count[22]. Spermatogenesis can be affected by several factors, including hormonal changes. In this regard, testicular lipid metabolism is crucial, not only as energy substrates but also as structural elements of the sperm to maintain spermatogenesis[9]. Storage as well as maturation of sperm takes place in the epididymis. Besides this, epididymis also regulates defence response mechanisms and apoptosis in sperm[23].

At present, paucity of studies specifically on the treatment of reproductive dysfunction in obese conditions. In the present study, an obese animal model was established to find out whether HFD-induced NT-mediated disruption in testicular and epididymal morphology. Li and co-workers suggested that NTR1 antagonist SR48692 inhibits fat absorption by disrupting normal NT signalling[7]. A biologically active compound, SR48692 can decrease cell viability in several cancer cell lines. According to earlier research, cancer of the lung, breast, pancreas, pituitary, and prostate have been shown to upregulate NTR expression[24,25]. A report on prostate cancer cell lines, on androgen-insensitive (PC3 cells) and androgen-sensitive (LnCaP cells) cells revealed that treatment with SR48692 significantly decreased the growth of LnCaP cells in the prostate cancer cell line[24,26]. Based on previous reports, we hypothesized that the co-treatment of SR48692 with HFD could inhibit NT-mediated lipid dysregulation and also improve testicular and epididymal histoarchitecture. Therefore, our present study principally focused on the crucial role of SR48692 against impaired testicular and epididymal histopathological architecture as well as imbalanced reproductive hormones in HFD-induced male mice.

2. Materials and Methods

2.1 Animals and Study Design

8 weeks old, swiss mice (male) were purchased from the IITR, Lucknow, India, and placed in polyvinyl chloride cages with *ad libitum* access to rodent Chow Diet (CD) and drinking water under standard conditions, 12 h day/night cycle, 40-60 % of humidity and 22–24°C temp. After acclimatization of one week, the mice were arbitrarily assigned into the control group (n = 12) that received CD, and the High-Fat Diet (HFD) group (n = 12) that received HFD (60% energy from the fat), containing lard as a fat source, prepared in our laboratory[27]. After four weeks of feeding, the CD and HFD groups were further divided into the following four groups (n = 6/per group); Group-1 CD, Group-2 HFD, Group-3 HFD+SR48692 and Group-4 CD+SR48692. Group-1 and 2 were administered intraperitoneally (i.p.) physiological saline and kept as vehicle control groups (CD and HFD). Group-3 and 4 were administered NTR1 antagonist SR48692 (HPLC: ≥98% purity Sigma Aldrich, Bengaluru, India) at the dose 100 µg/kg b.w. given i.p. for four weeks taking into the reference of previous reports[28]. Over the entire experimental duration, the body weight of

animals was recorded regularly. Quantification of the daily food intake/mice of each group was done by subtracting the food left by the food given and further dividing by six. At the end of the experiment, by using pentobarbital (100 mg/kg) mice were euthanized, and blood samples for plasma were collected in heparinized microcentrifuge tubes. The experiments were performed by the guidelines of the Committee for Control and Supervision of Experimental Animals (CPCSEA), Government of India, approved by the Institutional Animal Ethics Committee (IAEC) of the University of Allahabad, Prayagraj, India [Ethical Approval No: IAEC/AU/2019(1)/03].

2.2 Measurement of Lipid Profiles

The collected blood samples were centrifuged for 15 minutes at 3000 rpm for plasma separation. Lipid profiles, i.e., TC, TG, LDL-c, and HDL-c, were quantified as per the manufacturer's protocol provided with the diagnostic kits (Arkray Healthcare, Pvt. Ltd., India).

2.3 Measurement of Reproductive Hormones

Plasma concentrations of LH, FSH and testosterone were measured via ELISA kits according to the commercially available manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany). The findings were expressed in ng/ml for testosterone and in IU/L for LH and FSH.

2.4 Histopathology of Testis and Epididymis

For histological analysis, after euthanizing mice, testis and epididymis were quickly collected and fixed in 4% paraformaldehyde solution for 24 hours. After proper fixation, the tissue samples were dehydrated using alcohol series, followed by paraffin embedding, sectioned into 5-6 µm thick sections, and stained using hematoxylin and eosin (H and E) for the analysis of histopathology. The morphological changes in sections were examined under a light microscope (Leica DM 2500, Germany), and photomicrographs were obtained. In brief, nearly twenty-five Seminiferous Tubules (ST) were evaluated following Johnsen's scoring method, every tubule was evaluated from 1 (absence of germinal and Sertoli cells) to 10 (complete spermatogenesis) and the mean scores per mouse were stated as the Johnsen's score[29]. The epididymal epithelial thickness was measured using the image analysis software package, Image J 1.32J (NIH, Bethesda, MD).

2.5 Statistical Analysis

For statistical analysis, Graph Pad Prism 9 (Graph Software Inc., USA) was applied. The difference between the control and experimental groups was compared by one-way ANOVA followed by the Dunnett test and the results were expressed as Mean ± SEM. The results were analyzed following Burnning and Knitz, 1977, indicating statistical significance with a p-value of less than 0.05[30].

3. Results

3.1 Food Intake and Body Weight

In HFD-consumed mice, the food intake was significantly lower ($p<0.001$) compared to CD mice. SR48692 co-treatment with HFD mice, increased the consumption of food from 5 to 8 weeks, compared to HFD, however, remained significantly less ($p<0.001$) compared to CD. HFD intake mice showed a significant increase ($p<0.01$) in % body weight to that of CD. SR48692 co-treatment with HFD mice showed that % body weight significantly decreased ($p<0.05$) less compared to HFD. Only SR48692 treated group % body weight gain remains equivalent to CD (Figure 1).

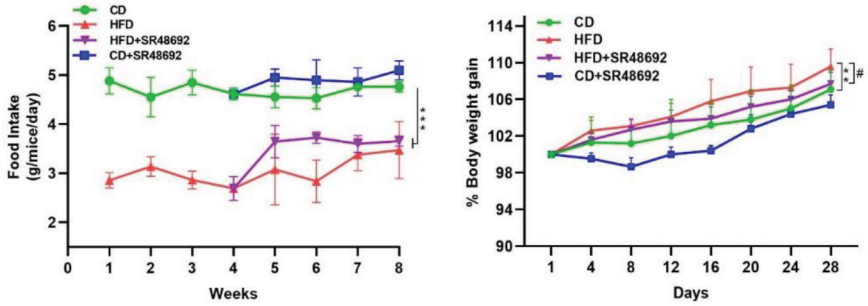


Figure 1. Graph showing food intake and body weight gain (%) experimental groups as well as vehicle controls. The values were given as the Mean ± SEM. Statistical significance was assigned as CD Vehicle control vs Experimental groups **p<0.01 and ***p<0.001; HFD Vehicle control vs Experimental groups #p<0.05.

Table 1. Plasma lipid profiles

S. No.	Parameters	CD	HFD	HFD+SR48692	CD+SR48692
1.	TG (mg/dl)	136.8±0.23	182.7±17.39[c]	158.2±7.06[a, d]	132.3±1.38
2.	TC (mg/dl)	96.62±1.16	112.7±2.42[c]	101.7±1.91[c, f]	92.62±0.99
3.	HDL-c (mg/dl)	14.51±0.66	11.80±0.30[c]	13.74±0.35[a, f]	13.47±0.12[b]
4.	LDL-c (mg/dl)	54.74±1.69	64.34±1.92[c]	56.30±2.69[f]	52.69±0.14

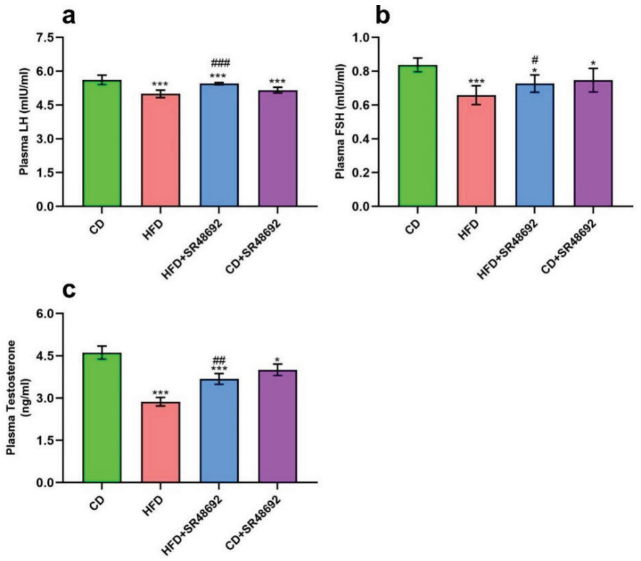


Figure 2. Graph showing reproductive hormone levels; LH **(a)**, FSH **(b)** and Testosterone **(c)** of experimental groups as well as vehicle controls. The values were given as the Mean ± SEM. Statistical significance was assigned as CD Vehicle control vs Experimental groups **p<0.05 and ***p<0.001; HFD Vehicle control vs Experimental groups #p<0.05, ##p<0.01 and, ###p<0.001.

3.2 Plasma Lipid Profiles Quantification

In HFD-consumed mice, the plasma concentration of TG, TC, and LDL-c were significantly enhanced (p<0.001) and HDL-c significantly decreased (p<0.001), compared to CD mice. However, the co-treated group (HFD+SR48692) TG, TC, and LDL-c were significantly lower (p<0.05, p<0.001 and p<0.001, respectively), and HDL-c was higher (p<0.001) than that of HFD, however, compared to CD the TG and TC were high (p<0.05 and p<0.001, respectively), and HDL-c less (p<0.05). In only SR48692 treated group, both the TC and HDL-c were decreased (p<0.01), while TG and LDL-c remained equivalent to CD (Table 1).

Table showing the lipid profiles of experimental groups as well as controls. The values were given as the Mean ± SEM. Statistical significance was assigned as CD Vehicle control vs Experimental groups [a]p<0.05, [b]p<0.01 and [c]p<0.001; HFD Vehicle control vs Experimental groups [d]p<0.05, [e]p<0.01 and [f]p<0.001.

3.3 Plasma Reproductive Hormone Quantification

Plasma concentrations of LH, FSH, and testosterone decreased significantly (p<0.001) in HFD-consumed mice compared to CD mice. With the co-treatment of SR48962 to HFD mice concentrations of LH, FSH, and testosterone were significantly higher (p<0.001, p<0.05, and p<0.01, respectively) than HFD. Still, compared to CD the FSH and testosterone concentrations were significantly less (p<0.05), however, the LH remained equivalent. The only SR48692 treated group showed significantly lower LH (p<0.001), FSH (p<0.05), and testosterone (p<0.01) than that of CD (Figure 2).

3.4 Histological Analysis of the Testis and Epididymis

Testicular sections from CD mice revealed normal ST and an organized interstitium with multilayer spermatogonial cells, suggesting active spermatogenesis. In contrast, distinct structural disorder and loss of compactness in the ST, reduced number of germinal epithelial cells; dilatations between the cells of germinal epithelial, and numerous immature spermatocytes in the lumen were observed in HFD. The antagonist co-treated (HFD+SR48692) group showed comparatively restored ST and increased compactness among the germinal epithelial cells than that of HFD. Only the SR48692 treated group showed increased structural disorder and decreased compactness in the ST compared to CD. The histopathologic Johnson's score was significantly less (p<0.001) in the HFD group than that of CD. However, in the co-treatment group, the histopathologic Johnson's score was significantly

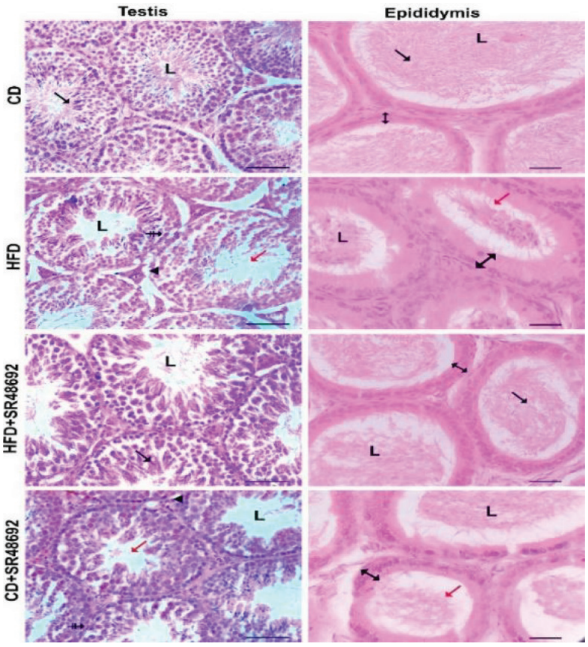


Figure 3. Representative photomicrographs of H and E stained cross-sections of testis and cauda epididymis of CD, HFD, HFD+SR48692 and CD+SR48692 groups showing lumen of the tubules (**L**), sloughing germinal epithelium and irregular basement membrane of testis ( ), vacuoles and widening of the interstitial space ( ), epididymal epithelial thickness ( ), increased sperm density (black arrows), and decreased sperm density (red arrows), magnification ×40; Scale bar 50µm.

higher ($p<0.01$) as compared to HFD but significantly lower ($p<0.001$) compared to CD. The only antagonist-treated group significantly decreased ($p<0.001$) than that of CD.

Normal and compactly arranged circular tubules with a well-organized stratified columnar epithelium were observed in CD with densely packed sperm cells in the tubular lumen. However, reduced lumen area and sperm density with significantly higher ($p<0.001$) epididymal epithelial thickness was observed in HFD than in CD. With the antagonist co-treatment (HFD+SR48692), increased lumen area with densely packed sperm cells, reflected in significantly decreased ($p<0.001$) epididymal epithelium thickness compared to HFD, still significantly increased compared to CD. In the only antagonist-treated group the lumen and sperm density decreased with significantly increased ($p<0.001$) epithelial thickness than that of CD (Figures 3 and 4).

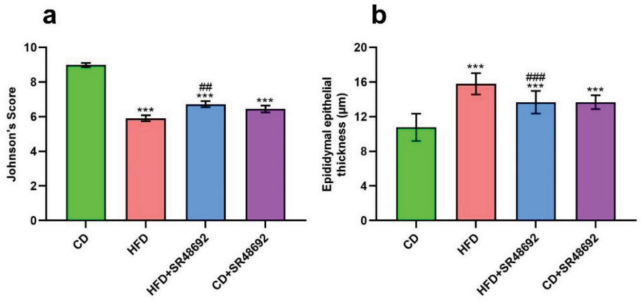


Figure 4. Graph showing Johnson's score **(a)** and epididymal epithelial thickness **(b)** of experimental groups as well as vehicle controls. The values were given as the Mean ± SEM. Statistical significance was assigned as CD Vehicle control vs Experimental groups ***$p<0.001$; HFD Vehicle control vs Experimental groups ##$p<0.01$ and, ###$p<0.001$.

4. Discussion

The current study focused on the ameliorative effect of NTR1 antagonist (SR48692) against HFD-induced impaired testicular and epididymal outcomes was observed, through modulating plasma lipid profile as well as reproductive hormones. Plenty of lard in HFD may be an essential ingredient in causing lipid profile alteration because of its high saturated fatty acids content that might be reflected in reduced food intake and body weight gain (%)[2,10]. The improper regulation of lipid metabolism exhibiting imbalanced TG, TC, LDL-c, HDL-c and reduced food intake observed in the present study might be related to NT-mediated increased fat absorption through binding to its cognate receptors under HFD condition[7,8]. Earlier studies investigated the association between plasma cholesterol with a significant decline in semen quality and sperm epididymal maturation, have found that enhanced cholesterol alters the functionality of the epididymis[31,32]. The findings were in agreement with previous research that increased cholesterol might influence the organization of the plasma membrane of the testis consequences in the altered availability of gonadotropin receptors, such as LH and FSH which ultimately impaired testosterone production and spermatogenesis indicates the disruption of the HPG axis[33]. The level of reproduction-related hormones is governed by GnRH secretion through the hypothalamus, which acts on the pituitary to promote the secretion of LH and FSH [12]. Alterations in gonadotropins are intricately associated with obesity-induced physiological changes, indicating an inverse relation between obesity and gonadal hormones[9].

Under HFD conditions decreased LH and FSH secretion due to alteration in GnRH secretions reflected in histopathology of the ST and epididymal degeneration, as earlier reported[22]. It is well documented that cannabis consumption has evidenced a deleterious effect on reproductive performance via impaired reproductive hormones as well as histoarchitechture of the gonads in both males and females[34]. HFD consumption suppresses reproductive outcomes with alterations in the testicular and epididymal histopathology that could affect spermatogenesis are consistent with earlier studies[12,35]. Histological changes, such as a significant reduction in lumen diameter and increased epididymal epithelial thickness, often suggest an excessive accumulation of interstitial fluid and may be an indicator of reduced sperm production[35,36]. Furthermore, the increased epithelial thickness of the tubules in the epididymis, with clearly observed necrotic epithelial cells, and interstitial edema in the HFD, are corroborated with previous reports[37]. The histopathological findings of our study suggest a decline in spermatogenesis evidenced by Johonson's score and were consistent with the epididymal histopathology, which was either devoid of spermatozoa or had decreased spermatozoan density, as reported previously[22,38]. The data obtained from lipid profiles, reproductive hormones and histological assessment could be associated with functional deficiency and epididymis after the consumption of HFD.

In this study, the co-treatment of SR48692 with HFD resulted in an improvement in plasma lipid profiles along with increased food intake which is consistent with a previous study that represented increased gut motility in response to SR48692 treatment resulting in decreased NT-mediated lipid absorption[39,40]. Miller and coworkers reported that blockade of NT evoked mast cell-mediated histamine release by SR48692, which could be responsible for decreased bile acid absorption[41]. The decreased lipid absorption is subsequently reflected in the lipid profiles as well as body weight gain observed[12]. A negative correlation between the serum triglycerides and testicular histopathology, conversely, a positive correlation was seen between the blood HDL-c level and testicular weight, testosterone level, seminiferous tubule area, and the quantity of viable and normal sperm[42,43].

SR48692 administration to HFD mice, showed improvement in testosterone, FSH, and LH levels, which might explain the modulation in testicular and epididymal characteristics due to amelioration in spermatogenic profile and lipid profiles[22,35]. An endogenous NT at the physiological level participates in the regulation of various biological processes, including lipid absorption via NTRs, the imbalance in the NT level leads to the development of many diseases[8]. Endogenous NT was upregulated under HFD intake, and by inhibiting NTR1 activity with SR48692, food intake, and lipid profiles were improved and might be the reason for restored reproductive outcomes. In this study improvements in the functional property of the epididymis for the storage and maturation of sperm are reflected in the dilation of the epididymis and a rise in the number of cells along with decreased epididymal epithelial thickness, corroborated with previous studies[22,31,44]. The analysis of the histomorphometry of the testis revealed upgraded spermatogenesis, resulting in an improved Johnsen score[22]. The only SR48692 treated group showed decreased Johnsen's score, resulting in impaired spermatogenesis and with a more detrimental effect on epididymis and all studied parameters, similar to that of observed histopathological alterations in epididymis with molybdenum exposure[45]. Also supported by the earlier study the SR48692 (80 µg/kg i.p.) can cross the blood-brain barrier and can antagonize the NT-induced effect[46]. Collectively, in this study, lipid profiles, reproductive hormone level, Johnson's score, and epithelial thickness of the epididymis were significantly ameliorated by the co-treatment of SR48692 with HFD, suggesting its therapeutic efficacy in HFD-mediated testicular and epididymal impairments.

5. Conclusion

In conclusion, HFD intake impaired reproductive outcomes by affecting lipid profiles, and reproductive hormones as well as the histoarchitecture of the testis and epididymis. The SR48692 administration revealed that significant ameliorative effect against HFD-induced impaired testicular and epididymal histopathological architecture along with prejudiced reproductive hormones. It could be suggested that the SR48692 administration has a potential therapeutic role in HFD-induced impairments in testicular and epididymal histopathology.

6. Acknowledgment

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