The Effect of Vitamin D And Swimming Exercise on The Reproductive System in High Fat-Fed Adult Male Rats

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Abstract

Background: Diet has a significant impact on regular functioning, and the negative consequences of high-fat diets and obesity on male reproductive health are becoming more well-known.

Objective: The present study aims at studying the beneficial effects of vitamin D and exercise against high fat diet induced insulin resistance and male subfertility in male albino rats.

METHODS: 35 male albino rats treated with high fat diet for 12 successful weeks and divided into 5 equal groups; each group is 7 rats; (i) normal control rats, (ii) high fat diet (HFD) group, (iii) received HFD with vitamin D, (iv) received HFD and swimming exercise, (v) received HFD with vitamin D and swimming exercise. Blood samples were withdrawn from the experimental animals for analysis of Glucose, Insulin, Testosterone, FSH, LH and semen analysis where testis also was excised to measure antioxidant markers (Glutathione, SOD, MDA and NO).

RESULTS: HFD resulted in insulin resistance characterized by high HOMA IR values and induced diminished semen quality with alteration in male sex hormones profile. Treatment with vitamin D and swimming exercise individually showed marked alleviation of the HFD deleterious effect while combined therapy of both vitamin D and swimming exercise showed synergistic effects.

CONCLUSION: The use of vitamin D and swimming exercise in the treatment of insulin resistance and diminished male fertility offered preventive and curative effect against HFD, especially when combined together.

Keywords: HFD, vitamin D, swimming exercise, insulin resistance, male fertility.
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1. Introduction

Obesity, which is largely caused by high-fat meals and lack of physical activity, is still a major public health issue globally. Despite much has been discovered about the link between obesity, metabolic abnormalities, and chronic illness, the specific physiological and molecular processes underlying these relationships have yet to be completely investigated [1].

In both genders, being overweight or obese in adulthood affects gonadal steroidogenesis and fertility. Obesity affects the hypothalamic-pituitary-gonadal axis in men, lowering testosterone levels and impairing sperm production. While male obesity is well

acknowledged as a major problem in reproductive medicine, the possible effects of obesity on male fertility are a serious warning sign that more study is needed [2].

Vitamin D (vitD) has recently been investigated for its importance in a number of systems and organs. The regulation of vitD receptor in the testis and spermatozoa has been established in the testis and spermatozoa. In both animals and humans, vitamin D deprivation has a detrimental influence on sperm and hormone function [3].

Moderate exercise enhanced adiposity index and sperm function without altering glucose tolerance, indicating a conflict between metabolism and adiposity in determining reproductive failure [4].

2. Materials and Methods

Normal balanced diet (NBD), containing 67% carbohydrates, 10% fat and 23% protein as the energy sources (overall calories 3.6 Kcal/g), and high fat diet (HFD), containing 52% carbohydrates, 30% fat and 18% protein, overall calories 4.8 Kcal/g were prepared [5]. Vitamin D (Vidrop) was purchased from Medical Union Pharmaceuticals (MUP)Egypt, swimming pool (30 × 30 × 90 cm) filled with 32±1°C water.

Research design

A total of 35 adult male albino rats, weighing 150-250 grams and aged 8-10 weeks, were utilized in this investigation. This study compared five groups (7 per each group): normal diet, HFD, HFD with vitamin D, HFD with exercise, HFD with swimming exercise and vitamin D.

Generating rat models with HFD

This study used male albino rats, which aged 8-10 weeks' old weighing 150-250gm BW. Seven rat groups were adapted in a hygienic polypropylene cage for 3 months by controlling light condition (12 h/day) and 22°C ± 0.5°C temperature. The control group received a standard food containing 67% carbohydrates, 10% fat and 23% protein as the energy sources providing (overall calories 3.6 Kcal/g). The HFD groups received a pelleted food, which was adopted from a previous study containing 52% carbohydrates, 30% fat and 18% protein, overall calories 4.8 Kcal/g for 12 weeks.

Administration of vitamin D in Rats with HFD

This group consisted of seven rats receiving HFD mentioned above with the administration of vitamin D (oral gavage 10 ug/kg/day) for 12 weeks orally. [6]

Administration of exercise in rats with HFD

This group consisted of seven rats receiving HFD mentioned above and subjected to swimming exercise sessions (five sessions of 30-min swimming/week) in a pool (30 × 30 × 90 cm) filled with 32±1°C water for 12 weeks.

Administration of vitamin D and exercise in Rats with HFD

This group consisted of seven rats receiving HFD mentioned above and subjected to vitamin D and swimming exercise with same protocol mentioned above.

Measurement of insulin resistance and male fertility parameters

At the end of the experiment, rats were anesthetized for blood collection via retro-orbital venous plexus and tissue and semen collection then euthanized by cervical dislocation. The blood was centrifuged at 1000 g for 10 minutes. The serum was removed and stored at -80-degree C for future analysis. The serum was separated and used for estimation of Glucose, Insulin, Testosterone, FSH, LH, testis was excised to measure antioxidant markers (Glutathione, SOD, MDA and NO).

Data collection and statistical analysis

Data were coded and inputted using "SPSS version 25". Number of instances and relative frequencies (percentages) were used to summarize quantitative data. ANOVA with multiple comparisons post hoc test was used to compare groups. The Pearson correlation coefficient was used to compare quantitative data. Statistical significance was defined as 0.05 or less.

3. Results

Administration of vitamin D and exercise significantly reduced insulin resistance in rats with HFD

The use of vitamin D combined with exercise induction (Group V) attenuated the deleterious effects of HFD on insulin resistance evidenced by significant decrease (p value <0.05) in serum glucose, insulin and HOMA IR as compared to HFD fed group (Group II) as shown in table 1.

Administration of HFD significantly reduced testosterone in rats with HFD

Serum sex hormones (FSH, LH, Testosterone)

As observed in table 2, **induction of HFD in (Group II)** resulted in a significant increase (p value <0.05) in the serum FSH, LH and a significant decrease (p value <0.05) in serum testosterone levels compared to their corresponding values in the **control group (Group I).**

Contrarily to what observed in **Group 2**, supplementation of **vitamin D** alone **(Group III)** or in combination with exercise **(Group V)** alleviated the deleterious effect of **HFD** evidenced by a significant decrease (p value <0.05) in serum FSH and LH levels and significant increase (p value <0.05) in serum testosterone levels.

However, **exercise induction in (Group IV)** resulted in significant decrease (p value <0.05) in FSH levels and a significant increase (p value <0.05) in serum testosterone levels in comparison to their corresponding values in **HFD fed group (Group II),** yet there is no significant difference in LH serum levels.

Administration of Semen analysis (sperm count, morphology and motility)

As shown in table (3), it is observed that induction of HFD in **Group II** was associated with a remarkable deleterious effect on semen analysis evidenced by a significant decrease (P<0.05) in the sperm count, normal sperm morphology and sperm motility compared to its corresponding values in **control group (Group I).**

Remarkably, rats exposed to **vitamin D supplementation and/or exercise (Groups III , IV, V)** showed improve in their semen analysis proven by a significant increase (P<0.05) in sperm count, morphology and motility compared to rats in **HFD group (group II)**.

Testicular oxidative stress markers

Notably, **vitamin D supplementation and/or exercise (Groups III, IV, V)** ameliorates the effect of HFD on testicular oxidative stress markers evidenced by significant decrease (P<0.05) in testicular MDA, NO and a significant increase (P<0.05) in testicular GPX, G reductase and SOD in comparison to its corresponding values in **HFD group (Group II)**, yet there was a substantial reduction (P<0.05) in glutathione levels.

Table (1): mean values of serum glucose, serum insulin and HOMA IR among the studied groups

	Group I Control	Group II HFD	Group III HFD+Vit D	Group IV HFD+exercise	Group V HFD+vitD+exercise
Serum Glucose (mg/dl)	67.71±3.55	157.86±11.85 *	114±10.1 *#	111.57±6.08 *#	79.43±4.58 #$@
Serum Insulin (ng/ml)	5.27±0.45	6.54±0.44 *	6.56±0.68 *	5.87±0.67	4.59±0.43 #$@
HOMA IR	0.88±0.1	2.55±0.23 *	1.85±0.12 *#	1.62±0.24 *#	0.9±0.11 #$@

(Table 1), induction of HFD resulted in a significant increase (p value <0.05) in the serum glucose, insulin and HOMA IR in HFD fed group (Group II), the use of vitamin D combined with exercise induction (Group V) attenuated the deleterious effects of HFD on insulin resistance evidenced by significant decrease (p value <0.05) in serum glucose, insulin and HOMA IR as compared to HFD fed group (Group II).

Table 2: Effect of vit D and exercise on serum FSH, LH and testosterone among the studied group:

	Group I Control	Group II HFD	Group III HFD+vit D	Group IV HFD+exercise	Group V HFD+vitD+exercise
Serum FSH (mlU/ml)	2±0.36	5.87±0.39 *	3.67±0.19 *#	4.99±0.24 *#	2.63±0.32 *#$@
Serum LH (mlU/ml)	2.14±0.39	11.24±1.29 *	6.4±0.62 *#	9.76±1.6 *$	3.3±0.24 #$@
Serum Testosterone (ng/ml)	4.73±0.7	0.88±0.15 *	2.97±0.3 *#	1.59±0.18 *#$	4.53±0.26 *#$@

Table 3: Vit D and exercise ameliorated sperm count, motility, and morphology in comparison to HFD group

	Group I Control	Group II HFD	Group III HFD+vit D	Group IV HFD+ exercise	Group V HFD+vitD+ exercise
sperm count million /ml	15.79±2.23	8.21±1.87 *	11.5±2.25 *#	11.74±1.8 *#	15.64±1.14 #$@
sperm motility%	71.43±9	47.86±6.36 *	66.14±12.01 #	75.29±7.11 #	76.43±7.89 #
sperm morphology%	76.86±3.93	47.86±7.56 *	58.57±6.27 *#	65.86±6.04 *#	74.14±6.72 #$

Table 4: testicular MDA, glutathione, Gpx, G reductase, SOD, and NO among the studied groups.

	Group I Control	Group II HFD	Group III HFD+vit D	Group IV HFD+ exercise	Group V HFD+vitD+ exercise
Testis MDA (nmol/mg ptn)	0.72±0.06	2.35±0.06 *	1.33±0.07 *#	1.69±0.15 *#$	0.54±0.05 *#$@
Testis Glutathione (ng/mg ptn)	6.81±0.39	3.34±1.25 *	8.74±1.26 *#	11.4±1.26 *#$	9.45±0.22 *#$@
Testis Gpx (nmol/min/mg ptn)	31.77±0.67	13.33±1.42 *	22±4.39 *#	19.8±2.06 *#	30.37±1.93 #$@
Testis G Reductase (µg/mg ptn)	3.43±0.31	1.57±0.18 *	2.52±0.26 *#	2.12±0.38 *#	3.34±0.17 #$@
Testis SOD (U/mg ptn)	6.23±0.21	1.91±0.16 *	4.01±0.3 *#	2.73±0.22 *#$	5.56±0.39 *#$@

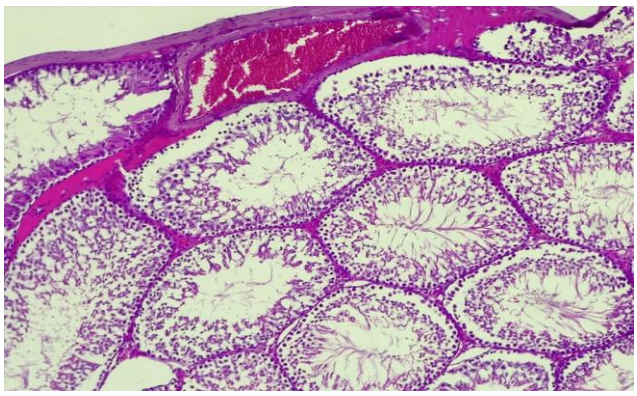

Fig (1) Histology of the testis of the HFD group (Group II) showing seminiferous tubules with interepithelial vacuoles, distortion of the spermatogenic cells. There is congested and dilated blood vessels with celluar infilteration in between the tubules (H&E x 400)

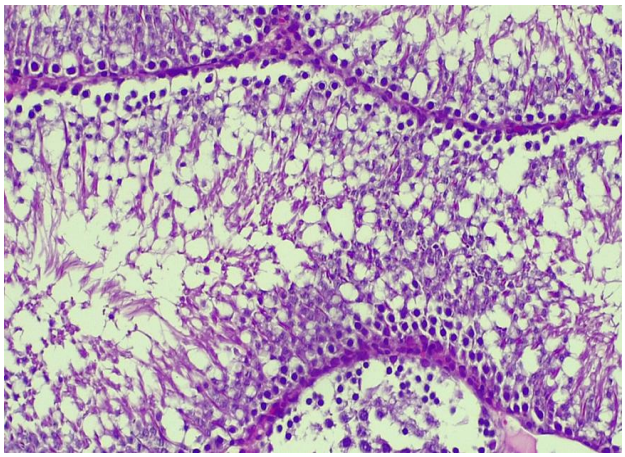

Fig (2) Histology of the testis of HFD + vit D + exercise group (Group V) showing some seminiferous tubules restored there lining spermatogenic cells, the lumen filled with sperms. The tubules are more or less similar to control group (H&E X 400)

4. Discussion

In this study, results showed that 12 weeks of HFD successfully establish the mouse insulin resistance model, and induce negative effects on male rat fertility, where rats were provided with HFD

In this study we have documented the effects of high fat diet on insulin resistance in male rats which agrees with Yan and his colleagues who revealed that a high-fat diet (HFD) induces cellular damage, with the reactive oxygen species (ROS) and AMP triggered protein kinase pathways created by redox processes being strongly linked to insulin resistance [7].

Additionally, it was reported that the effects of vitamin D administration and swimming exercise on serum glucose, insulin resistance, testosterone and semen analysis in male rats with high fat diet. Vitamin D administration and exercise significantly enhanced insulin resistance in rats with HFD which is consistent with Poret and his colleagues who stated that low blood vitamin D was linked to insulin resistance in high fat diets. The possible function of vitamin D supplementation in enhancing glucose homeostasis has excited scientists and physicians worldwide [8].

Furthermore, prior research has demonstrated that SOD, MDA, and GSH-Px may accurately represent the status of oxidative stress in the body. SOD may eliminate damaging free radicals from the body, protect cells from oxygen free radical damage, and quickly repair damaged cells which comes inconsistence with our results as it was observed that HFD induced a change in the oxidative stress parameters[9].

Our study showed that HFD causes an increase in the ROS that induces insulin resistance which agrees with a study done by Bouabout and his colleagues[10]. Our research also found that a long-term HFD reduces the body's antioxidant capacity and leads to insufficient removal of ROS, resulting in an oxidative stress state that increases cell membrane permeability, causing oxidative damage to cells, which is consistent with a similar study on rats' skeletal muscles[9].

A high-fat diet can cause systemic oxidative stress, which can lead to a decrease in testosterone production, spermatogenesis, and sperm quality, as well as testicular and sperm oxidative stress. This study found that rats on a high-fat diet had lower serum testosterone levels and sperm quality indicators, but ROS levels were higher; NO and MDA levels in the testicular tissue were significantly higher, while GSH and SOD activities were significantly lower[11].

Exercise's advantages for weight loss and body fat reduction are well-known, but its effects on male reproductive function are equivocal[12]. While both moderate and high-load exercise reduced body fat, only moderate-load exercise reduced oxidative stress and enhanced male reproductive health[13].

Swimming exercise increases free radicals associated with increased oxygen intake; as a positive adaptive response, it can also stimulate the expression and activity of antioxidant enzymes[14].

On the other hand, other researchers claim that heavy load exercise increases oxygen consumption and produces a significant number of free radicals through diverse methods. Free radicals attack biological macromolecules and membrane structures, producing oxidative damage to the body that may be linked to exercise-induced hypoandrogenemia and sperm quality deterioration[15].

Increases in free radicals, on the other hand, can promote enhanced antioxidant enzyme activity, avoiding cell damage from excessive free radical generation. The impact of this positive adaptation response on male reproductive function is consistent with our findings, which show a substantial rise in blood testosterone levels as well as sperm quality, count, and motility[16].

Insulin resistance and male infertility induced by HFD where vitamin D administration was associated with a decrease in the insulin resistance caused by HFD in the current study disagrees with Gulseth and his colleagues who found no effect of vitamin D supplementation on insulin sensitivity[17].

Previous studies stated that vitamin D use enhanced testicular antioxidant markers as it was associated with significant increase in SOD and glutathione level and activities and significant decrease in MDA for lipid peroxidation[18][19].

5. Conclusion:

Induction of HFD for 12 weeks caused a significant dyslipidemia, elevation in insulin resistance with deterioration in male rats' fertility evidenced by significant decrease in testosterone levels with a deterioration in semen analysis (sperm count, motility and morphology) associated with a reduction in testicular and prostatic antioxidant markers.

Vitamin D supplementation for successive 12 weeks solely ameliorated the deleterious effect of HFD mainly by improving the antioxidant markers activity in testicular and prostatic tissue and enhancing the insulin resistance by decreasing serum glucose levels.

Interestingly, swimming exercise during the 12 weeks of our study had an improving effect on insulin resistance to a great extent and on the male fertility profile to a lesser extent.

The combination of vitamin D administration with exercise gave the optimum results in enhancing the insulin resistance by resetting the HOMA IR results back to the normal values, in addition to ameliorating the deleterious effect of HFD on male fertility profile by improving the semen analysis parameters and the antioxidant markers in testicular and prostatic tissue.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Conflicting Interest (If present, give more details): No Conflict of Interest

No financial disclosure

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Not applicable

-Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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