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Effects of Orlistat on Testicular Redox Markers and Reproductive Parameters in High-Fat-Fed Male Rats

Asmaa T. Z. Taha[1], Hussein Kamil Awad[2], Radwa Donia[1], Salwa M. A. Dahesh[3], Maha Fadaly[4], Eman Sultan[1], Rasha F. Bakry[5], Marwa Bekheet[1], Arafa Ahmed Lotfy[6] and Asmaa Sayed Abd elwahed[1]

[1]National Nutrition Institute, General Organization for Teaching Hospitals and Institutes, Cairo, Egypt.
[2]Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.
[3]Research Institute of Medical Entomology, General Organization for Teaching Hospitals and Institutes, Egypt.
[4]Central Public Health Laboratories, Ministry of Health and Population, Cairo, Egypt.
[5]Pathology Department, Ahmed Maher Teaching Hospital, GOTHI, Cairo, Egypt.
[6]National Heart Institute, General Organization for Teaching Hospitals and Institutes, Cairo, Egypt.

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ABSTRACT

Dietary fat intake can modify the nutritional, hormonal, and oxidative environment supporting male reproduction. Orlistat reduces fat absorption, but its effects on testicular function and sperm characteristics require evaluation alongside these metabolic changes. This study investigated testicular redox markers, serum testosterone, epididymal sperm characteristics, and testicular histology during concurrent high-fat feeding and orlistat administration. Forty male rats were assigned to five groups receiving a standard diet, a high-fat diet, or a high-fat diet with oral orlistat at reported doses of 360, 480, or 600 mg/kg/day for 60 days. Compared with the untreated high-fat group, all orlistat groups showed lower testicular malondialdehyde and thiobarbituric acid-reactive substances, indicating lower measured lipid peroxidation markers. Testosterone decreased progressively across treatment doses, reaching 1.44 ng/mL at the highest dose, compared with 2.50 ng/mL in the high-fat control and 1.43 ng/mL in the standard-diet control. Mean sperm viability declined from 75.6% in the high-fat control to 62.8% at the highest dose, although this remained an exploratory observation. Motility showed no clear between-group difference. Histological appearances varied across doses, with apparently preserved spermatogenic architecture at the highest dose despite lower mean sperm viability. These findings suggest that biochemical, hormonal, and sperm responses to orlistat do not necessarily change in parallel. Altered nutrient availability and endocrine–redox interactions provide a plausible framework for interpreting this pattern. Measurements of energy substrates, sperm ATP, reproductive hormones, and functional fertility are needed to determine the underlying pathways and clarify whether lower lipid peroxidation markers translate into improved reproductive function under comparable conditions.

Keywords: orlistat; high-fat diet; testis; redox markers; sperm viability; testosterone

1. Introduction

Diet-related metabolic change has effects across organ systems. The wider literature links obesity with altered hormonal, inflammatory, and nutrient-signalling environments, including pathways discussed in cancer research (Munteanu and Dhanasekaran, 2026; Pati *et al*., 2023). A prospective cohort examined body size and composition in relation to gastric and oesophageal adenocarcinoma (MacInnis *et al*., 2006), while a population study in women linked dietary and lifestyle factors with

Corresponding Author: Salwa M. A. Dahesh, Research Institute of Medical Entomology, General Organization for Teaching Hospitals and Institutes, Dokki, Giza, Egypt.
E-mail: salwamohamed970@gmail.com, ORCID: 0000-0001-8084-8025

central adiposity (Azadbakht and Esmaillzadeh, 2008). This broader context supports distinguishing dietary exposure, body composition, and metabolic phenotype when examining reproductive outcomes.

Obesity and metabolic disease can alter the hormonal and nutritional environment supporting male reproduction. Clinical and mechanistic reviews describe effects on semen characteristics and reproductive endocrine function (Cannarella *et al*., 2024; Du Plessis *et al*., 2010; Pereira *et al*., 2020). A study of overweight men assessed semen quality together with serum sex hormones (Aggerholm *et al*., 2008), illustrating the value of evaluating these outcomes jointly while retaining their distinct biological meanings.

High-fat feeding can affect male reproduction through changes in testicular redox balance, endocrine function, and sperm characteristics. Rat experiments have documented oxidative injury, inflammation, and apoptosis under high-fat feeding (Suleiman *et al*., 2020a), together with altered reproductive hormones and steroidogenic pathways (Suleiman *et al*., 2020b). These processes offer a biological framework for studying dietary effects, but they do not predict that every reproductive endpoint will respond in the same direction.

Macronutrient composition also warrants consideration independently of adiposity. Experiments in mice have shown that the relative contributions of dietary protein, fat, and carbohydrate differ among reproductive traits (Crean *et al*., 2023). Consequently, sperm motility, membrane viability, circulating testosterone, and tissue architecture should be assessed as distinct outcomes. Changes in one cannot establish the net effect on fertility.

Clinical trials have established orlistat as a treatment that supports weight loss and weight maintenance (Rössner *et al*., 2000). These metabolic effects provide a rationale for examining reproductive responses experimentally, while clinical weight-loss outcomes alone do not establish reproductive efficacy.

Beyond gastrointestinal lipase inhibition, orlistat has been investigated as an inhibitor of fatty acid synthase in experimental oncology (Azadbakht *et al*., 2015; Hao *et al*., 2023). Chuang *et al*. (2011) reported caspase-3-associated apoptosis in a colorectal carcinoma model. These findings identify lipid metabolism and cell-survival pathways as subjects for mechanistic investigation; a response in tumour cells does not establish the corresponding pathway in testicular tissue.

Orlistat, marketed as Xenical, reduces gastrointestinal fat absorption and has been investigated experimentally in high-fat-fed rats. Previous studies reported attenuation of testicular oxidative injury (Suleiman *et al*., 2020a) and improvement of steroidogenic measures (Suleiman *et al*., 2020b), although responses depend on the dietary and treatment conditions. The present study examined three reported orlistat doses administered concurrently with high-fat feeding for 60 days. Its objective was to characterize biochemical, hormonal, sperm, and histological responses and examine whether they changed in parallel. Because treatment began with the diet, the experiment addresses concurrent exposure rather than reversal of established obesity-related reproductive injury.

2. Materials and Methods

2.1. Animals and experimental design

Forty male albino rats, approximately two months old and weighing about 190 g, were acclimatized for two weeks at the Animal House of the National Nutrition Institute. Animals were housed in stainless-steel cages at 22–25 °C under a 12-hour light cycle. Eight rats were allocated to each of five groups: standard diet plus 0.9% saline (Control A); high-fat diet without orlistat (Control B); or high-fat diet plus orlistat at 360, 480, or 600 mg/kg/day (XE-360, XE-480, and XE-600, respectively). Diet exposure and treatment were concurrent for 60 days.

2.2. Diet and drug administration

The experimental diet was prepared by incorporating animal fat into basal feed at a reported proportion of 30%, with the dietary approach attributed to Altunkaynak (2005). Orlistat (Xenical) was purchased from a pharmacy in Diwaniyah. The reported daily doses were 360, 480, and 600 mg/kg body weight, administered orally in 1 mL using a dosing syringe. Distilled water was used as the vehicle. Complete nutrient composition and formulation details were unavailable, as addressed under Limitations.

2.3. Sample collection

At the end of the 60-day experiment, rats were anesthetized with isoflurane and terminal blood samples were collected. Samples were centrifuged at 3,000 rpm for 15 minutes, and serum was stored at −80 °C for testosterone measurement. Testes were excised, weighed, rinsed with saline, and stored at −80 °C for biochemical analyses. Separate tissue portions were fixed in 10% formalin for histological examination. Further details of terminal procedures were not available in the study documentation used for this reanalysis.

2.4. Testicular biochemical measurements

Testicular homogenates were assessed for glutathione S-transferase (GST), malondialdehyde (MDA), the outcome originally labelled reactive oxygen species (ROS), peroxiredoxin, thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione (GSH). The reported TBARS method used a tetramethoxypropane standard (Draper and Hadley, 1990). SOD, GPx, and GST were measured using colorimetric kits (Bio Diagnostic, Egypt), following the reported manufacturer instructions. After protein precipitation, GSH was assessed using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at 412 nm. The documentation attributes the ROS-labelled outcome to Erel's (2005) method, which measures total oxidant status. The original label is retained for traceability and does not identify individual ROS. Incomplete MDA-specific procedures, assay normalization, and characterization of the peroxiredoxin measurement are addressed under Limitations.

2.5. Epididymal sperm assessment

Epididymal sperm samples were evaluated using computer-assisted sperm analysis and an Olympus microscope. Reported outcomes were motility, viability, and abnormalities of the neck, tail, and head. Two hundred spermatozoa per rat were assessed morphologically using the classification approach attributed to Wyrobek and Bruce (1975). The cited method was developed in mice; rat-specific adaptations and complete viability and motility protocols were not available. These reporting limitations are considered when interpreting the sperm outcomes.

2.6. Serum testosterone

Serum testosterone was measured using a DRG International ELISA kit according to the reported manufacturer protocol. Kit identification, analytical precision, and sampling-time details were unavailable.

2.7. Testicular histology

Formalin-fixed testicular specimens were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin using the reported histological approach (Drury and Wallington, 1980). Histological findings were evaluated qualitatively. Animal-level scores, morphometry, and documentation of blinded assessment were unavailable.

2.8. Statistical analysis

Results are presented as mean ± standard deviation (SD), with eight rats per group. Statistical analyses used the group means, SDs, and sample sizes. The animal was the experimental unit.

Exploratory analyses were performed from the available group summaries, treating the animal as the experimental unit. Welch's one-way ANOVA compared the five groups without pooling variances. Holm adjustment covered 14 endpoint-level omnibus tests. Games–Howell comparisons were calculated for all ten group pairs within each analyzed endpoint, with approximate 95% simultaneous confidence intervals. Selected diet-versus-control and treatment-versus-high-fat contrasts are reported in the statistical supplement. The pairwise adjustment applies within each endpoint; findings in endpoints that did not pass Holm screening are hypothesis-generating.

Analyses were conditional on eight independent observations per group and used no reconstructed or imputed animal-level data. Nonsignificance was not interpreted as equivalence. Calculations used Python, NumPy, and SciPy 1.17.0. The consequences of unavailable individual data and source output are described under Limitations.

3. Results

3.1. Testicular biochemical markers

Group means are presented in Tables 1 and 2, with exploratory omnibus results in Table 4. GST, MDA, the reported ROS measure, TBARS, and testosterone retained evidence of between-group differences after Holm adjustment across the 14 analyzed endpoints (all adjusted $P < 0.001$). SOD, GPx, GSH, peroxiredoxin, viability, and the three morphology categories did not pass that adjustment.

Mean MDA was 36.98 nmol/g in the standard-diet control and 37.99 nmol/g in the high-fat diet control. The high-fat minus standard-diet difference was 1.01 nmol/g (Games–Howell $P = 0.362$). Compared with the high-fat diet control, MDA was lower at 360, 480, and 600 mg/kg/day by 2.29, 3.24, and 3.19 nmol/g, respectively (within-endpoint adjusted $P = 0.008$, <0.001, and <0.001). TBARS was higher in the high-fat diet control than in the standard-diet control and lower in all three treated groups than in the high-fat diet control (all selected pairwise $P \leq 0.002$). At the highest dose, the TBARS mean was 1.40 compared with 1.34 in the standard-diet control in the reported units.

GST was lower than the high-fat diet control at 360 and 600 mg/kg/day (Games–Howell $P < 0.001$ and $P = 0.013$), but evidence for the 480 mg/kg/day contrast was insufficient ($P = 0.748$). The reported ROS mean at 480 mg/kg/day was higher than the high-fat diet control by 0.56 units (approximate 95% simultaneous CI 0.41 to 0.71; $P < 0.001$), whereas the other treatment-versus-high-fat contrasts were not supported. The ROS result was sensitive to the variance model: pooled-variance ANOVA gave $P = 0.065$, whereas Welch ANOVA gave $P < 0.001$, reflecting the large differences in reported precision. The assay interpretation is constrained by the oxidant-status method described above. Lower SOD and GPx means in treated groups were descriptive; their unadjusted Welch P values were 0.345 and 0.171. GSH and peroxiredoxin showed nominal omnibus differences that did not remain after Holm adjustment.

Table 1: Testicular GST MDA ROS and peroxiredoxin

Group	GST	MDA (nmol/g)	ROS	Peroxiredoxin
Control A	20.07 ± 2.40	36.98 ± 0.99	15.95 ± 0.28	3.90 ± 0.113
Control B	22.82 ± 0.48	37.99 ± 1.13	15.71 ± 0.06	3.74 ± 0.085
XE-360	17.44 ± 0.65	35.70 ± 1.10	15.40 ± 1.13	3.44 ± 0.396
XE-480	19.79 ± 7.07	34.75 ± 1.02	16.27 ± 0.11	3.37 ± 0.651
XE-600	12.11 ± 6.42	34.80 ± 0.96	16.38 ± 1.13	3.65 ± 0.424

Values are mean ± SD (n = 8 rats/group). Control A, standard diet; Control B, high-fat diet; XE, high-fat diet plus orlistat at the stated dose in mg/kg/day. See Table 4 and the statistical supplement for between-group comparisons.

Table 2: Testicular biochemical markers and serum testosterone

Group	TBARS (mmol/mg)	SOD (U/mg)	GPx (mmol/min/ml)	GSH (mg/dl)	Serum testosterone (ng/ml)
Control A	1.34 ± 0.12	10.28 ± 4.10	51.89 ± 13.21	185.17 ± 8.43	1.43 ± 0.057
Control B	3.40 ± 0.16	11.35 ± 9.90	50.93 ± 16.12	206.19 ± 14.09	2.50 ± 0.170
XE-360	2.90 ± 0.22	8.30 ± 7.01	48.85 ± 12.87	198.18 ± 11.17	1.80 ± 0.141
XE-480	2.60 ± 0.13	8.20 ± 4.10	45.79 ± 12.73	190.13 ± 10.97	1.60 ± 0.113
XE-600	1.40 ± 0.17	5.90 ± 4.10	40.56 ± 6.02	188.13 ± 15.75	1.44 ± 0.085

Values are mean ± SD (n = 8 rats/group). Control A, standard diet; Control B, high-fat diet; XE, high-fat diet plus orlistat at the stated dose in mg/kg/day. See Table 4 and the statistical supplement for between-group comparisons.

Table 3: Epididymal sperm characteristics

Group	Sperm motility(%)	Sperm viability (%)	Neck (%)	Tail (%)	Head (%)
Control A	90.1 ± 12.64	73.8 ± 2.26	1.6 ± 3.68	7.1 ± 8.77	2.6 ± 3.11
Control B	95.3 ± 9.55	75.6 ± 4.70	3.5 ± 4.53	9.2 ± 11.60	6.7 ± 13.01
XE-360	87.3 ± 18.10	72.6 ± 3.85	2.5 ± 4.81	12.2 ± 15.84	8.8 ± 13.29
XE-480	85.6 ± 15.27	67.6 ± 6.17	2.4 ± 4.24	10.3 ± 18.38	7.7 ± 12.45
XE-600	84.5 ± 12.16	62.8 ± 8.15	2.3 ± 3.96	9.3 ± 18.67	4.3 ± 11.60

Values are mean ± SD (n = 8 rats/group). Control A, standard diet; Control B, high-fat diet; XE, high-fat diet plus orlistat at the stated dose in mg/kg/day. See Table 4 and the statistical supplement for between-group comparisons.

Table 4: Exploratory omnibus comparisons from reported summaries

Endpoint	Welch F	Degrees of freedom	P	Holm P
GST	82.18	4, 15.95	<0.001	<0.001
MDA	12.93	4, 17.48	<0.001	<0.001
Reported ROS	35.72	4, 15.41	<0.001	<0.001
Peroxiredoxin	4.58	4, 16.11	0.012	0.093
TBARS	276.75	4, 17.33	<0.001	<0.001
SOD	1.20	4, 17.20	0.345	1.000
GPx	1.83	4, 16.66	0.171	1.000
GSH	3.70	4, 17.29	0.024	0.166
Testosterone	72.95	4, 16.79	<0.001	<0.001
Motility	1.11	4, 17.30	0.385	1.000
Viability	4.95	4, 16.60	0.008	0.074
Neck abnormalities	0.19	4, 17.46	0.939	1.000
Tail abnormalities	0.17	4, 17.07	0.953	1.000
Head abnormalities	0.74	4, 15.33	0.576	1.000

Welch ANOVA compares the five groups using their means, SDs, and sample sizes. Holm adjustment covers 14 endpoint-level tests. The animal was the experimental unit (n = 8/group). The ROS-labelled endpoint is interpreted as an aggregate oxidant-status measure, as described in Methods.

3.2. Serum testosterone

Mean serum testosterone (mean ± SD) was 1.43 ± 0.057 ng/mL in the standard-diet control and 2.50 ± 0.170 ng/mL in the high-fat diet control. The difference was 1.07 ng/mL (approximate 95% simultaneous CI 0.85 to 1.29; Games–Howell $P < 0.001$). All treated groups had lower means than the high-fat diet control (all selected pairwise $P < 0.001$). Means at 360 and 480 mg/kg/day remained above the standard-diet mean in within-endpoint comparisons ($P < 0.001$ and $P = 0.022$). At 600 mg/kg/day, the mean was 1.44 ± 0.085 ng/mL; its difference from the standard-diet control was 0.01 ng/mL (approximate 95% simultaneous CI −0.10 to 0.12; $P = 0.999$). These findings indicate numerical proximity at the highest dose without establishing equivalence or physiological normalization.

3.3. Epididymal sperm characteristics

Mean motility was 90.1% in the standard-diet control, 95.3% in the high-fat diet control, and 87.3%, 85.6%, and 84.5% in the treated groups. Welch ANOVA did not support a between-group difference ($F = 1.106$; df = 4, 17.298; $P = 0.385$; Holm-adjusted $P = 1.000$). The higher high-fat control mean therefore remains a descriptive observation.

Mean viability was 73.8% and 75.6% in the two controls and 72.6%, 67.6%, and 62.8% with increasing orlistat dose. The unadjusted Welch omnibus P value was 0.008, whereas the Holm-adjusted value was 0.074. The highest-dose versus high-fat control contrast was −12.80 percentage points (approximate 95% simultaneous CI −23.52 to −2.08; Games–Howell $P = 0.018$). Because the endpoint did not pass across-endpoint screening, this pairwise finding is exploratory rather than confirmatory. The high-fat versus standard-diet viability contrast was not supported ($P = 0.860$). No clear omnibus

differences were detected for neck, tail, or head abnormalities (unadjusted Welch P = 0.939, 0.953, and 0.576, respectively); wide uncertainty precludes concluding that morphology was unaffected.

3.4. Qualitative testicular histology

Qualitative histological examination indicated preserved architecture in the standard-diet control and seminiferous tubular degeneration in the high-fat diet control. Less pronounced atrophy was described at 360 mg/kg/day, disorganized germinal layers and necrosis at 480 mg/kg/day, and apparently preserved spermatogenic architecture at 600 mg/kg/day. The pattern was nonuniform across doses. No quantitative between-group histological test was performed because animal-level scores and morphometric data were unavailable.

Histological figures

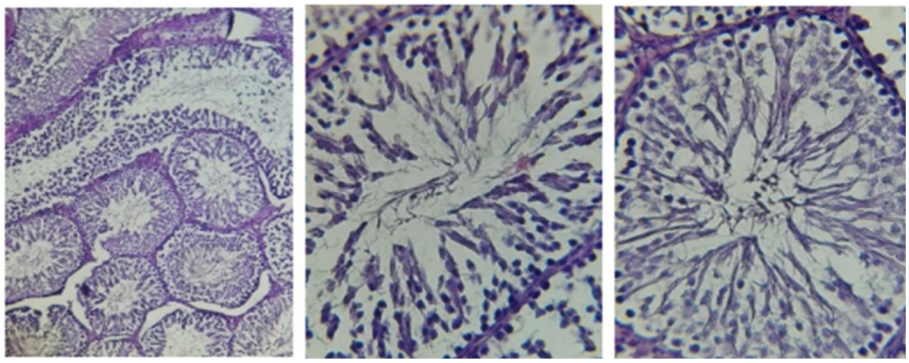


Fig. 1: Control A. Preserved testicular architecture with Leydig cells, spermatocytes, seminiferous epithelium, and spermatids. Hematoxylin and eosin staining.

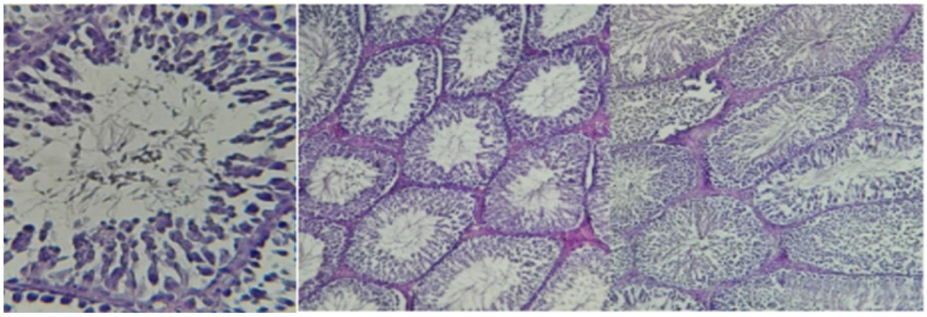


Fig. 2: Control B. Seminiferous tubular degeneration with variable tubular size and shape, reduced germ-cell layers, and widened lumina. Hematoxylin and eosin staining.

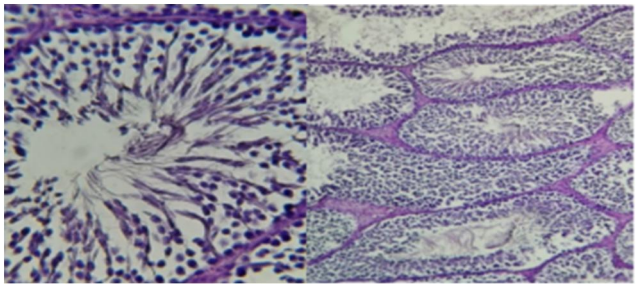


Fig. 3: XE-360. Less pronounced seminiferous tubular atrophy in the group receiving orlistat at 360 mg/kg/day during high-fat feeding. Hematoxylin and eosin staining.

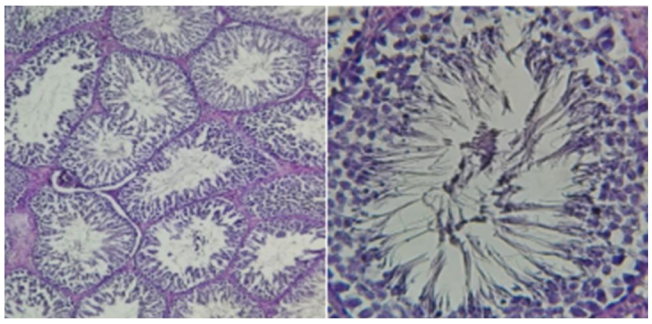


Fig. 4: XE-480. Disorganized germ cells, reduced germinal layers, and necrosis as described in the histological assessment of the 480 mg/kg/day group. Hematoxylin and eosin staining.

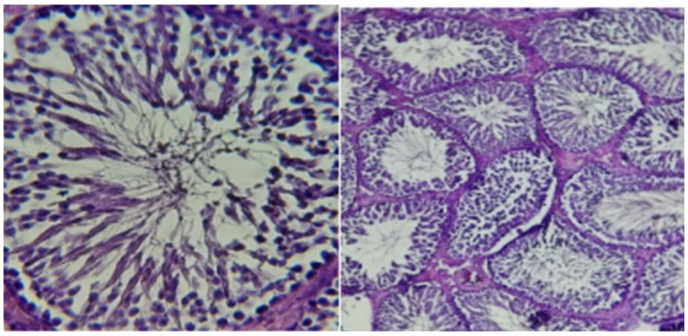


Fig. 5: XE-600. Apparently preserved seminiferous tubules with spermatogenic layers and mature sperm in the 600 mg/kg/day group. Hematoxylin and eosin staining.

4. Discussion

4.1. Principal findings and biological interpretation

The findings indicate that reproductive responses to high-fat feeding and concurrent orlistat administration differed among endpoints. Compared with the untreated high-fat diet group, all three treated groups had lower MDA and TBARS and lower serum testosterone, whereas sperm viability showed an exploratory downward pattern and the qualitative histological appearances were nonuniform (Tables 1–4; Figures 1–5). The strongest statistical evidence concerned selected biochemical markers and testosterone. Viability did not retain omnibus significance after adjustment across endpoints, and motility showed no supported omnibus difference in the summary-level analysis. These distinctions define the evidence available for the biological interpretation.

A plausible working explanation is that diet and treatment influenced nutrient handling, endocrine regulation, and local redox conditions to different degrees. Such responses could affect sperm movement, membrane integrity, and tissue architecture differently. The proposed scenario accommodates divergence among endpoints, but the intervening metabolic pathways were not measured. It should therefore be regarded as a framework for testing mechanisms.

4.2. Diet composition and the proposed energy-substrate scenario

Mean motility was higher in the high-fat diet control than in the standard-diet control (95.3% versus 90.1%), while viability differed little between these controls (75.6% versus 73.8%). The motility omnibus comparison was nonsignificant ($P = 0.385$). The higher mean is therefore descriptive and does not establish stimulation of sperm activity. Nevertheless, trait-specific dietary responses have experimental precedent. In mice, Crean *et al*. (2023) found that dietary macronutrient balance affected reproductive traits differently and reported a modest positive association of dietary fat with total motility. A separate mouse study found greater motility but lower sperm count with low-fat, high-carbohydrate diets (Crean *et al*., 2024). Together, these findings support considering nutrient balance and individual sperm traits separately; they do not establish that high-fat feeding generally improves reproduction.

Diet and activity studies also illustrate why movement and tissue outcomes need separate interpretation. Nematollahi *et al*. (2019) assessed sperm function, testicular morphology, and sperm lipid peroxidation in obese and nonobese mice exposed to dietary and exercise interventions; the

responses differed among endpoints. In another high-fat-fed mouse model that included an orlistat comparator, Wu *et al*. (2022) reported that sperm motility in the orlistat group did not differ significantly from the untreated high-fat group. These findings support interpreting each functional outcome within its own experimental context.

The suggested energy-substrate mechanism is biologically plausible but remains untested here. In rats, supplementation with particular dietary oils altered sperm functional and energetic measures, indicating that fatty-acid composition deserves consideration alongside total fat exposure (Ferramosca *et al*., 2017). More directly relevant to orlistat, Suleiman *et al*. (2020c) reported changes in testicular glucose- and lactate-transporter expression and intratesticular substrate concentrations during concurrent orlistat treatment in high-fat-fed rats. That study supports investigating nutrient transport and utilization as a possible pathway; its intratesticular findings do not demonstrate increased energy supply to epididymal sperm in the present experiment.

The proposed measurements also have an experimental basis beyond drug treatment. Fayyazian *et al*. (2025) jointly assessed testicular glucose, lactate, redox markers, and testosterone in rats exposed to diet and exercise interventions. Applying an integrated measurement strategy of this kind would help test whether substrate handling accompanies the endocrine and redox pattern observed here.

Fructose is one candidate substrate for future testing, rather than an established explanation for the observed means. The reported diet contained added animal fat, but increased sugar intake was not documented. Neither fructose nor glucose, lactate, ATP, or mitochondrial activity was measured in reproductive samples. Moreover, the sperm examined were epididymal, before mixing with accessory-gland secretions. Rat experiments have shown that added substrates can stimulate sperm respiration without necessarily activating movement (Murdoch *et al*., 1999). Consequently, substrate availability, substrate utilization, and motility activation should be tested separately. The current findings are compatible with a broader energy-availability hypothesis, but do not identify fructose as its mediator.

4.3. Lipid peroxidation and antioxidant responses

Lower MDA and TBARS in all treated groups than in the high-fat diet control constitute the clearest treatment-associated redox pattern. However, the two untreated controls did not differ clearly in MDA, whereas TBARS was higher with high-fat feeding. The treatment findings therefore support lower measured lipid peroxidation markers relative to the high-fat comparator, with stronger evidence for a diet-associated increase in TBARS than in MDA. Incomplete documentation of the analytical relationship between MDA and TBARS limits treating these outcomes as independent evidence of a single mechanism.

The antioxidant-enzyme findings further qualify this interpretation. GST was lower than the high-fat diet control at 360 and 600 mg/kg/day, but the intermediate-dose contrast was not supported. Lower mean SOD and GPx activities did not have supported omnibus differences, and the GSH and peroxiredoxin findings did not remain after adjustment across endpoints. A lower enzyme activity could reflect altered regulation or oxidative demand, or diminished defense capacity; these measurements cannot distinguish those possibilities. The higher reported ROS measure at 480 mg/kg/day also argues against describing a uniform reduction of oxidative stress, particularly because its inference was sensitive to the variance model and the cited assay measures total oxidant status (Erel, 2005).

GST participates in glutathione-dependent conjugation reactions, providing a functional context for interpreting its activity alongside other redox measures (Habig *et al*., 1974). MDA is discussed as a lipid-peroxidation marker across several disease settings, including allergy-oriented research (Cordiano *et al*., 2023). Its broader use supports interpreting the present MDA findings as a biochemical outcome, while testicular function and sperm competence require their own measurements.

Previous rat experiments found that orlistat reduced testicular oxidative injury, inflammation, and apoptosis under high-fat feeding (Suleiman *et al*., 2020a). This provides a relevant comparison for the lower lipid peroxidation markers, while the present enzyme pattern does not reproduce a general enhancement of antioxidant defenses. That earlier work used 10 mg/kg/day and preventive or delayed-treatment schedules (Suleiman *et al*., 2020a), whereas the present study reports concurrent administration at 360–600 mg/kg/day. Dose, duration, dietary formulation, and metabolic phenotype limit direct comparison. Inflammation and apoptosis were not measured here, so they remain candidate pathways rather than demonstrated mediators.

Earlier work by Galaly *et al*. (2014) examined orlistat alongside a herbal intervention in high-fat-fed rats and reported improvements in selected testicular and oxidative-stress measures. Chen *et al*. (2013) assessed antioxidant measures and sperm outcomes during probiotic supplementation in high-fat-fed rats, showing the value of linking reproductive function with the local redox environment. In obese patients, Yesilbursa *et al*. (2005) investigated lipid peroxidation during orlistat-associated weight reduction. The human findings provide systemic metabolic context; they do not establish the testicular or sperm-specific mechanism in this rat experiment.

4.4. Testosterone in relation to both dietary controls

The endocrine result should be interpreted against both controls. Testosterone was highest in the untreated high-fat diet group (2.50 ng/mL), compared with 1.43 ng/mL in the standard-diet group. Treatment means declined across the reported doses to 1.80, 1.60, and 1.44 ng/mL. Thus, the treatment-associated decrease relative to high-fat feeding does not establish testosterone deficiency relative to the standard-diet condition. At the highest dose, the mean was numerically close to the standard-diet value, although the nonsignificant comparison does not establish physiological equivalence. Neither the higher high-fat control value nor numerical proximity to the standard-diet value alone demonstrates superior reproductive function.

Exposure conditions are particularly relevant when comparing hormonal and sperm responses. Sabik *et al*. (2022) administered orlistat at 500 mg/kg/day to adult rats and evaluated outcomes after 45 and 90 days; at 90 days they reported lower testosterone and deterioration in selected sperm measures. This provides a comparison at an exposure closer to the reported doses here, while differences in duration and metabolic conditions prevent treating the two experiments as interchangeable. It also supports examining exposure duration alongside the apparent dose pattern.

The direction differs from a rat study in which high-fat-induced obesity lowered circulating and intratesticular reproductive hormones and orlistat improved steroidogenic measures (Suleiman *et al*., 2020b). That study also assessed LH, FSH, steroidogenic proteins, and relevant gene expression (Suleiman *et al*., 2020b), measurements absent here. Its findings identify useful pathways to investigate without overriding the current endocrine pattern. Modulation of the diet-associated hormonal state is a reasonable hypothesis, but suppression of steroidogenesis, altered central regulation, and altered hormone disposition cannot be distinguished from a single serum testosterone measurement.

Other experimental and clinical work also separates metabolic and endocrine outcomes. Abdel-Baky and Abdel-Rahman (2021) evaluated biochemical and hormonal responses to orlistat in high-fat-fed male rats. Ghandi *et al*. (2011) compared orlistat with metformin in obese women with polycystic ovary syndrome. The latter provides clinical endocrine context in a different sex and disorder; the direction of an androgen response must therefore be interpreted against the baseline hormonal condition of each model.

4.5. Sperm viability and histology assess different levels of function

Mean viability declined from 75.6% in the high-fat diet control to 62.8% at the highest dose. The highest-dose pairwise contrast was nominally supported within that endpoint, but the viability omnibus result did not pass Holm adjustment across endpoints (adjusted $P = 0.074$). This is an exploratory signal requiring confirmation. Similarly, the absence of supported morphology differences does not prove that sperm morphology was unaffected. These levels of uncertainty should accompany any explanation of sperm responses.

The apparently preserved testicular architecture at 600 mg/kg/day can coexist with lower mean epididymal sperm viability because qualitative tissue structure and mature-sperm membrane integrity assess different compartments and functions. The sections do not quantify the competence of the epididymal sperm population, and the viability result does not locate the source of a possible defect. The intermediate-dose histological abnormalities and the more preserved highest-dose appearance also preclude a simple monotonic histological dose–response claim. Blinded animal-level scoring and matched sperm functional measurements would clarify this relationship.

4.6. Integrated interpretation and testable predictions

Taken together, the results support a working model in which high-fat feeding and orlistat exposure influence reproductive biology through partially separable metabolic, endocrine, and redox responses.

Altered substrate transport or utilization could contribute to sperm movement; changes in the hormonal environment could influence testicular function; and lower lipid peroxidation markers could occur without proportional improvement in mature-sperm viability. This model accommodates the observed pattern but does not demonstrate compensation, causation, or net fertility benefit. It predicts that substrate and ATP measurements would track a true energetic contribution, that LH/FSH and intratesticular steroidogenic measurements would help localize the hormonal change, and that sperm-specific redox and membrane assays would clarify the viability signal. These measurements, together with quantitative histology and fertility outcomes, are the main requirements for testing the proposed internal sequence, as detailed below.

4.7. Limitations

This study evaluated concurrent dietary and drug exposure over 60 days. Longitudinal metabolic measurements, a standard-diet orlistat group, and intake-matched controls were unavailable, limiting separation of dietary, nutritional, and drug effects. Details of randomization, blinding, diet composition, and delivered drug concentrations were incompletely documented. Analysis relied on group summaries, preventing assessment of individual distributions, outliers, and cage effects. The reported doses also differed substantially from those in previous rat experiments, requiring caution in direct comparisons (Suleiman *et al*., 2020a, 2020b, 2020c). The proposed nutritional and endocrine mechanisms require measurements of reproductive substrates, sperm ATP, mitochondrial function, LH, FSH, and intratesticular testosterone. Sugar intake was not documented, and epididymal sampling cannot establish a seminal-fructose mechanism. The ROS-labelled assay represents total oxidant status (Erel, 2005); incomplete assay descriptions and measurement units also constrain biochemical comparisons. GSH/GSSG profiling and defined peroxiredoxin activity measurements would extend redox characterization (Griffith, 1980; Mohsin *et al*., 2025). Complete sperm-assessment protocols were unavailable, and motility exceeding viability requires clarification of assay timing and denominators. Histology was qualitative, without quantitative scoring, documented blinding, or calibrated image scales. Published methods for sperm concentration, acrosomal staining, and steroid assays provide precedents but require validation for the present rat samples (Eljarah *et al*., 2013; Bryan and Akruk, 1977; Nash *et al*., 2000). Functional fertility, sperm DNA integrity, and repeated assessments would help determine the reproductive consequences and reversibility of the observed changes. The findings therefore apply to the studied male-rat conditions and do not establish human reproductive effects.

5. Conclusion

During concurrent high-fat feeding, orlistat was associated with lower MDA and TBARS and a testosterone pattern that approached the standard-diet control at the highest reported dose. These changes did not establish uniform improvement in reproductive function. Sperm viability showed an exploratory downward pattern, motility remained a descriptive finding, and histology was nonuniform across doses. Nutrient availability and endocrine–redox regulation provide testable interpretations, but the missing metabolic, energetic, cellular, and functional measurements prevent identification of a complete mechanistic pathway. The findings support targeted follow-up rather than a general claim of reproductive protection, reproductive toxicity, or human safety.

Abbreviation	Full term
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CASA	Computer-assisted sperm analysis
CI	Confidence interval
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle-stimulating hormone

Abbreviation	Full term
GOTHI	General Organization for Teaching Hospitals and Institutes
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
LH	Luteinizing hormone
MDA	Malondialdehyde
ORCID	Open Researcher and Contributor ID
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
TBARS	Thiobarbituric acid-reactive substances
XE	Xenical (orlistat) treatment group

Declarations

Ethics approval: The animal study was approved by the Ethical Committee of the General Organization for Teaching Hospitals and Institutes (approval number 1N00207, dated 17 December 2025) and conducted at the National Nutrition Institute.

Consent to participate and publish: Not applicable.

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Competing interests: The authors declare no competing interests.

Data availability: Group summaries and statistical results are included in the manuscript and supplementary document. Original animal-level observations and SPSS output were unavailable for this reanalysis.

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