

Proceeding Paper

High-Fat Diet Promotes a Pro-Inflammatory Environment in Testis and Inhibits Antioxidant Defenses in the Progeny [†]

Luís Crisóstomo ¹, Romeu A. Videira ², Ivana Jarak ³, Kristina Starčević ⁴, Tomislav Mašek ⁵, Luís Rato ⁶, João F. Raposo ^{7,8}, Pedro F. Oliveira ⁹ and Marco G. Alves ^{1,*}

- ¹ Department of Anatomy, and Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal; luis.d.m.crisostomo@gmail.com
- ² REQUINTE/LAQV, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; rvideira@ff.up.pt
- ³ Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal; jarak.ivana@gmail.com
- ⁴ Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia; kristina.starcevic@vef.hr
- ⁵ Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia; tomlav.masek@vef.hr
- ⁶ Health School of the Polytechnic Institute of Guarda, 6300-559 Guarda, Portugal; luis.pedro.rato@gmail.com
- ⁷ NOVA Medical School, New University Lisbon, 1169-056 Lisbon, Portugal; joao.raposo@nms.unl.pt
- ⁸ APDP—Diabetes Portugal, 1250-189 Lisbon, Portugal
- ⁹ QOPNA & LAQV, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; pfbbox@gmail.com
- * Correspondence: alves.marc@gmail.com; Tel.: +351-967-245-248
- [†] Presented at the 1st International e-Conference on Antioxidants in Health and Disease, 1–15 December 2020; Available online: <https://cahd2020.sciforum.net/>.



Citation: Crisóstomo, L.; Videira, R.A.; Jarak, I.; Starčević, K.; Mašek, T.; Rato, L.; Raposo, J.F.; Oliveira, P.F.; Alves, M.G. High-Fat Diet Promotes a Pro-Inflammatory Environment in Testis and Inhibits Antioxidant Defenses in the Progeny. *Med. Sci. Forum* **2021**, *2*, 20. <https://doi.org/10.3390/CAHD2020-08623>

Academic Editor: Mihalisa I. Panagiotidis

Published: 30 November 2020

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The adoption of high-fat diets (HFDs) is a major contributor to the increasing prevalence of obesity worldwide. Herein, we study the impact of an HFD from early age in testicular physiology and sperm parameters in two generations of mice, with a focus on testicular oxidative status. Mice of the diet-challenged generation (F0; n = 36) were randomly fed after weaning with standard chow (CTRL), a high-fat diet (HFD) for 200 days, or a transient high-fat diet (HFDt) (60 days of HFD + 140 days of standard chow). The offspring generation (F1; n = 36) was obtained by mating normoponderal females with those fed a chow diet 120 days post-weaning. Mice fed an HFD for a lifetime presented impaired insulin tolerance, a trait inherited by their sons. The sons of mice fed HFD inherited decreased catalase activity and displayed lower activities of mitochondrial complexes I and IV. Similar to their progenitors, the sons of HFD mice had a higher prevalence of pinhead and bent neck defects than that of the sons of the CTRL mice. The adoption of HFD impaired testicular antioxidant defenses and mitochondrial function in the progeny, which is detrimental to sperm morphology.

Keywords: high-fat diet; intergenerational effects; pro-inflammatory state; antioxidant defenses; testis

1. Introduction

Overweight and obesity have achieved epidemic proportions worldwide, mostly due to lifestyle choices, such as low levels of physical activity and the adoption of a high-fat diet (HFD) [1,2]. These numbers raise concerns about the consequences of sexual health in men of reproductive age suffering from excess adiposity and common comorbidities, such as type 2 diabetes (T2D) [3,4]. These concerns were aggravated by recent evidence of the intergenerational effects of acquired traits, i.e., by the evidence that acquired traits can compromise the health of offspring, especially in male reproductive health [5,6].

We have previously described the effects of an HFD, even if temporary, in sperm parameters and testicular composition of mice [7,8]. Hereby we investigate the effects of an HFD on sperm parameters and testicular physiology of the offspring (sons), with a focus on testicular antioxidative status.

2. Experiments

2.1. Animal Model

This study was performed in 2 generations of *Mus musculus* C57BL6/J mice. The first generation (Generation F0) was originated from normoponderal progenitors (both male and female) fed standard chow (#F4031, BioServ, Flemington, NJ, USA—carbohydrate: 61.6%, protein: 20.5%, and fat: 7.2% (16.3% Kcals)) and water ad libitum. After weaning (21–23 days), F0 mice (n = 36) were randomly divided in three groups: control (CTRL) (n = 12), HFD (n = 12), and HFDt (n = 12). The mice from the CTRL group were fed with a standard chow. Mice from the HFD group received a fat-enriched diet (#F3282, BioServ, Flemington, NJ, USA—carbohydrate: 35.7%, protein: 20.5%, and fat: 36.0% (59.0% Kcals)). The mice from the HFDt group were fed a fat-enriched diet for 60 days (#F3282, BioServ, New Jersey, USA) and then switched to standard chow (#F4031, BioServ, NJ, USA). F0 mice were mated at 120 days of age with normoponderal, chow-fed, same-age randomly selected females to generate the F1 generation. Mating lasted for 8 days and consisted of placing a male and a female in the same cage for 6 h each day without water or food supply. After weaning, F1 mice were assigned to the same experimental group as their fathers: CTRL—sons of CTRL (n = 12); HFD—sons of HFD (n = 12); HFDt—sons of HFDt (n = 12). Litters were generated until the target number of mice per group (n = 12) was achieved. In this generation (F1), all mice were fed with standard chow. Food and water were supplied without restrictions. The mating of F1 mice was performed under the same conditions as those used for their progenitors (Generation F0). Mice from both generations were killed by cervical dislocation 200 days after weaning, and tissues were collected for further analysis. Total body weight, water, and food intake were monitored weekly from weaning to sacrifice. The animal model is compliant with the ARRIVE guidelines and was licensed by the Portuguese Veterinarian and Food Department (0421/000/000/2016).

2.2. Glucose Homeostasis Assessment

One week before sacrifice, glucose homeostasis was evaluated using the intraperitoneal glucose tolerance test (ipGTT) and intraperitoneal insulin resistance test (ipITT) according to the previously described protocol [8]. Fasting glucose was measured before sacrifice, as previously described [7,8]. Serum was separated from blood obtained by cardiac puncture at sacrifice, and insulin was measured via ELISA [7]. HOMA2 indexes were calculated based on the serum insulin and glucose at sacrifice, using the HOMA2 Calculator [9].

2.3. Enzymatic Activity of Antioxidant Enzymes and Mitochondrial Complexes

The enzymatic activity of antioxidant enzymes and mitochondrial complexes was evaluated from testicular extracts. A phase separation protocol was used to obtain a mitochondria-rich fraction and a cytosolic, mitochondria-free fraction according to a protocol previously described [7]. Lipid peroxidation was measured in the cytosolic fraction of the testicular extract by the TBARS assay [7]. Enzymatic activities of glutathione peroxidase (GPx), glutathione S-reductase (GSR), and mitochondrial complex I were measured using fluoroscopic methods in 96-well plates, as previously described [7]. Enzymatic activities of superoxide dismutase (SOD), citrate synthase, mitochondrial complex II and mitochondrial complex IV were measured by colorimetric methods in 96-well plates, as previously described [7]. All colorimetric and fluorometric readings were obtained using a Biotek Synergy H1 plate reader (Winooski, VT, USA). Catalase (CAT) activity was polarographically determined following oxygen production resulting from H₂O₂ decomposition using a Clark-type oxygen electrode (Hansatech, Norfolk, UK), as previously described [7].

2.4. Assessment of Sperm Parameters

Sperm was collected from the right epididymis of each mice after sacrifice. Sperm count and motility were immediately assessed as previously described [7,8]. Sperm viability and morphology were evaluated using specific staining techniques and optical microscopy as previously described [7,8].

2.5. Untargeted Metabolomics and Lipidomics

A combined extraction of polar and nonpolar metabolites from testicular tissue (50 mg) was performed, as previously described [7,8]. Two fractions resulted from the method—an aqueous, polar fraction, and an organic, nonpolar fraction. ¹H-NMR was performed to analyze and quantify the metabolites in the polar fraction according to our methods [7,8]. GC-MS was used to analyze and quantify the nonpolar metabolites soluble in the organic fraction [7].

2.6. Statistics

Univariate parametric statistics were the preferred statistical methods. The assumptions of normality and homoscedasticity requested for parametric statistics were tested for each variable using the Kolmogorov–Smirnov test with Lillefor’s correction and Levene’s test, respectively. Univariate ANOVA was corrected for pairwise comparisons by Tukey’s honest significant difference (HSD). ipGTT and ipITT data were tested using repeated-measures (RM) ANOVA corrected by Šidak’s method for pairwise comparisons. Data were previously tested for sphericity using Bartlett’s test. The distribution of sperm defects was tested using the χ^2 test, and column proportions were tested by the Z-test corrected for pairwise comparisons using Bonferroni’s method. The significance cutoff was set when $p < 0.05$. All methods were performed using IBM SPSS Statistics v26 (Armonk, NY, USA).

3. Results

3.1. The Offspring of HFD-Fed Mice Display Abnormal Insulin Tolerance

Glucose homeostasis was assessed by ipGTT, ipITT, and HOMA2 indexes. The sons of the mice fed a lifelong HFD displayed higher serum glucose at 90 and 120 min of the ipITT than that of the sons of CTRL and HFDt (Figure 1). Regarding HOMA2 indexes, no differences were found between the sons of diet-challenged mice.

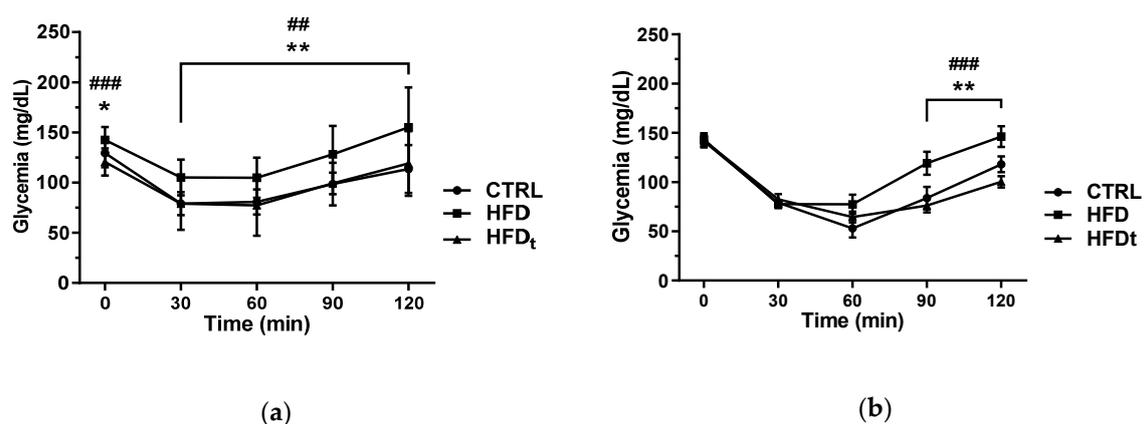


Figure 1. Glycemia during the ipITT in (a) mice fed standard chow (CTRL) and a life-long high-fat diet (HFD), those subjected to diet correction after 60 days (HFDt) (generation F0), and (b) their progeny (generation F1). Results are expressed as the mean (mg/dL) \pm SD in function of time (minutes). Data were tested by two-way ANOVA corrected by Šidak’s method. Significance was considered when $p < 0.05$. * CTRL vs. HFD; # HFD vs. HFDt. * $p < 0.05$; ** $p < 0.01$; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$.

3.2. The Adoption of HFD Inhibits Testicular Antioxidant Defences Even in Offspring

The enzymatic activity of antioxidant enzymes was measured in testes. Mice fed an HFD for a lifetime had decreased activity of GSR and catalase (Figure 2). Interestingly, this phenotype was partially inherited by their sons, which had decreased testicular catalase activity, when compared to the sons of CTRL.

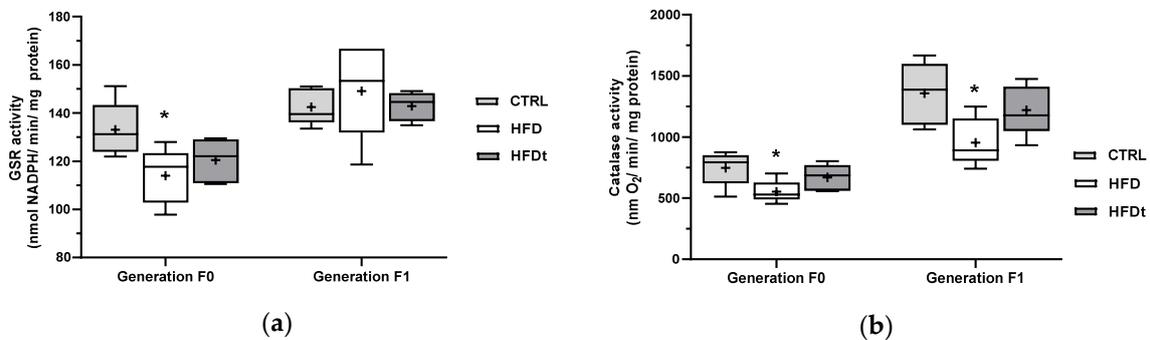


Figure 2. Enzymatic activity of the antioxidant enzymes (a) GSR and (b) catalase in testes of mice fed standard chow (CTRL) and a life-long high-fat diet (HFD), those subjected to diet correction after 60 days (HFDt), and their progeny (generation F1). Results are expressed as mean \pm standard deviation. Experimental groups were compared by one-way ANOVA with Tukey's HSD. Significance was considered when $p < 0.05$. * vs. CTRL; # vs. HFD. *** $p < 0.001$.

3.3. Testicular Mitochondrial Defects Are Only Detected in Offspring of HFD-Fed Mice

The enzymatic activity of mitochondrial complexes was measured in testes. No changes were found between groups of diet-challenged mice (generation F0) (Figure 3). The sons of lifelong HFD-fed mice showed reduced activity of mitochondrial complex I and complex IV.

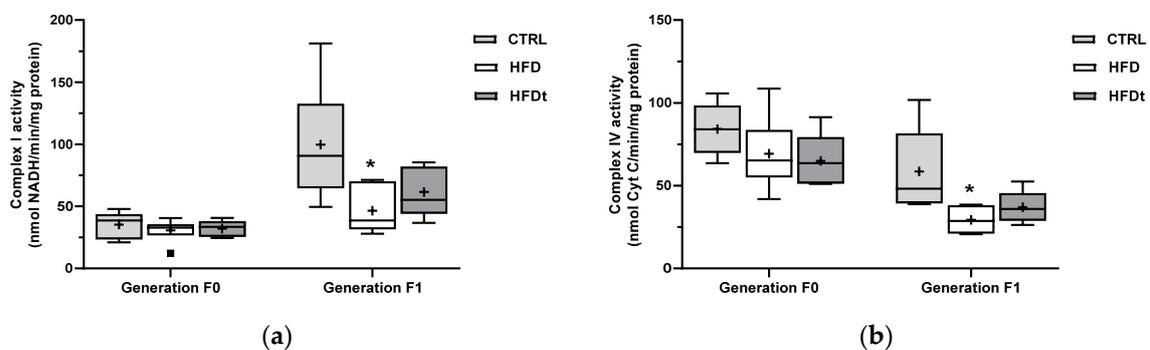


Figure 3. Enzymatic activity of the mitochondrial (a) complex I and (b) complex IV in testes of mice fed standard chow (CTRL) and life-long high-fat diet (HFD), those subjected to diet correction after 60 days (HFDt), and their progeny (generation F1). Results are expressed as mean \pm standard deviation. Experimental groups were compared by one-way ANOVA with Tukey's HSD. Significance was considered when $p < 0.05$. * vs. CTRL; # vs. HFD. * $p < 0.05$.

3.4. Paternal HFD Causes Intergenerational Sperm Defects

Epididymal sperm parameters were evaluated after sacrifice. Sperm count and motility were assessed immediately after collection. Contrary to their progenitors, the sons of HFD and HFDt did not show differences in sperm motility and viability when compared to sons of CTRL. However, regarding sperm morphology, the sons of HFD mice had a greater prevalence of pinhead defects than that of the sons of CTRL and a greater prevalence of bent neck defects than that of the sons of HFDt (Figure 4).

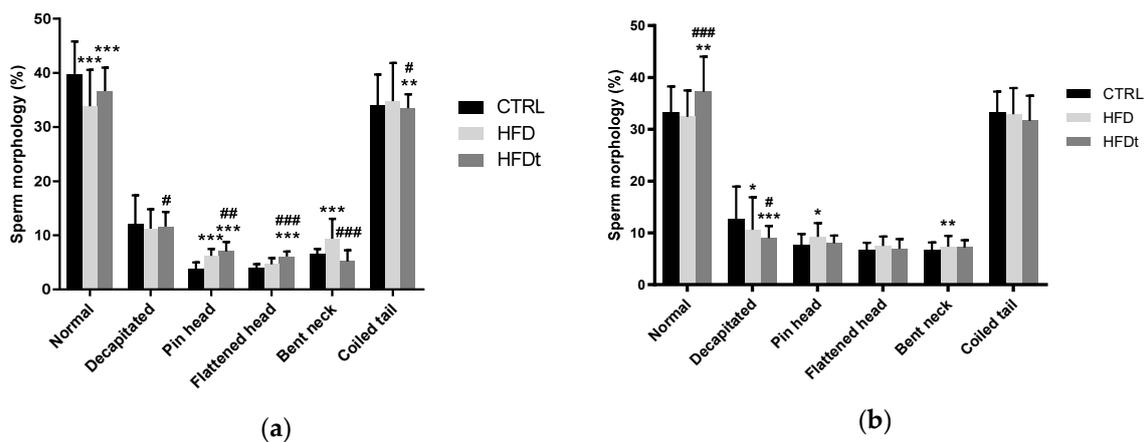


Figure 4. Distribution of sperm morphology per experimental group in the (a) diet-challenged generation (F0) and their offspring (Generation F1). Data are presented as the mean (%) \pm SD. Independence of experimental groups was tested by the χ^2 test, and column proportions were tested with the Z-test corrected for pairwise comparisons using Bonferroni's method. Significance was considered when $p < 0.05$. * vs. CTRL; # vs. HFD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$.

3.5. Testicular Metabolic and Lipidomic Signatures of HFD Are Not Inherited by Direct Offspring

Testicular polar and nonpolar metabolites were extracted and quantified by either $^1\text{H-NMR}$ or GC-MS. However, no significant changes were found in the testicular content of the sons of the diet-challenged mice.

4. Discussion

In this work, we evaluated the impact of an ancestral paternal exposure to an HFD, either lifelong or up to early adulthood, on testicular metabolism and sperm parameters of the direct offspring (sons). To do so, we evaluated the oxidative status, the mitochondrial function, and the metabolite content in the testis of mice whose parents were fed standard chow (CTRL), a lifelong HFD (HFD), or an HFD from weaning to early adulthood and then replaced with standard chow (HFDt).

We also evaluated the whole-body glucose homeostasis to search for cues of metabolic syndrome that may be inherited by the offspring. Indeed, the sons of HFD mice showed signs of insulin intolerance regarding their performance in the ipITT (Figure 1). We previously described that the inhibition of testicular antioxidant enzyme catalase and GSR and the increased testicular content of $\omega 6$ -polyunsaturated fatty acids elicit that a lifelong HFD promotes a pro-inflammatory environment in testis [7]. To balance this HFD-induced inflammation, mice fed with HFD have increased testicular glutathione (GSH) and taurine levels [8]. Similarly to their progenitors, the sons of HFD mice showed lower enzymatic activity of catalase in testes, suggesting an inheritable trait caused by the pro-inflammatory testicular environment of the progenitor. However, no differences in lipid peroxidation in both diet-challenged mice and their sons were found. Notwithstanding, no changes in testicular metabolome were found in the sons of HFD when compared to the sons of CTRL and HFDt. Therefore, it is unclear whether the testicular oxidative balance is being balanced by other mechanisms. This is even more interesting when considering the decrease in the mitochondrial activity of complex I and IV found in the sons of HFD. In contrast, the sons of HFD mice have the highest prevalence of sperm head defects (compared to sons of CTRL) and sperm neck defects (compared to sons of HFDt). Indeed, oxidative damage in the testis has been linked to a higher prevalence of abnormal sperm [10]. Hence, the changes in antioxidant defenses and mitochondrial activity observed in the testis of the sons of HFD mice might be associated with the increase in abnormal sperm.

Interestingly, the differences found in sons of diet-challenged mice in all evaluated parameters are restricted to the sons of HFD. These mice were the only group receiving an

HFD at the moment of conception. Several reports mention that sperm carries non-genomic factors, such as small non-coding RNA (sncRNA) and epigenetic modifications, that are sensitive to diet [11,12]. Therefore, the phenotypes observed in the sons of HFD mice are likely manifestations of intergenerational effects of an HFD; i.e., they are the result of direct exposure of the male gamete to the toxicant (HFD) [13].

5. Conclusions

The adoption of HFD by fathers causes intergenerational signatures in testis, notably in antioxidant defenses and mitochondrial activity. Those signatures, in turn, are associated with a higher prevalence of sperm head and neck defects. The negative impact of paternal HFD is more evident if it is continued at the moment of conception.

Author Contributions: L.C., P.F.O., M.G.A., and R.L.B. contributed to study design, analysis, and interpretation of data. L.R., I.J., K.S., T.M., R.A.V., and L.C. performed experimental work. L.C. edited the images and tables, performed the statistics, and contributed to the analysis and interpretation of data. J.F.R., and R.A.V. critically reviewed the manuscript and suggested modifications. All of the authors contributed to manuscript writing/editing and approved the final version.

Institutional Review Board Statement: The animal model is compliant with the ARRIVE guidelines and was licensed by the Portuguese Veterinarian and Food Department (0421/000/000/2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data included in this report is available upon reasonable request.

Acknowledgments: This work was supported by the Portuguese Foundation for Science and Technology: L. Crisóstomo (SFRH/BD/128584/2017), M.G. Alves (IFCT2015 and PTDC/MEC-AND/28691/2017), P.F. Oliveira (IFCT2015), UMIB (UID/Multi/00215/2019), and QOPNA (UID/QUI/00062/2019) co-funded by FEDER funds (POCI/COMPETE 2020); by the Portuguese Society of Diabetology: L. Crisóstomo and M.G. Alves (“Nuno Castel-Branco” research grant and Group of Fundamental and Translational Research); and by the Croatian Science Foundation: K. Starčević (IP-2016-06-3163). NMR data were collected at the UC-NMR facility, which is supported in part by FEDER—European Regional Development Fund through the COMPETE Programme (Operational Programme for Competitiveness) and by National Funds through FCT—Fundação para a Ciência e a Tecnologia (Portuguese Foundation for Science and Technology) through grants REEQ/481/QUI/2006, RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012, and Rede Nacional de Ressonância Magnética Nuclear (RNRMN).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

¹ H-NMR	proton nuclear magnetic resonance
ANOVA	analysis of variance
CTRL	standard diet (standard chow)
GC-MS	gaseous chromatography–mass spectroscopy
GPx	glutathione peroxidase
GSH	glutathione
GSR	glutathione S-reductase
HFD	high-fat diet
HFDt	transient high-fat diet
HSD	honest significant difference
RM	repeated measures
SD	standard deviation
sncRNA	small non-coding RNA
SOD	superoxide dismutase

References

1. World Health Organization. *Global Status Report on Noncommunicable Diseases 2014*; WHO Press: Geneva, Switzerland, 2014; p. 280.
2. Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res. Clin. Pract.* **2019**, *157*, 107843. [[CrossRef](#)] [[PubMed](#)]
3. Imani, M.; Talebi, A.R.; Fesahat, F.; Rahiminia, T.; Seifati, S.M.; Dehghanpour, F. Sperm parameters, DNA integrity, and protamine expression in patients with type II diabetes mellitus. *J. Obstet. Gynaecol.* **2021**, *41*, 439–446. [[CrossRef](#)] [[PubMed](#)]
4. Salas-Huetos, A.; Maghsoumi-Norouzabad, L.; James, E.R.; Carrell, D.T.; Aston, K.I.; Jenkins, T.G.; Becerra-Tomás, N.; Javid, A.Z.; Abed, R.; Torres, P.J.; et al. Male adiposity, sperm parameters and reproductive hormones: An updated systematic review and collaborative meta-analysis. *Obes. Rev.* **2021**, *22*. [[CrossRef](#)] [[PubMed](#)]
5. Pavlinkova, G.; Margaryan, H.; Zatecka, E.; Valaskova, E.; Elzeinova, F.; Kubatova, A.; Bohuslavova, R.; Peknicova, J. Transgenerational inheritance of susceptibility to diabetes-induced male subfertility. *Sci. Rep.* **2017**, *7*, 4940. [[CrossRef](#)] [[PubMed](#)]
6. Craig, J.R.; Jenkins, T.G.; Carrell, D.T.; Hotaling, J.M. Obesity, male infertility, and the sperm epigenome. *Fertil. Steril.* **2017**, *107*, 848–859. [[CrossRef](#)] [[PubMed](#)]
7. Crisóstomo, L.; Videira, R.A.; Jarak, I.; Starčević, K.; Mašek, T.; Rato, L.P.; Raposo, J.F.; Batterham, R.L.; Oliveira, P.F.; Alves, M.G. Diet during early life defines testicular lipid content and sperm quality in adulthood. *Am. J. Physiol. Metab.* **2020**, *319*, E1061–E1073. [[CrossRef](#)] [[PubMed](#)]
8. Crisóstomo, L.; Rato, L.; Jarak, I.; Silva, B.M.; Raposo, J.F.; Batterham, R.L.; Oliveira, P.F.; Alves, M.G. A switch from high-fat to normal diet does not restore sperm quality but prevents metabolic syndrome. *Reproduction* **2019**, *158*, 377–387. [[CrossRef](#)] [[PubMed](#)]
9. Levy, J.C.; Matthews, D.R.; Hermans, M.P. Correct Homeostasis Model Assessment (HOMA) Evaluation Uses the Computer Program. *Diabetes Care* **1998**, *21*, 2191–2192. [[CrossRef](#)] [[PubMed](#)]
10. Oliveira, P.F.; Tomás, G.D.; Dias, T.R.; Martins, A.D.; Rato, L.; Alves, M.G.; Silva, B.M. White tea consumption restores sperm quality in prediabetic rats preventing testicular oxidative damage. *Reprod. Biomed. Online* **2015**, *31*, 544–556. [[CrossRef](#)] [[PubMed](#)]
11. Nätt, D.; Kugelberg, U.; Casas, E.; Nedstrand, E.; Zalavary, S.; Henriksson, P.; Nijm, C.; Jäderquist, J.; Sandborg, J.; Flinke, E. Human sperm displays rapid responses to diet. *PLoS Biol.* **2019**, *17*, e3000559. [[CrossRef](#)]
12. Watkins, A.J.; Dias, I.; Tsuro, H.; Allen, D.; Emes, R.D.; Moreton, J.; Wilson, R.; Ingram, R.J.M.; Sinclair, K.D. Paternal diet programs offspring health through sperm- and seminal plasma-specific pathways in mice. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 10064–10069. [[CrossRef](#)] [[PubMed](#)]
13. Nilsson, E.; Ben Maamar, M.; Skinner, M.K. Chapter 2—Definition of epigenetic transgenerational inheritance and biological impacts. In *Transgenerational Epigenetics*, 2nd ed.; Tollefsbol, T.O., Ed.; Academic Press: Cambridge, MA, USA, 2019; Volume 13, pp. 13–24.