

Exploring the effect of exercise training on testicular function

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ACKNOWLEDGMENTS

We are thankful to the Portuguese Foundation for Science and Technology (FCT), European Union, QREN, FEDER and COMPETE for funding the iBiMED (UID/BIM/04501/2013 and POCI-01-0145-FEDER-007628), QOPNA (UID/QUI/00062/2013) and CIAFEL (UID/DTP/00617/2013) research units, and the research project RUNawayPCa (POCI-01-0145-FEDER-006958 and PTDC/DTP-DES/6077/2014).

ABSTRACT

Purpose: The impact of exercise training on testicular function is relatively ill-defined. To gain new insights into this important topic, published data, deriving from both human and animal studies, were critically analyzed.

Results and Conclusions: The effects of exercise on the hypothalamus–pituitary–gonadal axis, influenced by the type, intensity and duration of the exercise program, can be evaluated in terms of total and free testosterone and/or luteinizing hormone (LH), serum levels of follicle-stimulating hormone (FSH) and sperm parameters. High intensity exercise promotes a common decrease in these parameters and so negatively impacts upon testicular function. However, published data for moderate-intensity exercise training are inconsistent. Conversely, there is consistent evidence to support the benefits of exercise training to prevent and/or counteract the impairment of testis function caused by aging, obesity and doxorubicin treatment. This positive effect is

likely the consequence of decreased oxidative stress and inflammatory status. In the future it will be important to clarify the molecular mechanisms which explain these reported discrepancies and to establish guidelines for an active lifestyle to promote healthy testicular function.

Keywords: physical activity, hypothalamus–pituitary–gonadal axis, testosterone, aging, obesity.

LIST OF ABBREVIATIONS

3 β -HSD - 3 β -hydroxysteroid dehydrogenase
17 β -HSD - 17 β -hydroxysteroid dehydrogenase
BMI - body mass index
CAT – catalase
DOX - doxorubicin
FSH – follicle-stimulating hormone
G6PDH - glucose-6-phosphate dehydrogenase
GPx - glutathione peroxidase
GST - glutathione S-transferase
HCR - high capacity runners
HPG - hypothalamus–pituitary–gonadal
HSP70 - heat shock protein 70
IL-1 β - interleukin-1 β
IL-10 – interleukin-10
JAK2 - Janus kinase 2
LCR - Low intrinsic capacity runners
LDH - lactate dehydrogenase
LEP - leptin
LH – luteinizing hormone
MDA - malondialdehyde
METs - metabolic equivalents
NADPH - nicotinamide adenine dinucleotide phosphate
NF-kB - nuclear factor kappa B

Nrf2 - nuclear factor erythroid 2p45-related factor 2
ODF-1 - outer dense fiber protein 1
PO_{2m} - microvascular oxygen partial pressure
SAMP8 - senescence-accelerated prone mouse model
SHBG – sex hormone binding globulin
SOD -superoxide dismutase
StAR - steroidogenic acute regulatory protein
STAT3 - signal transducer and activator of transcription 3
TGF- α - transforming growth factor alpha
TNF- α - tumor necrosis factor alpha
TUNEL - transferase nick-end labelling
VO_{2max} - maximal oxygen uptake

INTRODUCTION

There is consensus amongst the scientific and clinical communities that exercise improves an individual's health and quality of life. Increasingly, exercise training is being promoted by physicians as a primary life-style prevention for several diseases, particularly those of the cardiovascular system (Xu et al. 2010; Boyle et al. 2012; Wu et al. 2013; Aune et al. 2015; Vasankari et al. 2017). However, the recommendation of exercise training as a preventative or therapeutic strategy for testicular dysfunction is more problematic since data relating the impact of exercise training on testicular function are controversial.

Impairment of testicular function has increased in industrialized countries with a consequential decrease in fertility rates coupled with additional negative clinical outcomes that compromise life quality. Indeed, additional physiological functions may be influenced by chronic changes in the levels of testosterone, a steroid hormone with a panoply of essential systemic roles that include the stabilization of blood sugar and maintenance of cardiovascular function (Köhn 2006). Thus, decreased levels of testosterone may increase the propensity of developing serious disease states which include type 2 diabetes and cardiovascular pathologies (Traish et al. 2009; Corona et al. 2011).

Lifestyle and behavioral causes of testicular dysfunction include modifiable factors

such as obesity that can be altered or avoided (Sharpe 1974). Indeed, men with a body mass index (BMI) higher than 25 have an average 25% reduction in sperm parameters, number and motility, compared with those exhibiting a normal BMI (Kort et al. 2006). In this case, exercise training has been recommended to reduce and control body mass (Swift et al. 2014); however, it might not be an appropriate intervention to manage testis dysfunction in these patients. Moreover, aging is also recognized as a cause of testicular dysfunction since it is associated with decreased free testosterone levels (Orwoll et al. 2006).

Herein, we rigorously review the current knowledge on the cross-talk between exercise and testis function with particular reference to the systemic and molecular alterations promoted by exercise training of different types and intensities. **Most of these studies focused upon aerobic exercise training, running and swimming programmes of moderate- and high-intensity and employ animal models to evaluate the molecular alterations in testis. In human studies, the molecular markers of testicular dysfunction were generally evaluated in blood-derived fluids.** Subsequently, we discuss the effects of exercise training in both healthy individuals and those with impaired testicular function caused by distinct pathophysiological conditions is discussed. These findings support the definition of guidelines for the prescription of exercise training in the case of individuals with or at risk of testicular dysfunction.

MODERATE-INTENSITY EXERCISE TRAINING

Several population based-studies reported a positive association between moderate-intensity aerobic exercise training and testis function parameters such as sperm concentration and hormonal serum levels. Indeed, increased serum levels of total and free testosterone, sperm count, motility and morphology were observed in trained subjects (Grandys et al. 2009; Vaamonde et al. 2012; Gaskins et al. 2015; Priskorn et al. 2016). The HPG axis may be responsible for the observed alterations, since LH and FSH were also increased, suggesting an increased stimulation of Leydig and Sertoli cells' functions (Grandys et al. 2009; Vaamonde et al. 2012; Gaskins et al. 2015; Priskorn et al. 2016). Alterations in sex hormone binding globulin (SHBG) serum levels were also observed after exercise training; however, there is no consensus about

the trend of such alterations since both a decrease and an increase in its levels were reported. **This globulin binds to testosterone, reducing its bioavailability** (Grandys et al. 2009; Priskorn et al. 2016). **Resistance training, despite being less intensively studied, seems also to have a beneficial influence upon testicular function. This exercise regime was associated with increased levels of total and free testosterone serum levels (Ahtiainen et al. 2003), similar to those verified for aerobic training. Endurance activities have been the most studied, particularly those of moderate intensity. A maximal oxygen uptake (VO_{2max}) of 50% (range 40-60%) (Grandys et al. 2009; Vaamonde et al. 2012), or 3-6 metabolic equivalents (~~approximately 3-6~~ METs), a measure of energy consumption, is usually considered in the definition of moderate-intensity exercise programs** (Gaskins et al. 2015; Priskorn et al. 2016).

Contrary findings were described in other studies, with no impact or negative effects of moderate-intensity exercise training on testicular function (Safarinejad et al. 2009; Dominguez et al. 2011; Wise et al. 2011; Torma et al. 2014). **In a longitudinal study**, lower serum levels of free and total testosterone, LH and FSH were observed in moderate-intensity trained men, suggesting decreased testis function. Higher levels of SHBG were also observed in trained subjects, accordingly with reduced levels of free testosterone serum levels, ~~since SHBG binds to testosterone, reducing its bioavailability~~ (Safarinejad et al. 2009). Wise et al. (Wise et al. 2011) demonstrated a lack of association between exercise training of diverse types, including running and cycling (**aerobic**) and weightlifting (**resistance**), at different hours *per* week, and semen parameters. One exception was observed in a population of men who cycled as a primary form of exercise. These subjects presented a decreased sperm count and motility if they cycled for five or more hours *per* week when compared with a cohort who did not exercised regularly. So, the effect of exercise training seems to depend not only on the type but also on the duration of the activity. Furthermore, long-term cycling seems to be associated with a negative outcome **though the precise underlying mechanisms responsible are unknown. Testicular mechanical trauma, the result of compression against the bicycle saddle, and increased scrotal temperature may both induce an inflammatory response with negative outcomes in testis function** (Behzad et al. 2015). **Several saddle designs that reduce the pressure in the testis have been proposed to minimize the influence of this confounding factor when analyzing the**

effect of this type of exercise training on testicular function (Rodano et al. 2002; Lowe et al. 2004).

To better comprehend the molecular basis of the physiological alterations in testis promoted by exercise training in men, animal model-based studies have been designed and performed (figure 1). Moderate-intensity treadmill running was reported to induce a weakened oxygen supply to rat testis tissue, since a decreased microvascular oxygen partial pressure (PO_{2m}) value was observed in the testis of trained rats. These lower levels of PO_{2m} may cause vascular dysfunction which contributes to the generation of an anoxic environment. The resulting perturbations in intracellular milieu in turn impact upon the health and function of the testis (Dominguez et al. 2011). An impaired spermatogenesis was also suggested, since decreased levels of outer dense fiber protein 1 (ODF-1) were also observed following treadmill training. ODF-1 is required for the correct morphology of sperm, being essential for a rigid head-tail junction (Yang et al. 2012; Torma et al. 2014).

HIGH-INTENSITY EXERCISE TRAINING

There is a general awareness that high-intensity exercise training is harmful to testicular function (figure 1). A high intensity exercise program is defined by a VO_{2max} higher than 60% or more than 6 METs (Fletcher et al. 2001). One randomized controlled longitudinal study involved 362 habitual aerobic male athletes, aged 20-40 years, submitted to 60 weeks of a running program (5 sessions/week, 120 min/session at a speed adjusted to VO_{2max} of 80%). In this study lower serum levels of total and free testosterone, higher levels of SHBG, and decreased semen parameters like sperm count, motility and morphology were observed (Safarinejad et al. 2009). These hormonal and physiological alterations are suggestive of impaired testicular function, which may be associated with an effect in HPG axis, since decreased serum levels of LH and FSH were also observed. Lower levels of LH and FSH, together with decreased testosterone levels and sperm parameters indicate that either there is an impairment of GnRH secretion by the hypothalamus or GnRH did not stimulate the anterior pituitary to secrete LH and FSH.

Studies with animal models indicate a negative impact of high-intensity exercise training on hormonal and seminal parameters (Manna et al. 2003, 2004; Jana et al.

2008; Chigrinskiy and Conway 2011). The decrease in testosterone serum levels was corroborated by a decreased testis activity of some steroidogenic enzymes, including 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD), responsible for several steps of testosterone production. Yet the involvement of the HPG axis in these alterations is controversial, with some studies reporting no significant alterations in FSH and decreased LH serum levels (Manna et al. 2003, 2004), and others reporting increased LH serum levels (Chigrinskiy and Conway 2011). Despite considering the same type of exercise, the duration and frequency of exercise training programs differed amongst studies and these factors may, at least in part, explain the contradictory results.

Several hypotheses have been proposed to explain the harmful effects of high-intensity exercise training upon testicular function. One such hypothesis is predicated upon the detrimental influences of exercise-induced oxidative stress. Several authors have described a decreased activity of enzymatic antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST), plus a reduced content of the non-enzymatic antioxidant defenses glutathione (GSH) and ascorbic acid in testis after **swimming programs lasting 4 weeks (3 hours/day, 5 days/week), 5 weeks (daily, until exhausting) or 10 weeks (4 hours/day, 6 days/week) exercise-training** (Manna et al. 2003, 2004; Jana et al. 2008; Chigrinskiy and Conway 2011). In agreement with data showing a decrease in antioxidant defenses, increased levels of some markers of oxidative damage were also observed, especially markers of lipid peroxidation (malondialdehyde (MDA) and conjugated dienes), suggesting that lipid peroxidation may contribute to the testicular dysfunction caused by intensive exercise training (Manna et al. 2003, 2004; Jana et al. 2008; Chigrinskiy and Conway 2011). Ribose deficiency was also suggested as a possible mechanism that contributes to testis dysfunction in exercised subjects. A decrease in ribose was confirmed by reduced levels of testis glucose-6-phosphate dehydrogenase (G6PDH), the enzyme that catalyzes the first step of the pentose phosphate pathway. Reduced levels of this enzyme may be associated with increased oxidative stress since decreased G6PDH inhibits nicotinamide adenine dinucleotide phosphate (NADPH) generation to reduce the activity of glutathione reductase, the enzyme that regenerates GSH from GSSG. Accordingly, oral administration of D-ribose

caused a restoration of testis MDA, GSH and ascorbic acid, and increased testosterone and LH serum levels (Chigrinskiy and Conway 2011). Finally, the lower levels of heat shock protein 70 (HSP70) observed in spermatogenic cells after high-intensity exercise training may be responsible, at least in part, for impaired spermatogenesis (Koeva et al. 2005). Decreased levels of HSP70 were associated with maturation arrest in the germinative cell populations, which in some cases causes low sperm count and may be associated with male infertility (Feng et al. 2001).

EFFECT OF EXERCISE TRAINING IN TESTICULAR DYSFUNCTION ASSOCIATED WITH:

AGING

Longitudinal studies strongly associate aging ~~has been strongly associated~~ with testicular dysfunction, the likely consequence of age-related increases in oxidative stress and inflammation (Wang et al. 2005; Luo et al. 2006). **In older men, acute exercise was associated with a transient increase in testosterone serum levels, counteracting age-related testicular dysfunction (Zmuda et al. 1996).** Studies using a senescence-accelerated prone mouse model (SAMP8) submitted, from two months of age, to a moderate-intensity swimming exercise program lasting 6 months, reported a protection and/or reversion of age-associated impairments of testicular function (figure 2) (Chigurupati et al. 2008; Zhao et al. 2013; Joseph et al. 2014). Exercised mice presented higher levels of nuclear factor erythroid 2p45-related factor 2 (Nrf-2) in testis, which leads to an increased expression of antioxidant enzymes, confirmed by the increased mRNA levels of SOD and GPx. The activities of these two enzymes, like CAT, were also increased in the testis of exercised mice. In agreement with these results, a decreased oxidative damage in the testis of exercised animals, determined by lower levels of protein carbonyls, 8-isoprostanes, MDA and 8-hydroxy-20-deoxyguanosine, was observed (Zhao et al. 2013). In addition to the modulation of oxidative stress, a reduction in testis inflammation after exercise training was also observed based on a decreased levels of nuclear factor kappa B (NF- κ B), a transcription factor which induces the expression of pro-inflammatory cytokines, chemokines and additional inflammatory mediators. Accordingly, reduced levels of pro-inflammatory

mediators, interleukin-1 β (IL-1 β) and tumor necrosis factor alpha (TNF- α), and increased levels of anti-inflammatory mediators, interleukin-10 (IL-10) and transforming growth factor alpha (TGF- α), confirmed the anti-inflammatory effect of lifelong exercise training in the testis (Zhao et al. 2013). The exercise training-related reduction of oxidative stress and inflammation in testis improved testicular function. Indeed, lifelong exercised rats presented increased levels of serum and testis testosterone. These findings were corroborated by the observed higher levels of steroidogenic enzymes, steroidogenic acute regulatory protein (StAR) and Cyp11a, which are responsible for the first steps of steroidogenesis. In addition, an increased number of Leydig cells and spermatogonia in seminiferous tubules were reported, observations which suggest increased testosterone and sperm production respectively (Chigurupati et al. 2008; Zhao et al. 2013). Exercised older rats showed attenuated age-related testicular atrophy and reduced levels of testis DNA damage, measured by the levels of phosphorylated histone H2AX (Joseph et al. 2014). The benefits of exercise training in counteracting age-related testis dysfunction are more pronounced when performed early in life (Zhao et al. 2013).

OBESITY

Obesity is also correlated with testicular dysfunction, highlighted by decreased serum levels of free testosterone, a result of decreased gonadotropins, coupled with impaired spermatogenesis (Stokes et al. 2015). **In obese men, weight loss as a result of exercise training improves semen quality and the production of reproductive hormones (Hakonsen et al. 2011).** Obese male mice with impaired spermatogenesis evidenced increased levels of serum leptin (LEP), a finding indicative of leptin resistance. Indeed, reduced testis expression of leptin receptors and of two precursors of leptin signal transduction, Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), were observed in obese mice, resulting in the reduced activation of the LEP/JAK2/STAT3 signal transduction pathway (Yi et al. 2017). To the best of our knowledge, only one study has evaluated the effect of exercise training in obesity-induced testis dysfunction using a mice model submitted to eight weeks of swimming training. This exercise training program restored the LEP-JAK-STAT signaling pathway in testis. (figure 2). The same study revealed an increased expression of steroidogenic

factor-1 which controls the transcription of some steroidogenic enzymes, including StAR and Cyp11a, also elevated in trained obese mice. Accordingly, an increased level of serum total testosterone, an increased sperm count and an improvement in sperm motility were also observed (Yi et al. 2017).

DOXORUBICIN TREATMENT

Testicular dysfunction is a reported side-effect of some drugs. One example is doxorubicin (DOX), a chemotherapeutic agent used to treat several types of cancer. DOX treatment impairs testis function, confirmed by both a reduced number and diameter of testicular seminiferous tubules and the depletion of spermatids in DOX-treated rats. To date, there are two main mechanisms proposed to be responsible for DOX-induced testis dysfunction: lipid peroxidation and cellular apoptosis. Indeed, increased MDA levels, decreased SOD, GPx and GSH levels, and increased terminal deoxynucleotidyl transferase nick-end labelling (TUNEL)-positive cells were observed in testis tissue of DOX-treated rats (Yeh et al. 2009; Magalhães et al. 2017). A recent study, using rats sub-chronically treated with DOX (2mg/kg per week, for 7 weeks), 5 weeks after initiating a treadmill exercise program, showed no positive effect of exercise training to prevent the reduced sperm motility induced by DOX or to alleviate oxidative damage in the testis. Nevertheless, a beneficial influence of exercise training to inhibit the oxidative damage of proteins involved in metabolism and the stress response against DOX (figure 2) was suggested (Magalhães et al. 2017). To the best of our knowledge, there are no studies performed in human subjects treated with chemotherapeutic agents.

LOW INTRINSIC CAPACITY RUNNERS RATS

The low intrinsic capacity runners (LCR) rat exhibits impaired testicular function and some authors have used this model to evaluate the effect of exercise training in the modulation of testicular dysfunction (Torma et al. 2014). This animal model is characterized by lower sirtuin-1 levels in the testis associated with an attenuation of spermatogenesis (Coussens et al. 2008). Paradoxically to its known deacetylase function, sirtuin-1 was associated with the acetylation of histones. The morphological alterations necessary to generate spermatozoa from haploid spermatid cells include

the histone to protamine transition, a process that is initiated with post-translational modifications of histones, including acetylation. Thus, reduced sirtuin-1 levels reduce this acetylation process and, consequently, impair spermatogenesis (Bell et al. 2014).

After twelve weeks of treadmill running at 60% VO_{2max} (moderate-intensity exercise training), LCR rats presented an enhancement in markers of testis and sperm function, but sirtuin-1 remained unchanged (Torma et al. 2014). Decreased levels of testis reactive oxygen species (ROS) and increased ODF-1 were observed, suggesting an improvement in spermatogenesis (figure 2). An increase in lactate dehydrogenase (LDH) C levels were also observed in testis after exercise training. This testis-specific isoform of LDH is essential for germ cells energy metabolism since lactate is a key energetic substrate in these cells. Consequently, LDHC plays a key role in sperm function (Odet et al. 2008). Curiously, these apparent beneficial alterations caused by exercise training were not verified in high capacity runners (HCR), who did not suffer from testicular dysfunction. Hence, in comparison with LCR rats, a contrary effect was observed for ODF-1, which decreased with exercise training in HCR rats (Torma et al. 2014).

CONCLUSIONS AND FUTURE PERSPECTIVES

The andrological aspects of exercise training, taking into consideration the possible risks and benefits for reproductive and sexual health, should be considered when playing sport. Exercise training might positively or negatively influence andrological health depending upon the type, intensity and duration of the training program, and on individual health status. This is particularly relevant when a lifestyle-related disease influencing reproductive and sexual functions (e.g. obesity, metabolic syndrome, diabetes and cardiovascular diseases) is prevalent. Curiously, in the presence of a condition that impairs testicular function, like obesity or DOX treatment, data from the literature is consensual showing that exercise training prevents and/or counteracts the negative effect of the underlying condition on testis function. Decreased oxidative stress and, possibly, inflammation following an exercise training program seems to markedly contribute to this beneficial effect of exercise.

In conclusion, we recommend that future studies should seek to further clarify the many effects of exercise training on testicular function and elucidate their underlying

molecular mechanisms. We envision that such findings will provide guidelines to enable physicians to recommend a more appropriate active lifestyle to prevent or even treat testicular dysfunction associated with aging, obesity and drug treatments.

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FIGURE LEGENDS

Figure 1: Overview of the impact of moderate- and high-intensity exercise training in testicular function. The effect of moderate-intensity exercise training in testicular function is not consensual among the scientific community but a decrease in PO_{2m} and ODF-1 after moderate-intensity exercise training is proposed. High-intensity exercise training negatively affect testis function, decreasing testosterone levels and sperm count and impairing sperm morphology and motility. Increased levels of OS and decreased levels of ribose and HSP70 are proposed as responsible mechanisms.

Abbreviations: **GnRH:** gonadotropin-releasing hormone; **LH:** luteinizing hormone; **FSH:** follicle-stimulating hormone; **LHR:** LH receptor; **FSHR:** FSH receptor; **PO_{2m} :** microvascular oxygen partial pressure; **ODF-1:** outer dense fiber protein 1; **OS:** oxidative stress; **HSP70:** heat shock protein 70

Figure was produced using Servier Medical Art.

Figure 2: Overview of the impact of exercise training on testicular functions in the presence of a condition that negatively affects testis function. DOX treatment negatively affects testicular function and exercise training improves testosterone levels and sperm parameters by modulating the susceptibility to oxidative modifications. With aging, testicular function is affected and exercise training helps to counteract these negative alterations by decreasing oxidative stress and inflammation. LCR rats are characterized by an impaired testicular function and, when subject to exercise training, an improvement in testicular function is observed. Decreased OS and increased ODF-1 and LDC are responsible for the above effect. A restoration of LEP-JAK-STAT signaling pathway after exercise training is observed in obese men, with consequent improvement of testis function parameters.

Abbreviations: **GnRH:** gonadotropin-releasing hormone; **LH:** luteinizing hormone; **FSH:** follicle-stimulating hormone; **LHR:** LH receptor; **FSHR:** FSH receptor; **ET:** exercise training; **ODF-1:** outer dense fiber protein 1; **OS:** oxidative stress; **LDC:** lactate dehydrogenase C; **LEP-JAK-STAT:** leptin-Janus kinase 2-signal transducer and activator of transcription 3 signaling pathway; **DOX:** doxorubicin; **LCR:** low capacity runners.

Figure was produced using Servier Medical Art.

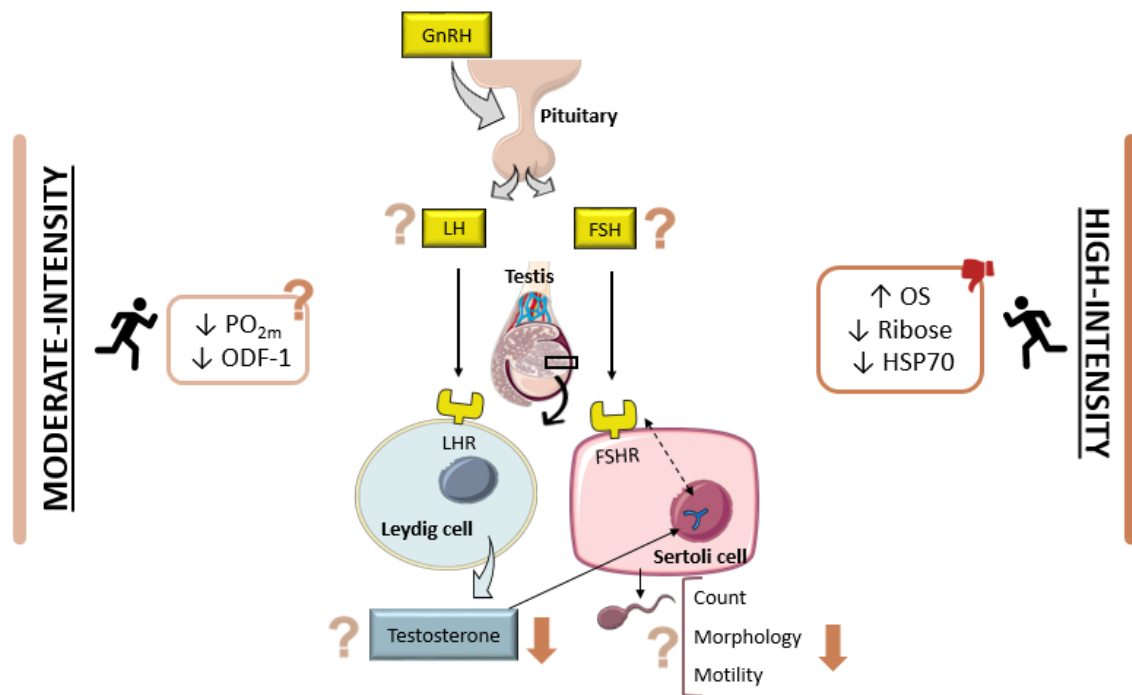


Figure 1: Overview of the impact of moderate- and high-intensity exercise training on testicular function. No consensus regarding the effects of moderate-intensity exercise training on testicular function has yet been reached within the scientific community, but a decrease in PO_{2m} and ODF-1 after moderate-intensity exercise training is proposed. High-intensity exercise training negatively affect testis function, decreasing testosterone levels and sperm count and impairing sperm morphology and motility. Increased levels of OS and decreased levels of ribose and HSP70 are proposed as the mechanisms underlying these observations.

Abbreviations: **GnRH:** gonadotropin-releasing hormone; **LH:** luteinizing hormone; **FSH:** follicle-stimulating hormone; **LHR:** LH receptor; **FSHR:** FSH receptor; **PO_{2m}:** microvascular oxygen partial pressure; **ODF-1:** outer dense fiber protein 1; **OS:**

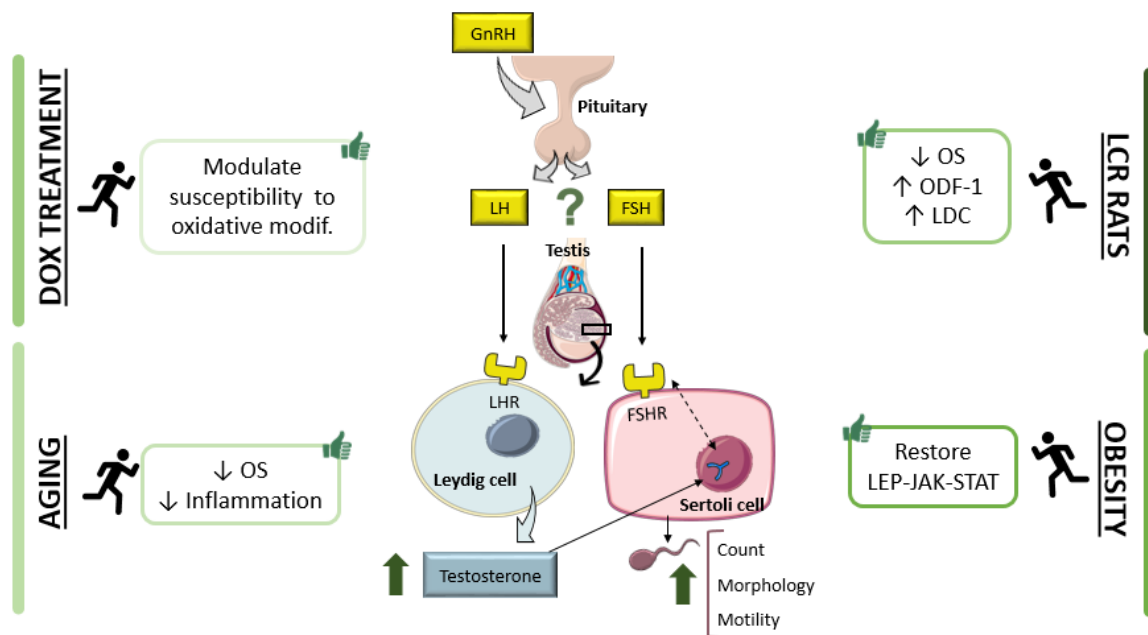


Figure 2: Overview of the impact of exercise training on testicular function in the presence of a condition that negatively affects these parameters. DOX treatment negatively affects testicular function and exercise training improves testosterone levels and sperm parameters by modulating the susceptibility to oxidative modifications. With aging, testicular function is compromised and exercise training helps to counteract these negative alterations by decreasing oxidative stress and inflammation. LCR rats are characterized by an impaired testicular function and, when subject to exercise training, an improvement in testicular function is observed. Decreased OS and increased ODF-1 and LDC are responsible for the above effect. A restoration of LEP-JAK-STAT signaling pathway after exercise training is observed in obese men, with consequent improvement of testis function parameters.

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