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Short communication

Could leptin be responsible for the reproductive dysfunction in obese men?

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ABSTRACT

Low sperm concentration, increased fraction of morphologically abnormal sperm, and raised levels of markers of oxidative stress are often reported in the seminal plasma of infertile obese males. The precise reason for changes remains unknown. This short review summarises evidence from human and animal studies linking leptin to the reproductive dysfunction reported in obese males and presents a possible mechanism for this based on the available data in the literature. Serum leptin concentrations correlate positively with body fat mass but its precise link to semen abnormalities reported in obese males has yet to be conclusively established. Decreased sperm concentration, increased fraction of morphologically abnormal sperm and increased markers of oxidative stress have been reported following six weeks of daily leptin treatment to normal weight rats. In addition, decreased expression of endogenous antioxidant enzymes and increased expression of respiratory chain enzymes noted in the testes of leptin treated rats increases the propensity to oxidative stress. Besides that, leptin's interference with histone to protamine transition in the DNA of sperm increases the susceptibility of sperm to free radical attack and may explain the often reported higher DNA fragmentation index in sperm of obese males. Concurrent supplementation of melatonin, a natural anti-oxidant, to these rats prevents the effects of leptin. The role of leptin in obesity-related reproductive dysfunction has to be considered seriously and these effects of leptin might involve increased oxidative stress.

1. Introduction

Leptin, a 167 amino acid non-glycosylated adipokine, was first identified through positional cloning in 1994. It has a molecular weight of 16 kDa and a tertiary structure similar to that of a cytokine [1]. Leptin in circulation is largely produced and secreted by white adipose tissue [2] but small quantities are also produced by a number of non-adipocyte tissues including the gastric mucosa [3], mammary epithelial cells [4], myocytes [5], anterior pituitary [6], placenta [7], and human ejaculated spermatozoa [8].

Circulating levels of leptin correlate positively with percentage body fat [9]. Besides that, serum leptin levels exhibit sexual dimorphism, where the levels are a little higher in women than in men [9,10]. This is due to the generally higher percentage of body fat present in women than in men. In addition, it may also be due to the stimulatory effects of oestrogen on leptin secretion in the female, and the inhibitory effects of testosterone on leptin secretion in the male [11]. Serum leptin levels increase with age in children of both sexes until puberty. The levels in females continue to increase after puberty but, in males, they tend to either remain unchanged or decrease a little following puberty. This is perhaps associated with the increased muscle mass and concurrent decrease in body fat that is usually seen in young males. Leptin levels tend to rise later in life in males as body fat mass increases.

The actions of leptin are mediated following its binding to membrane bound receptors. To date, six isoforms of this receptor have been identified [12,13]. These have been divided into long, short, and soluble isoforms [12]. The long-form mediates the cellular actions of leptin whereas the short-form is responsible for the transport of leptin across the blood brain barrier and cell membranes. The soluble form is found bound to leptin in the circulation [14–16]. Leptin binding to the long-form receptor activates one of five signalling pathways, depending on the target cell. These pathways include the (i) Janus kinase-signal transducer and activator of transcription (JAKSTAT) found in the hypothalamus, (ii) mitogen-activated protein kinase (MAPK) *e.g.* in monocytes and precursor osteoblasts, (iii) 5′adenosine monophosphate-activated protein kinase (AMPK) in the skeletal muscle and hypothalamus, (iv) mammalian target of rapamycin (mTOR) in intestinal epithelial cells, and (v) phosphoinositide 3-kinase (PI3K) found in the

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liver, pancreas, testes and some parts of the central nervous system, including the hypothalamus [16–18].

Apart from its role in the regulation of food intake and body weight, leptin has been shown to also have important roles in neuroendocrine, immune and reproductive functions [19]. The actions of leptin are mediated either through the central nervous system or directly on the peripheral tissues, as leptin receptors are found throughout the central nervous system and peripheral tissues. In view of its involvement in numerous physiological functions, leptin is now considered by many as a pleiotropic factor. For more information on the physiology of leptin, readers are referred to the following recent review [20].

2. Leptin in reproduction

Leptin's role in reproduction was first suspected from observations in leptin-deficient *ob/ob* mice. These mice are usually infertile, although some reproductive capability is present in some young *ob/ob* males. Leptin treatment restores fertility in these mice [21]. Besides, mice with leptin deficiency have ovaries and testes that are smaller compared with those in age-matched wild-type mice [22]. Seminiferous tubules of leptin-deficient mice show fewer sperm than those of their wild-type littermates. Leydig cells of leptin-deficient mice are also smaller and with less cytoplasmic content [21].

Obese humans with mutations in the leptin receptor or leptin gene also have reproductive dysfunction [23]. Two types of leptin signalling deficiencies have been reported in humans; mutations in leptin and leptin receptor genes. Obesity and infertility are associated with both of these mutations.

It has now become apparent that leptin has an important physiological role in reproduction and its deficiency leads to severe reproductive dysfunction. It increases the release of gonadotropins [24] but the precise mechanism for this remains uncertain. GnRH neurons do not express leptin receptors. Stimulation of gonadotropins by leptin must therefore involve some other indirect pathway/s. For this, the roles of kisspeptin neurons, the premammillary nucleus (PMN) and hypothalamic neuropeptides, including pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART) have been hypothesised [25]. Leptin receptors have also been identified on gonadotropes in the anterior pituitary [26]. Anyone of these pathways might be involved in leptin-stimulated increase in gonadotropin release.

3. Leptin in reproductive dysfunction in obese male

Despite its importance in the initiation and regulation of reproduction, there is accumulating evidence implicating elevated levels of leptin in reproductive dysfunction. High leptin levels may, in fact, be responsible for the reproductive dysfunction reported in obese hyperleptinaemic males and females. Although transgenic skinny female mice that overexpress leptin show signs of early sexual maturation, these mice also show early reproductive senescence. Thus it seems that, apart from advancing the onset of puberty, the hyperleptinemia also appears to promote early reproductive failure [27].

The role of leptin in obesity-related subfertility and infertility is becoming more evident in recent years. A number of reports from human studies point to the involvement of leptin in obesity-related male reproductive dysfunction. For example, obesity is three times more likely in sub-fertile men than in male partners of couples with idiopathic or female factor infertility [28]. Low sperm concentration, low number of motile sperm and even oligospermia are also frequently found in males with high BMI where leptin levels are also high [28,29]. Men with BMI of > 35 kg/m^2 have lower total sperm count and a higher fraction of sperm with DNA damage than normal-weight men [30]. Sperm DNA fragmentation Index has been found to correlate positively with BMI [28]. A case-control study on 42 obese and non-obese men found that obese men, in addition to having high leptin levels, low sperm concentrations, higher sperm DNA fragmentation, also had higher sperm mitochondrial membrane potential than that in normal-weight men [31].

The lower sperm concentration observed in obese men has in the past been attributed either to increased testicular temperature secondary to the extra fat in the lower abdomen, scrotal areas and thighs or to hormonal disturbances associated with obesity [32]. Circulating testosterone levels are lower in obese males [33]. Obese males when stimulated with human chorionic gonadotropin (hCG) show a higher 17-hydroxyprogesterone (17-OHP)/testosterone ratio compared with that in control lean individuals, indicating a dysfunction in the conversion of 17-OHP to testosterone in the Leydig cells of obese men [34]. The reason for this is not clear and whether leptin influences this conversion requires to be investigated further.

One other factor that is also often considered contributing to sperm dysfunction in the obese is inflammation. Chronic low-grade inflammation is associated with obesity, particularly involving the white adipose tissue (WAT) [35]. The precise trigger or factor responsible for the inflammation of WAT is unknown although hypoxia, endoplasmic reticulum stress, excess saturated fatty acids and stretching of the adipocytes have been implicated. Inflamed WAT increases its secretion of pro-inflammatory adipokines, which are then believed to trigger low-grade systemic inflammation, including in the testes. Apart from leptin, the other adipokines released from WAT are adiponectin, apelin, chemerin, resistin, vaspin, fibroblast growth factor 21 (FGF21), bone morphogenetic protein (BMP)-4, BMP-7, retinol-binding protein 4 (RBP4), dipeptidyl peptidase 4 (DPP-4), tumour necrosis factor-alpha (TNF-α), IL-6, MCP-1 and progranulin [36,37]. Of these, leptin, TNF-α and IL-6 are considered pro-inflammatory, while adiponectin is considered anti-inflammatory. The level of adiponectin incidentally correlates negatively with BMI and its serum levels are low in obese individuals. Raised levels of leptin, TNF-α and IL-6 together with lower levels of adiponectin predispose to an inflammatory state. It, however, remains to be established if the increased levels of these pro-inflammatory adipokines in circulation cause inflammation in the testes. If they do, then the neutrophil activation that occurs in inflammation will increase the liberation of reactive oxygen species. This will result in oxidative stress contributing to the reported abnormalities in the semen of obese males.

Whilst higher testicular temperature, low testosterone level, and inflammation may be responsible for the low sperm quality in obese individuals, recent evidence suggests that there might be more than just these three factors involved. Adipokines released from the enlarged, distended and distressed adipocytes may also be contributing to sperm abnormalities. Male Wistar rats fed a high-fat diet had increased body weight, raised serum leptin levels and lower sperm motility when compared with those of normal weight rats. Normal weight male Wistar rats treated with leptin for 42 days were found to have decreased fertility potential and increased preimplantation embryo loss after artificial insemination *in utero* [38].

Although adipocytes produce numerous adipokines, apart from leptin little is known about the impact of the others on sperm function and reproduction [25]. Even for leptin, its role in reproductive dysfunction in obese humans remains undetermined. There are no reports directly investigating the effects of leptin on human reproduction *per se*. The only evidence from human observations that links leptin to reproductive dysfunction is the reported correlation between leptin and BMI and that between BMI and altered semen parameters. Direct evidence linking leptin with reproductive dysfunction is mainly from animal studies, which first appeared about a decade ago. In these studies, it was noted that when normal weight Sprague-Dawley rats were given once daily intraperitoneal injections of leptin, in doses ranging from 5 to 60 μg kg^{-1} body weight for 6 weeks, they had significantly lower sperm count and higher fraction of sperm with abnormal morphology than those in normal-weight non-treated rats [24,39–41]. In addition, these leptin-treated rats also had lower seminiferous tubular epithelial

height and diameter than those in normal age-matched control rats. The precise mechanism responsible for these effects is still uncertain although leptin-induced oxidative stress is implicated. Evidence of increased levels of reactive oxygen species (ROS) [39], increased levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) (a marker of DNA damage due to oxidative stress), increased sperm DNA fragmentation and apoptosis [40,42], and impaired histone-to-protamine transition [43] following leptin treatment in rats has been documented. Incidentally, leptin has been shown to induce ROS formation in phagocytic and non-phagocytic cells [44] and in cells of the renal tubules by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [45]. Given the available evidence, it is conceivable that leptin may increase sperm damage by generating ROS in the seminiferous tubular cells or in the epididymis. The increased oxidative stress that is also observed in obese men might, in fact, be due to the high levels of leptin present in these men [46]. It must, however, be added here that although it is widely accepted that elevated levels of reactive oxygen species (ROS) are a major cause of male infertility, there are some men who are still fertile despite having high seminal ROS levels. This is attributed to their ability to greatly increase the expression of several antioxidant proteins and an effective proteasomal system for the degradation of defective proteins in seminal plasma and spermatozoa [47]. That oxidative stress might indeed be involved following leptin treatment is also supported by findings that melatonin, a powerful antioxidant, prevents these adverse effects when administered concurrently with leptin [42].

4. Possible mechanism of leptin induced reproductive dysfunction

Although there are no reports on human experimental studies investigating the role of leptin in male reproductive dysfunction, but when the abnormalities reported in the semen of obese males are taken together with data from studies in leptin treated mice and rats, there clearly emerges a link between leptin and obesity-related reproductive dysfunction in males. It seems that the low sperm count together with the high fraction of abnormal sperm and the high DNA Fragmentation Index reported in obese men may be due to the presence of persistently high leptin levels in these men. The effect of leptin most likely involves a direct action of leptin on sperm and the reproductive organs [24,48,49] involving leptin-induced increases in oxidative stress. This could result from a direct action of leptin on the testes itself, causing an increase in the production of reactive oxygen species (ROS). As leptin has proinflammatory tendencies, it is possible that it might also be causing inflammation within the testes and consequently oxidative stress. The oxidative stress that follows inflammation could then cause damage to the sperm and the seminiferous tubular cells leading to their subsequent loss through apoptosis. That leptin might indeed be directly increasing oxidative stress is supported by the findings of significant down-regulation of antioxidant enzyme gene expression and up-regulation of respiratory chain gene expressions in leptin treated rats [42]. In addition, studies *in vitro* on human Sertoli cells show that leptin directly disturbs the metabolism of these cells, which could increase the production of ROS causing either a decrease in nutritional support of spermatogenesis or apoptosis of these cells [50,51].

ROS's impact on sperm function can either be positive or negative; depending on the concentration, location, length of exposure, exposure to environmental factors such as temperature, the presence of ions, proteins, and ROS scavengers [52]. At physiological levels, ROS plays significant roles in sperm maturation, capacitation and acrosome reaction [53]. At pathological levels, ROS impairs testicular germ cell proliferation, negatively impact sperm plasma membrane fluidity, impair sperm motility and increase sperm DNA damage [52]. Infertile men with high ROS levels tend to have more sperm with abnormal morphology [54]. ROS has also been associated with increased apoptosis in sperm samples [55]. The somewhat higher susceptibility of spermatozoa to ROS attack is because sperm have less cytoplasm than somatic cells. They also have limited intrinsic antioxidant capability and a cell membrane that is rich in polyunsaturated fatty acids [56].

While high leptin levels evidently increase oxidative stress in the testes and sperm, the precise mechanisms and pathways responsible for this remain to be established. Of the five pathways associated with the action of leptin, those related to oxidative stress are AMPK, PI3K, MAPK, and mTOR pathways. These pathways have well-established roles in the mode of action of leptin [57]. Microarray analysis of the testes from leptin-treated Sprague-Dawley rats in our laboratory showed a 2-fold upregulation in the expression of genes of proteins associated with these pathways, particularly those of the PI3K pathway (unpublished data). In addition, the adverse effects of leptin on sperm are inhibited by LY294002 (a PI3K pathway inhibitor) but not by dorsomorphin (an AMPK inhibitor) [41]. This clearly indicates the role of the PI3K pathway in leptin-induced adverse effects on sperm.

Higher DNA fragmentation and indices of apoptosis are reported in seminiferous tubules of leptin treated rats. The expression of genes of apoptosis-inducing factor (*AIF)*, high temperature requirement protein A2 (*HTRA2*) and cathepsin D (*Ctsd*), factors involved in caspase-independent cell death, are significantly upregulated following leptin treatment [42]. Pro-survival and pro-death signals generated in response to multiple intracellular stress conditions converge into a mitochondrion-centred control mechanism. Lethal signals, including an attack by free radicals, cause permeabilization of the mitochondrial outer membrane [58]. The loss of mitochondrial transmembrane potential results in the release, into the cytosol, of *AIF* and HTRA2. These are normally confined within the mitochondrial intermembrane space. *AIF* is an active cell killer when released into the cytosol. From here it translocates into the nucleus and triggers peripheral chromatin condensation, DNA fragmentation and eventually cell death. [59]. Mitochondria are the main sources of superoxide in the cell [60]. The superoxide is rapidly converted into hydrogen peroxide by superoxide dismutase present in the mitochondria. Leptin modulates mitochondrial energy dynamics [50,51,61] and is associated with mitochondrial dysfunction in a number of cells including cardiomyocytes [62]. Dysfunction in the mitochondria secondary to high leptin levels could result in increased leakage of free radicals from the mitochondria, resulting in oxidative stress. This could indeed be the basis for most of the leptin-induced adverse effects on sperm.

5. Conclusion

The similarities between findings of low sperm count, increased sperm abnormalities, and oxidative stress reported in obese men and those reported in normal-weight rats given daily injections of leptin seem to point to leptin as a link between obesity and reproductive dysfunction. It seems that while leptin deficiency results in delayed puberty and poor functional development of the reproductive tract in males and females, high leptin levels in males meanwhile lead to low sperm count, increased sperm DNA fragmentation and increased production of morphologically abnormal sperm secondary to increased oxidative stress. Fig. 1 briefly summarises the hypothesised mechanism of leptin-induced adverse effects on sperm and male reproductive tissues. Binding of leptin to its receptors activates the PI3K pathway in the testes, which then disrupts mitochondrial function in the seminiferous tubular cells and the developing sperm. This disruption leads to disturbed energy metabolism and increased release of ROS from the mitochondria. The altered energy metabolism may be responsible for decreased sperm motility. Leptin at the same time decreases the expression of antioxidant enzymes, which together with increased ROS production results in oxidative stress. The activation of the PI3K pathway also disrupts the histone to protamine transition in the sperm during spermiogenesis. The higher histone to protamine ratio exposes the sperm DNA to attack from free radicals resulting in increased DNA fragmentation and apoptosis. This then leads to a decrease in sperm count and/or an increase in the formation of morphologically abnormal sperm. In addition, free radical attack on seminiferous tubular cells also

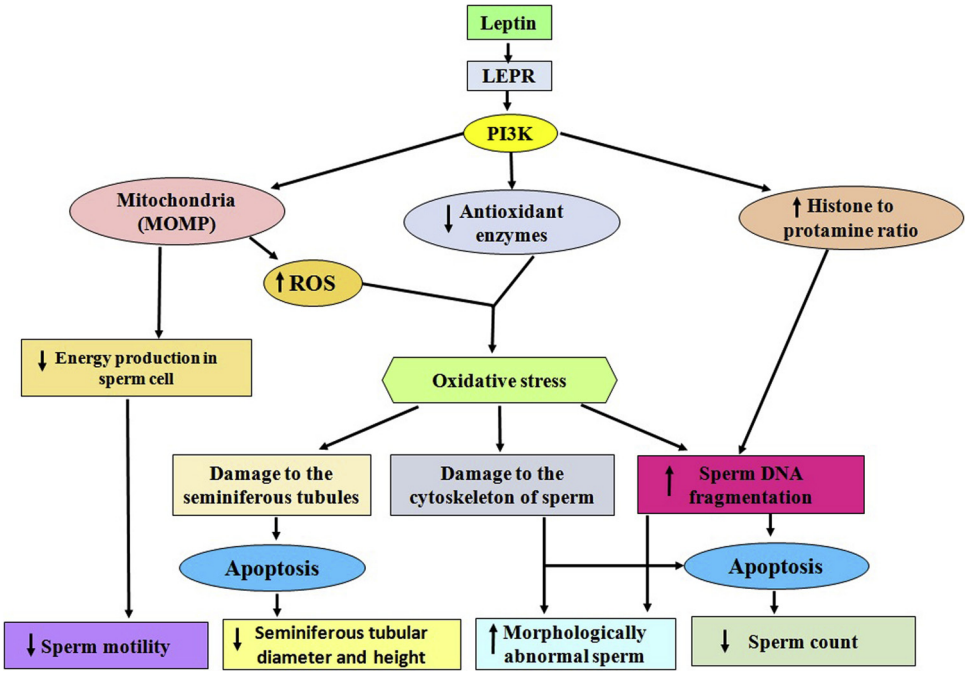

Fig. 1. A schematic diagram depicting the probable mechanism of leptin-induced effects on testes and sperm parameters.

triggers apoptosis in them resulting in decreased seminiferous tubule height and diameter. Damage to the cytoskeleton of the sperm following a free radical attack could account for the increased fraction of sperm with abnormal morphology and apoptosis.

It seems that although leptin is necessary for normal reproductive function, but when present in excess it has detrimental effects on the male reproductive system and has to be seriously considered as a link between obesity and infertility in males. Developing ways to reduce the negative impact of leptin on the reproductive system could help reduce the reproductive dysfunction observed in obese individuals.

Declaration of Competing Interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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