ORIGINAL ARTICLE

Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin

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Summary

Ghrelin has recently been reported to exert beneficial effects on various oxidative stresses as a result of its antioxidant properties. Therefore, we designed this study to explore the probable antioxidative effects of this peptide in the testis. Twenty-eight male adult Wistar rats were divided into equal control and treatment groups. In the treatment group, 1 nmol of ghrelin was administered as subcutaneous injection for 10 consecutive days or vehicle (physiological saline) to the control rats. The control and treated rats were killed on days 6 and 10 after beginning of ghrelin injection ($n = 7$ from each group on each day). The testes were taken and measured for antioxidant enzyme activity and malondialdehyde (MDA) content. Glutathione peroxidase activity significantly increased on day 10 in the treated animals compared with the control group ($P < 0.05$). Although the mean activity of glutathione peroxidase was greater on day 6 in the ghrelin-treated group than in the control animals, it was not statistically significant. There were no significant differences in superoxide dismutase and catalase activities between the groups. However, MDA level decreased by ghrelin treatment on day 10 compared with the control rats ($P < 0.05$). The results of this study indicate for the first time novel evidences for antioxidant properties of ghrelin in the rat testis.

Introduction

It has been discovered that ghrelin is an endogenous ligand for the growth hormone secretagogue receptor and that it is predominantly produced in the stomach. It potently stimulates growth hormone secretion and exerts various central and peripheral actions, such as regulation of food intake and control of energy balance (Kojima & Kanagawa, 2005). Compelling evidence has demonstrated the close connection between the systems governing energy homoeostasis and reproductive function (Fernandez-Fernandez et al., 2005). Expression of ghrelin has been demonstrated in mature Leydig cells of rat and human. In addition, expression of the functional ghrelin receptor, the GHS-R type 1a, has been shown in Sertoli and Leydig cells (Tena-Sempere et al., 2002; Barreiro &

Tena-Sempere, 2004). On the other hand, expression of GHS-R1a in the seminiferous tubules strongly suggests that the seminiferous epithelium might be a target for ghrelin action and directly regulates seminiferous tubule functions (Barreiro & Tena-Sempere, 2004).

It has been recently proposed that ghrelin may be an antioxidant and anti-inflammatory agent. For example, it has been proven that ghrelin prevents lipid peroxidation and reduction of antioxidant enzyme activities and glutathione level against pentylenetetrazole-induced oxidative stress in the erythrocytes, liver and brain of rats (Obay et al., 2008). Zwirska-Korczala et al. (2007) demonstrated that ghrelin significantly increases the activity of antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and that it decreases the concentration of malondialdehyde

(MDA), an end product of lipid peroxidation, in pre-adipocyte cell culture. Likewise, Iseri *et al.* (2005) showed that ghrelin treatment significantly increased GPx activities and reduced MDA levels in the alendronate-induced gastric tissue injury in rats. This same study found that ghrelin decreased formation of reactive oxygen species (ROS). Ghrelin has also been shown to inhibit vascular superoxide production and oxidative stress in hypertensive rats by inhibition of vascular NADPH oxidase (Kawczynska-Drozdz *et al.*, 2006) and to increase mRNA levels of SOD in trout phagocytic leucocytes (Yada *et al.*, 2006). Additional studies have echoed the proposal that ghrelin attenuates the oxidative stress response (Dong & Kaunitz, 2006).

Testicular cell membranes are rich in polyunsaturated fatty acids and thus susceptible to peroxidation injury, which leads the spermatozoa to infertility due to defective sperm function (Aitken *et al.*, 1989; Peltola *et al.*, 1992). Therefore, production of ROS in testicular tissue and control of these phenomena may have important physiological consequences. Various observations indicate that spermatozoa may be more exposed and vulnerable to oxidative stress than germ cells. First, epididymal spermatozoa are not protected, like pachytene spermatocytes (PS) and round spermatids (RS), by the microenvironment provided by the Sertoli cell barrier. Second, the membranes of spermatozoa may be particularly susceptible to free radical attack because of their high level of polyunsaturated fatty acids (Aitken *et al.*, 1989). Third, several groups have demonstrated that, in contrast to spermatogonia, PS and RS, elongated spermatids and spermatozoa have a reduced capability or are even unable to repair DNA damage caused by ROS (Van Loon *et al.*, 1991). It means that protection of spermatozoa by the antioxidant factors is necessary against free oxygen radicals.

In the light of these literatures one could hypothesise that ghrelin may affect the antioxidative enzyme activities or lipid peroxidation in the testis. To clarify the possible antioxidative properties of ghrelin, the activities of GPx, SOD and CAT as well as lipid peroxidation (MDA concentration) were measured following chronic administration of ghrelin in the rat testis.

Materials and methods

Drugs and chemicals

Rat lyophilised acylated ghrelin (n-octanoylated research grade) was purchased from Tocris Cookson Ltd (Bristol, UK). Ghrelin was dissolved in sterile physiological saline solution before injection. The kits used in the measurement of antioxidant enzyme activities were provided from Randox Laboratories Ltd (Antrim, UK).

Animals

All investigations were conducted in accordance with the Guiding Principles for the Care and Use of Research Animals. All animals were treated humanely and in compliance with the recommendations of Animal Care Committee for the Lorestan University of Medical Sciences (Khorram Abad, Iran). Experiment was carried out on 60-day adult male Wistar rats ($n = 28$) bred in the vivarium of the Pasture Institute in Iran. All animals were allowed free access to standard rat food and tap water *ad libitum*. All of rats were housed under standard conditions in a room in groups of seven rats per cage at temperature of 21–24 °C and constant 12 h light/dark cycle. All of experimental procedures were carried out between 08.00 hours and 11.00 hours.

Experimental design

The animals were divided into four groups ($n = 7$ per group) as two control and treatment groups. To verify the hypothesis that ghrelin treatment might alter the antioxidant enzyme activities, a general protocol of subcutaneous (S.C.) injection of ghrelin (1 nmol per 100 μl saline) or 100 μl vehicle (physiological saline) to the control group was applied once a day for 10 consecutive days. The dose of ghrelin used in our *in vivo* experiment was comparable with amounts of ghrelin secreted into the blood during starvation: exogenous administration of 1 nmol of ghrelin is able to induce a significant elevation (2.4–2.6 fold increase) in serum level of total ghrelin 1 h after injection (Fernandez-Fernandez *et al.*, 2005), whose magnitude is in the range of that induced by fasting (Wren *et al.*, 2001). The animals were injected under conscious conditions after careful handling to avoid any stressful influence. The rats from both groups were killed upon diethyl ether anaesthesia (May & Baker Ltd, Dagenham, UK) by decapitation 3 h after injection on days 6 ($n = 14$) and 10 ($n = 14$) from the first day of ghrelin injection respectively.

Sampling

Immediately after rat killing on days 6 and 10, both testes were removed and carefully cleaned of fat and adhering, then stored at liquid nitrogen prior to analysis for testicular antioxidant enzyme activity and MDA content.

Analysis and measurement

Tissue preparation for enzyme assay
Rat testes were rapidly thawed and manually homogenised in cold phosphate buffer (pH 7.4) and debris

removed by centrifugation at 3500 *g* for 10 min (Centrifuge 5415 R; Eppendorf AG, Hamburg, Germany). Supernatants were recovered and used for enzyme activity and protein assays.

Determination of GPx activity

The activity of GPx was evaluated with GPx detection kit according to the manufacturer's instructions. GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP$^+$. The decrease in absorbance was measured spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) against blank at 340 nm. One unit (U) of GPx was defined as μmol of oxidised NADPH per min per milligramme of tissue protein. The GPx activity was expressed as milliunit per milligramme of protein (mU mg^{-1} protein).

Determination of SOD activity

The activity of SOD was evaluated with SOD detection kit according to the manufacturer's instructions. The role of SOD is to accelerate the dismutation of the toxic superoxide (O_2^-) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity is then measured by degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. SOD levels were recorded at 505 nm and through a standard curve and expressed as unit per milligramme of protein (U mg^{-1} protein).

Determination of CAT activity

Tissue catalase activity was measured according to the method of Aebi (1984). Briefly, tissue sections were homogenised in triton X-100 1% (Merck, Darmstadt, Germany) and the homogenates were diluted with phosphate buffer (pH 7.0). The reaction was initiated by the addition of hydrogen peroxide to the reaction mixture and the level of enzyme activity was quantitated according to the ability of the tissue catalase to decompensate hydrogen peroxide by monitoring the decrease in absorbance at 240 nm against a blank contains phosphate buffer instead of substrate. The value of log A1/A2 for a measured interval was used for unit definition owing to the first-order reaction of enzyme. One unit of CAT is the amount of enzyme that decomposes 1.0 mmole of hydrogen peroxide per minute at pH 7.0 and 25 °C.

Measurement of lipid peroxidation

The level of lipid peroxidation was indicated by the content of MDA in testis. Tissue MDA was determined using the thiobarbituric acid reactive substance assay, as described by Buege & Aust (1978) with slight modifications. Briefly, a tissue specimen of 0.1 g was homogenised in 0.15 mol l^{-1} KCl at a ratio of 1–9 ml with a glass homogeniser. One volume of homogenate was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 mol l^{-1} hydrochloric acid. The solution was heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1000 *g* for 10 min. The absorbance of the clear supernatant was determined at 535 nm and MDA concentration calculated using 1.56×10^5 mol^{-1} cm^{-1} as molar absorbance coefficient. MDA results were expressed as nmol per gram of wet tissue.

Protein assay

Protein content of supernatants for enzyme analysis was determined using a colorimetric method of Lowry with bovine serum albumin as standard (Lowry *et al.*, 1951).

Statistical analysis

Results were analysed using the spss/pc computer program version 12.0 (SPSS Inc, Chicago, IL, USA). All data were subjected to *Levene's test* for homogeneity of variances. The activity of GPx, SOD, CAT as well as MDA level on days 6 and 10 between the control and treated groups were compared using *independent sample t-test* (Petrie & Watson, 1999). Data are presented as the mean ± SEM. Values were considered to be statistically significant at $P < 0.05$.

Results

Changes in biochemical parameters including GPx, SOD and CAT activities as well as MDA level on days 6 and 10 in the rat testicular tissue are shown in Tables 1 and 2. Chronic administration of ghrelin had a considerable influence on GPx activity and MDA content. The mean activity of testicular GPx was significantly ($P < 0.05$) higher in the treated group on day 10 compared with that in the control animals. The mean activity of this enzyme was 343.00 ± 72.75 versus 129.80 ± 37.40 mU mg^{-1} of tissue protein on day 10 in the treated and control groups, respectively. Although there was a tendency for the mean activity of GPx to be greater ($P = 0.08$) on day 6 in the ghrelin group (249.00 ± 54.58 versus 112.14 ± 49.44), the difference was not statistically significant.

Table 1 Mean ± SEM of testicular antioxidative enzyme activity (GPx, SOD and CAT) as well as MDA level on day 6 after the beginning of ghrelin injection in the control and treated rats

	GPx (mU mg^{-1} prot)	SOD (U mg^{-1} prot)	CAT (U mg^{-1} prot)	MDA (nmol g^{-1})
Control ($n = 7$)	112.14 ± 49.44[a]	19.53 ± 1.33[a]	3.36 ± 0.13[a]	33.60 ± 3.58[a]
Treatment ($n = 7$)	249.00 ± 54.58[a]	22.48 ± 1.09[a]	2.96 ± 0.24[a]	31.83 ± 2.29[a]

Means with same superscript (a) in each column do not significantly differ ($P < 0.05$).

Table 2 Mean ± SEM of testicular antioxidative enzyme activity (GPx, SOD and CAT) as well as MDA level on day 10 after the beginning of ghrelin injection in the control and treated rats

	GPx (mU mg^{-1} prot)	SOD (U mg^{-1} prot)	CAT (U mg^{-1} prot)	MDA (nmol g^{-1})
Control ($n = 7$)	129.80 ± 37.40[a]	16.92 ± 1.73[a]	3.07 ± 0.17[a]	36.44 ± 2.88[a]
Treatment ($n = 7$)	343.00 ± 72.75[b]	19.61 ± 1.50[a]	2.78 ± 0.17[a]	29.20 ± 1.08[b]

Means with different superscripts (a, b) in each column are significantly different ($P < 0.05$).

Significant differences in the testicular activities of SOD and CAT were neither observed at day 6 nor on day 10 between two experimental groups. By contrast, a marked reduction had occurred in lipid peroxidation, as MDA value, in the ghrelin-treated rats. The MDA level was significantly ($P < 0.05$) lower in the ghrelin group on day 10, in which it reached to 29.20 ± 1.08 nmol g^{-1} compared with 36.44 ± 2.88 in the control animals. However, ghrelin could not significantly affect MDA content on day 6 of the experiment.

Discussion

To our knowledge, this study examined for the first time, the ability of ghrelin antioxidant properties in the rat testicular tissue. There was a remarkable increase in GPx activity especially on day 10 in the treated rats. By contrast, ghrelin decreased MDA concentration of testis in this group which means a significant reduction in lipid peroxidation within the testis. However, in our in vivo setting, chronic repeated injection of ghrelin failed to induce overt changes in SOD or CAT activities.

It is well known that utilisation of oxygen represents an efficient mechanism for aerobic organisms to generate energy, but ROS are also produced within the biological systems (Cutler, 1991). Under normal conditions to protect cells against oxidative damage, the potential harmful effects of ROS and free radicals are effectively eliminated by the antioxidant defence systems such as antioxidant enzymes and nonenzymatic factors (Hu et al., 2005). Normally, cells possess a well developed biochemical defence system, comprising low-molecular weight free radical scavengers, i.e. glutathione (GSH), vitamin C, vitamin E and complex enzymes, including GPx, SOD and CAT (Yao et al., 2007). When the balance between the generation and elimination of ROS is broken, as a result of

these events, biomacromolecules including DNA, membrane lipids and proteins are damaged by ROS-mediated oxidative stress (Tian et al., 1998; Qian et al., 2008).

The sperm plasma membrane contains a high amount of unsaturated fatty acids which can be attacked by ROS and are therefore particularly susceptible to peroxidative damage with subsequent loss of membrane integrity, impaired cell function and decreased motility of spermatozoa. This oxidative stress is one of the factors associated with decline in fertility of spermatozoa (Aitken et al., 1989; Tramer et al., 1998). Furthermore, the high level of polyunsaturated fatty acids in mammalian testes previously reported (Aitken et al., 1989; Robinson et al., 1992) suggests that the plasma membranes of most testicular cells may be greatly susceptible to free radical attack. The fact that germ cells, despite a high GSH level, are not well equipped to combat oxidative stress or xenobiotic-mediated injury probably explains the high sensitivity of these cells to ionising radiation (Le Grande, 1970), to drugs (Gomes, 1970) and also, the extremely limited viability of isolated spermatogenetic cells in culture (Chapin & Phelps, 1990).

The results of this study demonstrated that ghrelin markedly increases GPx activity, as one of the key antioxidative enzymes against oxidative damage (Raes et al., 1987; Mirault et al., 1991), in the testicular tissue of treated animals. In addition, MDA, which functions as a marker of oxidative injury of cellular membranes (Husain et al., 2001; Yao et al., 2006), significantly decreased following daily administration of ghrelin for 10 days. The concentration of MDA is a direct evidence of toxic processes caused by free radicals (Sieja & Talerczyk, 2004). Therefore, it can be concluded that ghrelin preserves the membrane of spermatozoa against oxidative stress and lipid peroxidation. These findings support and are in agreement with our new investigation, in which we

proved that chronic administration of ghrelin increases the functional membrane integrity of rat spermatozoa (Kheradmand *et al.*, Anim. Biol., article in press). Likewise, the antioxidant properties of ghrelin in the rat testis are consistent with our other study, in which we have shown that ghrelin enhances the viability of rat spermatozoa during incubation at 37 °C up to 5 h, because of its antioxidative characteristics.

The presence of different antioxidative defence systems is well documented in the rat testis. Bauche *et al.* (1994) have shown a differential distribution of these enzymes among rat testicular cells so that Sertoli and peritubular cells had elevated SOD and GSH-dependent enzyme activities associated with a high GSH content. Pachytene spermatocytes and RS are characterised by higher SOD activity and GSH content associated with very low GSH-dependent enzyme activity. Spermatozoa exhibited the same enzymatic system as PS and RS but were devoid of GSH. Interstitial tissue displayed high GPx and GSH content and moderate SOD level. No detectable activity of CAT has been reported in the testicular cells in this study. It has been also indicated that the activity of CAT is low in the different testicular cells, in which the activity of CAT in the rat testis is very low (2–7%) compared with the level in the liver (Peltola *et al.*, 1992). A similar low testicular activity of CAT has been reported in the rabbit (Ihrig *et al.*, 1974). Thus, it was expectable to record a low level of CAT activity in our study and this is probably the reason why we did not observe significant changes in CAT activity in the treated animals.

Superoxide dismutase rapidly converts superoxide anion (O_2^-) to less dangerous hydrogen peroxide (H_2O_2). GPx and CAT can decompose H_2O_2 to water. H_2O_2 is not a particularly reactive product, but it may be reduced to the highly reactive metabolites hydroxyl radicals ($OH^.$) or single oxygen (Peltola *et al.*, 1992). It has been clearly indicated that the lipid peroxidation (evaluated by MDA value) significantly increases by accumulation of H_2O_2 in a concentration-dependent manner (Garcia *et al.*, 2005). The principal mechanism of H_2O_2 toxicity is thought to involve the generation of highly reactive $OH^.$ radical through its interaction with Fe^{2+} by the Fenton reaction (Sewerynek *et al.*, 1995). It seems that the increase of GPx activity in this study causes more rapid conversion of H_2O_2 to H_2O and preventing of H_2O_2 accumulation and availability to shift for lipid peroxide production. This is further supported by the fact that we detected lower MDA content in the rat testis following chronic treatment by ghrelin. In addition, it is believed that GPx functions in the detoxification of reactive lipid peroxides (Peltola *et al.*, 1992) and therefore, the reduction in MDA concentration in this work can be justified. We did not observe significant changes in SOD activity in our experi-

ment. Likely, a prolonged treatment by ghrelin or higher dose is needed to induce greater activity of SOD. Further researches are necessary to confirm this hypothesis.

In conclusion, chronic administration of ghrelin promotes the antioxidative defence system in the rat testis by increase in GPx activity and reduction of lipid peroxidation (MDA level). This finding confirms previous reports concerning the antioxidative properties of ghrelin in other tissues (Iseri *et al.*, 2005; Kawczynska-Drozdz *et al.*, 2006; Zwirska-Korczala *et al.*, 2007; Obay *et al.*, 2008).

Acknowledgement

References

Aebi H (1984) Catalase *in vitro*. *Methods Enzymol* 105: 121–126.

Aitken RJ, Clarkson JS, Fishel S (1989) Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biol Reprod* 41:183–197.

Barreiro ML, Tena-Sempere M (2004) Ghrelin and reproduction: a novel signal linking energy status and fertility? *Mol Cell Endocrinol* 226:1–9.

Bauche F, Fouchard MH, Jegou B (1994) Antioxidant system in rat testicular cells. *FEBS Lett* 349:392–396.

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. *Methods Enzymol* 52:302–310.

Chapin RE, Phelps J (1990) Recent advances in testicular cell culture: implications for toxicology. *Toxic In Vitro* 4: 543–559.

Cutler RG (1991) Human longevity and aging: possible role of reactive oxygen species. *Ann N Y Acad Sci* 621:1–28.

Dong MH, Kaunitz JD (2006) Gastroduodenal mucosal defense. *Curr Opin Gastroenterol* 22:599–606.

Fernandez-Fernandez R, Navarro VM, Barreiro ML, Vigo EM, Tovar S, Sirotkin AV, Casanueva FF, Aguilar E, Dieguez C, Pinilla L, Tene-Sempere M (2005) Effect of chronic hyperghrelinemia on puberty onset and pregnancy outcome in the rat. *Endocrinology* 146:3018–3025.

Garcia YJ, Rodriguez-Malaver AJ, Penaloza N (2005) Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. *J Neurosci Meth* 144:127–135.

Gomes WR (1970) Physiology of Reproduction. In: Johnson AD, Gomes WR, Vandemark NL (eds). The Testis. Vol. 3, Academic Press, New York, pp 483–545.

Hu Y, Rosen DJ, Zhou Y (2005) Mitochondrial manganese-superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *J Biol Chem* 280:39485–39492.

Husain K, Scott BR, Reddy SK, Somani SM (2001) Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. *Alcohol* 25:89–97.

Ihrig TJ, Renston RH, Renston JP, Gondos B (1974) Catalase activity in the developing rabbit testis. *J Reprod Fertil* 39:105–108.

Iseri SO, Sener G, Yuksel M, Contuk G, Cetinel S, Gedik N, Yegen BC (2005) Ghrelin against alendronate-induced gastric damage in rats. *J Endocrinol* 187:399–406.

Kawczynska-Drozdz A, Olszanecki R, Jawein J, Brzozowski T, Pawlik WW, Korbut R, Guzik TJ (2006) Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. *Am J Hypertens* 19:764–767.

Kojima M, Kanagawa K (2005) Ghrelin: structure and function. *Physiol Rev* 85:495–522.

Le Grande CE (1970) Radioprotectors and Anticarcinogens. In: Johnson AD, Gomes WR, Vandemark NL (eds). The Testis. Vol. 3, Academic Press, New York, pp 333–365.

Lowry O, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the folin phenol reagent. *J Biol Chem* 193:265–275.

Mirault ME, Tremblay A, Beaudoin N, Tremblay M (1991) Overexpression of seleno-glutathione peroxidase by gene transfer enhances the resistance of T47D human breast cells to clastogenic oxidants. *J Biol Chem* 266:20752–20760.

Obay BD, Tasdemir E, Tumer C, Bilgin HM, Atmaca M (2008) Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. *Peptides* 29:448–455.

Peltola V, Huhtaniemi I, Ahotupa M (1992) Antioxidant enzyme activity in the maturing rat testis. *J Androl* 13: 450–455.

Petrie A, Watson P (1999) Statistics for Veterinary and Animal Science. Blackwell Science, London, pp 93–102.

Qian ZJ, Jung WK, Kim SK (2008) Free radical scavenging activity of a novel antioxidant peptide purified from hydrolysate of bullfrog skin, *Rana catesbeiana shaw*. *Bioresource Technol* 99:1690–1698.

Raes M, Michiels C, Remacle J (1987) Comparative study of the enzymatic defense systems against oxygen-derived free radicals: the key role of glutathione peroxidase. *Free Radic Bio Med* 3:3–7.

Robinson BS, Johnson DW, Poulos A (1992) Novel molecular species of sphingomyelin containing 2-hydroxylated poly-enoic very-long-chain fatty acids in mammalian testes and spermatozoa. *J Biol Chem* 267:1746–1751.

Sewerynek E, Poeggeler B, Melchiorri D, Reiter RJ (1995) H_2O_2-induced lipid peroxidation in rat brain homogenates is greatly reduced by melatonin. *Neurosci Lett* 195:203–205.

Sieja K, Talerczyk M (2004) Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. *Gynecol Oncol* 93:320–327.

Tena-Sempere M, Barreiro ML, Gonzalez LC, Gaytan F, Zhang FP, Caminos JE, Pinilla L, Casanueva FF, Dieguez C, Aguilar E (2002) Novel expression and functional role of ghrelin in rat testis. *Endocrinology* 143:717–725.

Tian L, Cai Q, Wei H (1998) Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. *Free Radic Bio Med* 24:1477–1484.

Tramer F, Rocco F, Micali F, Sandri G, Panfili E (1998) Antioxidant systems in rat epididymal spermatozoa. *Biol Reprod* 59:753–758.

Van Loon AAWM, Den Boer PJ, van der Schans GP, Mackenbach P, Grootegoed JA, Baan RA, Lohman PHM (1991) Immunochemical detection of DNA damage induction and repair at different cellular stages of spermatogenesis of the hamster after *in vitro* or *in vivo* exposure to ionizing radiation. *Exp Cell Res* 193:303–309.

Wren AM, Small CJ, Thomas EL, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR (2001) Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 50:2540–2547.

Yada T, Kaiya H, Mutoh K, Azuma T, Hyodo S, Kangawa K (2006) Ghrelin stimulates phagocytosis and superoxide production in fish leukocytes. *J Endocrionol* 189:57–65.

Yao P, Li K, Jin Y, Song F, Zhou S, Sun X (2006) Oxidative damage after chronic ethanol intake in rat tissues: prophylaxis of *Ginkgo biloba* extract. *Food Chem* 99:305–314.

Yao P, Li K, Song F, Zhou S, Sun X, Zhang X, Nussler AK, Liu L (2007) Heme oxygenase-1 upregulated by *Ginkgo biloba* extract: potential protection against ethanol-induced oxidative liver damage. *Food Chem Toxicol* 45:1333–1342.

Zwirska-Korczala K, Adamczyk-Sowa M, Sowa P, Pilc K, Suchanek R, Pierzchala K, Namyslowski G, Misiolek M, Sodowski K, Kato I, Kuwahara A, Zabielski R (2007) Role of leptin, ghrelin, angiotensin Π and orexins in 3T3 L1 preadipocyte cells proliferation and oxidative metabolism. *J Physiol Pharmacol* 58:53–64.