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Regular moderate exercise alleviates gastric oxidative damage in rats via the contribution of oxytocin receptors

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Key points

- A moderate level of exercise has beneficial effects for the prevention of gastric ulcers.
- Although regular aerobic exercise was shown to elevate serum oxytocin levels and exogenously administered oxytocin exerts an anti-ulcer activity, the role of endogenous oxytocin in the gastroprotective effects of exercise has not yet been elucidated.
- We showed that increased anxiety and oxidative gastric damage induced by gastric ulcers were reversed in pre-exercised rats, while reduced hypothalamic oxytocin expression and decreased myenteric oxytocin receptor expression due to gastric ulcers were abolished by exercise.
- We also reported that the blockade of oxytocin receptors exaggerated gastric damage in exercised rats with ulcers.
- Our data establish that endogenous oxytocin is the key mediator in the beneficial effects of regular physical activity in alleviating gastric injury.

Abstract Exercise increases serum oxytocin levels and exogenous oxytocin exerts an anti-ulcer activity; but the role of oxytocin in the protective effects of exercise against gastric ulcers has not yet been evaluated. This study was designed to investigate the impact of regular swimming exercise on oxidative gastric injury, and the role of oxytocin receptor activity in the anxiolytic and anti-inflammatory actions of exercise. Adult Wistar albino rats of both sexes performed swimming exercise (30 min/day, 5 days) or stayed sedentary. At the end of the 6-week exercise/sedentary protocol, rats were injected intraperitoneally with atosiban (0.1 mg/kg/day) or saline for 4 days. On the 5th day, under anaesthesia, acetic acid (ulcer) or saline (sham) was applied onto the gastric serosa and the treatments were continued. On the 9th day, anxiety levels were determined; gastric blood flow was measured, and blood, gastric and brain tissues were obtained. Induction of ulcers in sedentary rats increased anxiety and serum corticosterone levels; but reduced

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gastric blood flow and resulted in apoptosis and oxidative gastric damage with increased cytokine expressions. However, when ulcers were induced in pre-exercised rats, behavioural and biochemical alterations due to gastric damage were reversed. The inhibition of oxytocin receptors by atosiban exaggerated pro-inflammatory cytokine expressions and gastric lipid peroxidation in the stomachs of exercised rats with ulcers. When rats had regularly exercised prior to ulcer induction, reductions in the immunolabelling of hypothalamic oxytocin and myenteric oxytocin receptors were abolished, suggesting that exercise-induced alleviation of gastric injury may involve the reversal of down-regulated oxytocinergic activity.

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Introduction

Peptic ulcer disease (PUD), characterized by lesions penetrating to the muscularis mucosae and lamina propria of the stomach or proximal duodenum (Najm, 2011), has a 4.1% prevalence (Aro *et al.* 2006) and affects 4 million people per year worldwide (Chung & Shelat, 2017). Increased secretion of gastric acid, increased production of reactive oxygen species (ROS) and pro-inflammatory cytokines, accompanied by a disrupted mucosal blood flow are involved in the pathophysiology of PUD (Sørbye & Svanes, 1994; Konturek *et al.* 2000; Szabo & Tarnawski, 2000; Liu *et al.* 2013*a*). Non-pharmacological treatment and prevention of PUD is mainly based on an active lifestyle with favourable health behaviours, including a healthy diet, stress-coping and regular exercise (Yegen, 2018). Apart from its anxiety-relieving effects (Bahrke & Morgan, 1978), the protective effects of exercise on ulcerogenesis is reported to occur by reducing gastric acid secretion (Markiewicz *et al.* 1977) and increasing gastric mucosal blood flow (Pique *et al.* 1989), along with the maintenance of an intact gastric mucosal barrier (Kwiecien *et al.* 2012). A moderate level of exercise was shown to exert beneficial effects by fine-tuning ROS production (Peternelj & Coombes, 2011; Bouzid *et al.* 2018) and by the activation of adaptive anti-oxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase, etc.), which prepare the gastric mucosa for any upcoming injurious events (Navarro *et al.* 2004; Ji *et al.* 2006; Bahadir *et al.* 2016; Simioni *et al.* 2018). However, hormonal mechanisms that could mediate the beneficial effects of exercise on the prevention of gastric ulcer have not been fully described yet.

In addition to its regulatory functions in several organ systems, the hypothalamic nonapeptide oxytocin is reported to attenuate gastric acid secretion (Asad *et al.* 2001*b*), and regulate gastrointestinal motility, intestinal inflammation and mucosal permeability (Qin *et al.* 2009; Welch *et al.* 2014). The central administration of oxytocin was shown to exhibit gastric cytoprotection and inhibit the development of PUD in rats (Grassi & Drago, 1993; Asad *et al.* 2001*a*). The anti-inflammatory actions of exogenously administered oxytocin involve the inhibition of neutrophil infiltration and pro-inflammatory cytokine production at the site of the tissue injury (Işeri *et al.* 2005; Al-Amran & Shahkolahi, 2014). Although a single injection of oxytocin was reported to stimulate the activity of the hypothalamo–pituitary–adrenal (HPA) axis, its long-term administration was shown to exert an anti-stress action via the inhibition of the HPA axis activity (Petersson *et al.* 1999). On the other hand, elevation of endogenous oxytocin levels under psychosocial stress was suggested to be associated with increased cortisol reactivity in humans, verifying its role as a stress-reducing hormone that aids the recovery from sustained stress (Heinrichs *et al.* 2009; Engert *et al.* 2016). Regular aerobic exercise, which has been reported to elevate plasma oxytocin levels in humans (Landgraf *et al.* 1982; Irianti *et al.* 2017), is universally acknowledged as an advantageous tool in stress reduction and for a better mood (Hamer *et al.* 2012). Similarly, exercise training in rodents has suppressed anxiety and enhanced oxytocin levels in the blood, cardiac and brain tissues (Gutkowska *et al.* 2007; Yüksel *et al.* 2019), implying that the beneficial effects of exercise could involve oxytocin-mediated mechanisms. However, the role of endogenous oxytocin in the gastroprotective effects of exercise has not yet been elucidated. This study was designed to investigate the possible protective effects of regular swimming exercise in alleviating oxidative gastric injury and the role of oxytocin receptor activity in the anxiolytic and anti-inflammatory actions of regular swimming exercise performed before gastric ulcer induction.

Materials and methods

Ethical approval

Experiments were designed and performed in compliance with the Turkish law on the use of animals in experiments and with the guidelines of the New York Academy of Sciences. All experimental protocols were approved by the Marmara University Animal Care and Use Committee

(approval code: 77.2017.mar; date: 6.11.2017). The ethical principles of the journal are understood, and the study complies with the checklist of animal ethics.

Animals

Adult Wistar albino rats of both sexes (220–280 g) were supplied from the Marmara University Animal Centre (DEHAMER) and housed in an air-conditioned room with controlled humidity, temperature (22 ± 0.5°C) and light/dark (12/12 h) cycles. The animals were given standard rat pellets and water *ad libitum*.

Experimental and surgical procedures

All rats were adapted to swimming training for 5 days (15 min/day) in a cylindrical plastic container (150 × 50 × 25 cm) filled with 50 cm deep water maintained at 30 ± 2°C temperature. Then, the rats were randomly chosen to either perform swimming exercise (30 min/day swimming, 5 days/week; $n = 32$) in the same container or to stay sedentary ($n = 32$) in a water-filled (30 ± 2°C) container that could only wet their feet (Radák *et al.* 2001) (Fig. 1). At the end of the 6-week exercise/sedentary protocol, half of the rats in each group were injected intraperitoneally with atosiban, an oxytocin/vasopressin V1A receptor antagonist (0.1 mg kg^{-1}/day in saline; Sigma-Aldrich, Missouri, USA), while the other half were injected with saline for 4 days. The rationale for the selected dose of atosiban was based on our earlier study (Memi & Yeğen, 2016). On the 5th day of treatment, rats were anaesthetized (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneally) for the application of either acetic acid (ulcer groups) or saline (sham groups) onto the serosa of the stomach. Intraperitoneal injections of atosiban and saline were continued for the following post-surgical 3 days. On the postsurgical 4th day, all rats were anaesthetized (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneally) for the measurement of gastric blood flow. Before killing, a cardiac puncture was made under anaesthesia to obtain trunk blood, gastric and brain tissues for the biochemical and histopathological analyses.

Ulcer induction

Gastric ulcers were induced using the method that was originally defined by Okabe *et al.* (1971). At the end of the 6-week swimming exercise or sedentary state, and after fasting overnight, an upper abdominal midline incision was made under anaesthesia (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneally). A half-millilitre of acetic acid (80%, vol/vol) in a 3 ml syringe was applied onto the serosal surface of the gastric corpus, exposing the acid content on a 60 mm^2 area. Following 1 min contact, acetic acid was aspirated and gently washed out from the serosal surface with saline and the incision was closed. In the sham groups, rats were subject to the same surgical procedure, but a saline-containing syringe was applied to the gastric serosa. Since it was reported that chronic mucosal ulcers occur within 2–3 days after the induction, and heal completely within 2–3 weeks without perforation or penetration to the surrounding organs (Okabe *et al.* 1971), we chose the postsurgical 4th day for the evaluation of blood flow and biochemical measurements of the experimental groups.

Evaluation of the anxiety level

At 3 h before killing, a hole-board test was applied to all rats, using a wooden box (100 × 100 × 50 cm) with 16 holes (3.8 cm diameter) equally distributed at the bottom. Each rat was put individually in one of the corners of the box and its movements were recorded by a video camera for 5 min. Before the next test, the box was cleaned with alcohol to eliminate the odour. In order to evaluate the anxiety level of rats based on their exploratory behaviour in the box (Brown & Nemes, 2008), the numbers of head-dips into the holes and rearing up on two hind legs were then counted from video recordings by an observer blinded to the experimental groups. Decreased numbers of head-dips and rearing up of rats indicated reduced exploratory behaviour, and hence increased anxiety.

Gastric blood flow measurement

On the day of killing, rats were anaesthetized (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneally) to evaluate the gastric serosal blood flow using a laser Doppler flow-meter (PeriFlux System 5000, Perimed, Sweden). The probe was fixed on the corpus of the stomach. After a 5 min recording for stabilization, a 10 min period was used to calculate the average blood flow, which was expressed in perfusion unit (Sarnik *et al.* 2007). Then cardiac puncture was performed to obtain blood and the experimental animals were killed.

Measurement of serum corticosterone and gastric 8-hydroxy-2′-deoxyguanosine levels

Both the serum levels of corticosterone and gastric levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Gastric tissue and serum samples were stored at −80°C. Genomic DNA was directly extracted from gastric samples using a commercial DNA extraction kit in accordance with the manufacturer's procedure (Invitrogen, USA). Gastric 8-OHdG levels were measured using competitive

ELISA (OxiSelect Oxidative DNA Damage ELISA Kit, Cell Biolabs, USA) and serum total corticosterone levels were also determined by ELISA (Shanghai Sunred Biological Technology, China), using the commercial kits in accordance with the manufacturers' directives.

Measurement of gastric malondialdehyde (MDA) and glutathatione (GSH) levels and myeloperoxidase (MPO) activity

Homogenized gastric samples (in 10% trichloracetic acid) were centrifuged (4°C, 3000 rpm, 15 min) and obtained supernatants were then re-centrifuged (4°C, 15,000 rpm, 8 min). The lipid peroxide levels were determined by spectrophotometry at 535 nm wave-length and expressed as nanomoles of MDA per gramme tissue. For the measurement of GSH levels, a modified Ellman protocol was used (Tuğtepe *et al.* 2007) and expressed in µmol per gramme tissue.

Homogenized gastric samples were centrifuged (4°C, 12,000 rpm, 10 min) in 50 mM potassium phosphate buffer. After the pellets were placed in 50 mM potassium phosphate buffer (mixed with 1 g of hexadecyltrimethylammonium bromide and 0.5 g of EDTA), MPO activity was determined based on H_2O_2-dependent oxidation of o-dianisidine.2HCl at 460 nm of the spectrophotometer and expressed as units per gramme tissue (Tuğtepe *et al.* 2007). Tissue MPO activity measured by this method was previously shown to correlate significantly with histochemically measured neutrophil infiltration to the inflamed tissues (Bradley *et al.* 1982).

Chemiluminescence assay

In order to assess the levels of ROS, luminol- (5-amino-2,3-dihydro 1,4 phthalazinedione) and lucigenin- (bis-N methylacridinium nitrate) enhanced chemiluminescence levels were measured at room temperature with a luminometer (Junior LB 9509, EG&G, Berthold, Germany). It is accepted that luminol will detect a group of ROS, i.e. UOH, H_2O_2, HOCl radicals, while lucigenin is mainly selective for superoxide anions. After the specimens were put into vials containing PBS-HEPES buffer (0.5 M PBS containing 20 mM HEPES at pH 7.2), lucigenin or luminol enhancers were added to reach a final concentration of 0.2 mM. Counts were obtained at 1 min intervals for 5 min and the results were given as the area under curve corrected for wet tissue weights (rlu/mg tissue) (Haklar *et al.* 2002).

Western blot analyses for protein expressions of cytokines

Western blot analyses and measurements were performed as previously described (Arabacı Tamer *et al.* 2019). The BCA assay (Thermo Scientific, USA) was used to detect protein concentrations in homogenized gastric samples. Afterwards, 25 µg of protein was resolved in 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and was transferred to PVDF membrane (sc-3718, Santa Cruz Biotechnology, USA), which was blocked with 3% BSA in Tris-buffered saline (TBS), washed twice in TBS plus Tween (TBS containing 0.1% Tween-20) and incubated overnight with the primary antibody (1:500 anti-TGF-β, anti-IL-6,

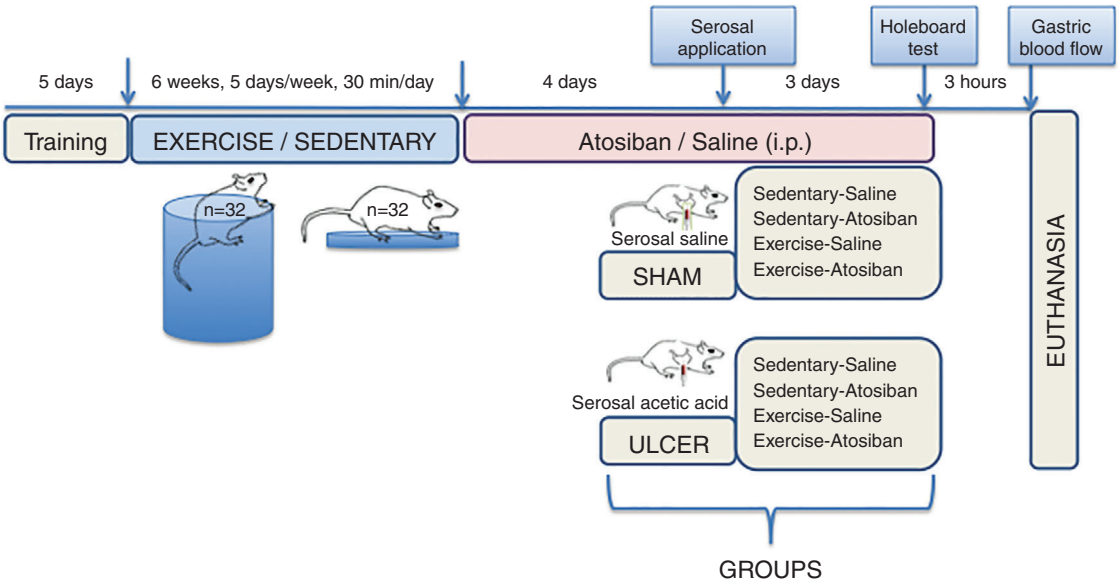

Figure 1. Experimental design
The timeline scheme of the procedures applied throughout the experimental protocol. [Colour figure can be viewed at wileyonlinelibrary.com]

anti-IL-8, anti-IL-10, anti-TNF-α-, anti-β-actin; Santa Cruz Biotechnology). The membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000 anti-mouse IgG1-HRP, sc-2060 and anti-rabbit IgG-HRP sc-2004, Santa Cruz Biotechnology) for 2 h. Chemiluminescence reagents (sc-2048, Santa Cruz Biotechnology) using a Chemiluminescent Imaging System (Syngene, USA) were used to detect the blot. Data were analysed using the ImageJ OD analysis software. Signals were normalized with respect to β-actin (Tamer *et al.* 2019).

Measurement of gastric caspase-3 activity

Caspase-3 activity assay was performed using the caspase-3 cellular activity assay kit (Calbiochem, San Diego, USA) according to the manufacturer's instructions. The DEVDpNA cleavage activity was calculated as pmol/min/mg protein. Protein concentration in gastric tissue samples was determined using the Bradford method.

Histopathological examination and immunohistochemistry

For light microscope investigations, gastric tissues were fixed in a 10% formaldehyde solution, processed by routine techniques, and were then embedded in paraffin. Tissue sections at 5 µm thickness were mounted on slides and stained by haematoxylin and eosin and examined under a microscope (Olympus Bx51, Tokyo, Japan). Histological assessment was made by an experienced histologist (FE), who was blinded to group names. Gastric injury was evaluated semi-quantitatively with a maximum score of 12 (0, none; 1, mild; 2, moderate; 3, severe) by using the criteria: desquamation of surface epithelium (0–3); haemorrhage, focal necrosis and mucosal congestion (0–3); degeneration of glandular cells (0–3); inflammatory cell infiltration (0–3) (Wang *et al.* 1999).

For immunohistochemistry, 3 µm thick paraffin sections were obtained from the stomach and brain tissues. After de-paraffinization and rehydration steps, endogenous peroxidase activity was blocked by incubating the sections in 3% H_2O_2 solution. Antigen retrieval was performed by keeping the sections in sodium citrate buffer solution (pH 6.0) in a microwave (200 watts) for 20 min. Stomach tissue sections were incubated in protein-blocking solution (R.T.U. Vectastain Kit, horse serum, Burlingame, CA) and then in goat polyclonal anti-oxytocin receptor primary antibody (1:400; Abcam, ab87312). Sections were then incubated in biotinylated secondary antibody and in streptavidin horseradish peroxidase complex (R.T.U. Vectastain Kit, Burlingame, CA). Sections from the brain tissues were incubated in blocking solution (Super Block, SensiTek HRP Anti-Polyvalent Lab Pack, SHP125) and then in rabbit monoclonal anti-oxytocin (1:8000; Abcam, ab212193) and rabbit polyclonal anti-corticotropin-releasing factor (CRF) antibodies (1:200; Abcam, ab216599) at 4°C overnight. After rinsing in phosphate buffer, the sections were incubated in biotinylated secondary antibody and streptavidin horseradish peroxidase complex (SensiTek HRP Anti-Polyvalent Lab Pack, SHP125). Reaction products were observed by using 3′-3′-diaminobenzidine (DAB, Abcam, ab80436). Mayer's haematoxylin was used for counterstaining. The sections were examined and photographed with an Olympus DP72 CCD camera attached to a BX51 light microscope. Staining intensity was scored using a semi-quantitative method (0: no staining, 1: mild staining, 2: moderate staining, 3: intense staining).

Statistical analysis

Statistical analyses were carried out using GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Each subgroup consisted of 6–8 (3–4 male and 3–4 female) rats. All data were expressed as means ± SD. Difference between groups was analysed by three-way ANOVA followed by the Bonferroni multiple comparisons test. $P < 0.05$ was considered to be statistically significant.

Results

Behavioural tests and the serum levels of corticosterone were performed to evaluate the degree of anxiety in the experimental groups. Reduction in the free exploratory behaviour (head-dipping and rearing up) of the rats indicated increased anxiety. When compared with the sedentary rats, regular swimming exercise performed for six weeks elevated the number of rearings up by ulcer-induced rats ($P < 0.05$; Fig. 2*A*), indicating the anxiolytic effect of exercise. Similarly, regular exercise increased the rearing up by the non-ulcerated rats, but this increase was not statistically significant. In ulcer-induced sedentary rats, the number of head-dips was significantly decreased ($P < 0.05$; Fig. 2*B*), showing enhanced anxiety by ulcer induction. On the other hand, having exercised before ulcer induction, prevented the reduction in head-dipping. When the exercised rats with ulcers were treated with the oxytocin antagonist atosiban, the exercise-induced increase in rearing up was abolished, suggesting that atosiban prevented the anxiolytic effects of exercise. In support of the aforementioned behavioural findings, serum corticosterone levels showed a tendency to rise in the ulcer-induced sedentary rats, but no statistical difference was reached (Fig. 2*C*). However, atosiban treatment given to sham rats with no ulcer induction resulted in elevated serum levels of corticosterone as compared to saline-treated sham groups ($P < 0.05$). On

the other hand, the elevation in serum corticosterone level due to atosiban treatment was abolished in the ulcer-induced rats ($P < 0.05$).

In an attempt to find any potential differences that could occur in gastric circulation, serosal blood flow was determined in the rats with gastric injury using laser Doppler. In the saline-treated sedentary groups, ulcer induction significantly decreased the mean serosal blood flow ($P < 0.05$), but no reduction in the blood flow was observed in the pre-exercised ulcer group (Fig. 3*A*). On the other hand, the blood flow in atosiban-treated non-ulcer or ulcer groups, either exercised or sedentary, was similar to that of the saline-treated sedentary sham group.

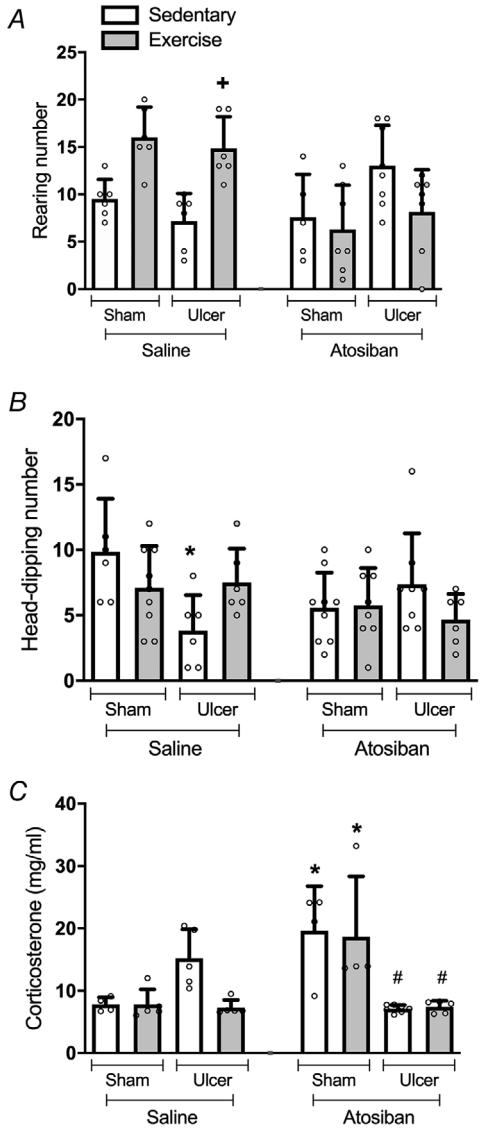

Figure 2. Numbers of rearing (*a*) and head-dipping (*b*) recorded for 5 min during the hole-board test and serum corticosterone levels (*c*) of all experimental groups
$n = 6$–8, with 3–4 females and 3–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. $^*P < 0.05$, compared to the saline-treated sedentary sham group; $+P < 0.05$, compared to the saline-treated sedentary ulcer group; # $P < 0.05$, compared to the atosiban-treated sedentary sham group.

Tissue levels of MDA, showing lipid peroxidation, as well as antioxidant GSH content were determined to evaluate the extent of oxidative damage, while the measured luminol- and lucigenin-enhanced chemiluminescences displayed the degree of ROS generation. In the sedentary rats, serosal application of acetic acid resulted in elevated gastric levels of MDA and MPO activity along with depleted GSH levels ($P < 0.001$; Fig. 3*B*–3*F*), while luminol- and lucigenin-enhanced chemiluminescences were not significantly changed. Having exercised prior to ulcer induction abolished the elevation in MDA level ($P < 0.001$), reversed the changes in MPO activity and GSH content. In intact rats with no ulcer induction, atosiban *per se* resulted in diminished GSH content and increased MPO activity ($P < 0.01$), while the induction of ulcers did not cause any additional effects. Moreover, exercise-induced suppression of the MDA level in ulcerated rats was not observed upon atosiban treatment.

Although protein expression levels of the pro-inflammatory and the anti-inflammatory cytokines (TGF-β, TNF-α, IL-8, IL-6 and IL-10) showed a tendency to increase in the stomachs of ulcer-induced rats, a statistical significance was reached only in the TNF-α levels of exercised rats with ulcers ($P < 0.05$; Fig. 4). Moreover, blockade of oxytocin receptors in the ulcerated rats also elevated TNF-α levels similar to those of the saline-treated ones ($P < 0.01$). However, when the exercised rats with ulcers had received atosiban injections, gastric protein expressions of TGF-β, TNF-α, IL-8 and IL-6 were highly elevated ($P < 0.01$–0.001), along with a non-significant elevation in IL-10 expression.

Gastric levels of caspase-3 activity and 8-OHdG, which are indicative of apoptosis and DNA damage, were both increased with ulcer induction ($P < 0.001$), while being sedentary or having exercised had no impact on these levels (Fig. 5*A* and *B*). Atosiban-treated sham rats also had a significantly elevated 8-OHdG level ($P < 0.001$), which was further increased in ulcer-induced rats that had received atosiban treatment ($P < 0.001$). Similarly, caspase-3 activity was increased more than twofold in atosiban-treated, sedentary and ulcerated rats ($P < 0.001$), but this elevation was abolished when rats had exercised.

Microscopic examination revealed a regular mucosa and a submucosa in all the sham groups, either sedentary/exercised or treated with saline/atosiban (Fig. 6). In parallel with the aforementioned biochemical findings, macroscopic observations and histological data demonstrated that ulcer induction in sedentary rats has resulted in high damage scores ($P < 0.001$), as evidenced by surface epithelial and glandular damage, severe mucosal haemorrhage and inflammatory cell infiltration in both

mucosal and submucosal layers. In the pre-exercised and saline-treated ulcer group, milder gastric damage was noted ($P < 0.05$). However, among the atosiban-treated groups, having previously exercised did not alter the severity of gastric injury.

A mild immunoreactivity of CRF was observed in the hypothalamus (supraoptic nucleus) of the saline-treated sedentary group with no ulcer (Fig. 7). Among the exercised groups, saline-treated sham, atosiban-treated sham and saline-treated ulcer groups showed moderate CRF immunoreactivity. Similarly, a moderate CRF immunoreactivity was observed in the sedentary sham group treated with atosiban. However, in contrast to other exercised groups, CRF immunoreactivity was mild in the atosiban-treated and pre-exercised ulcer group.

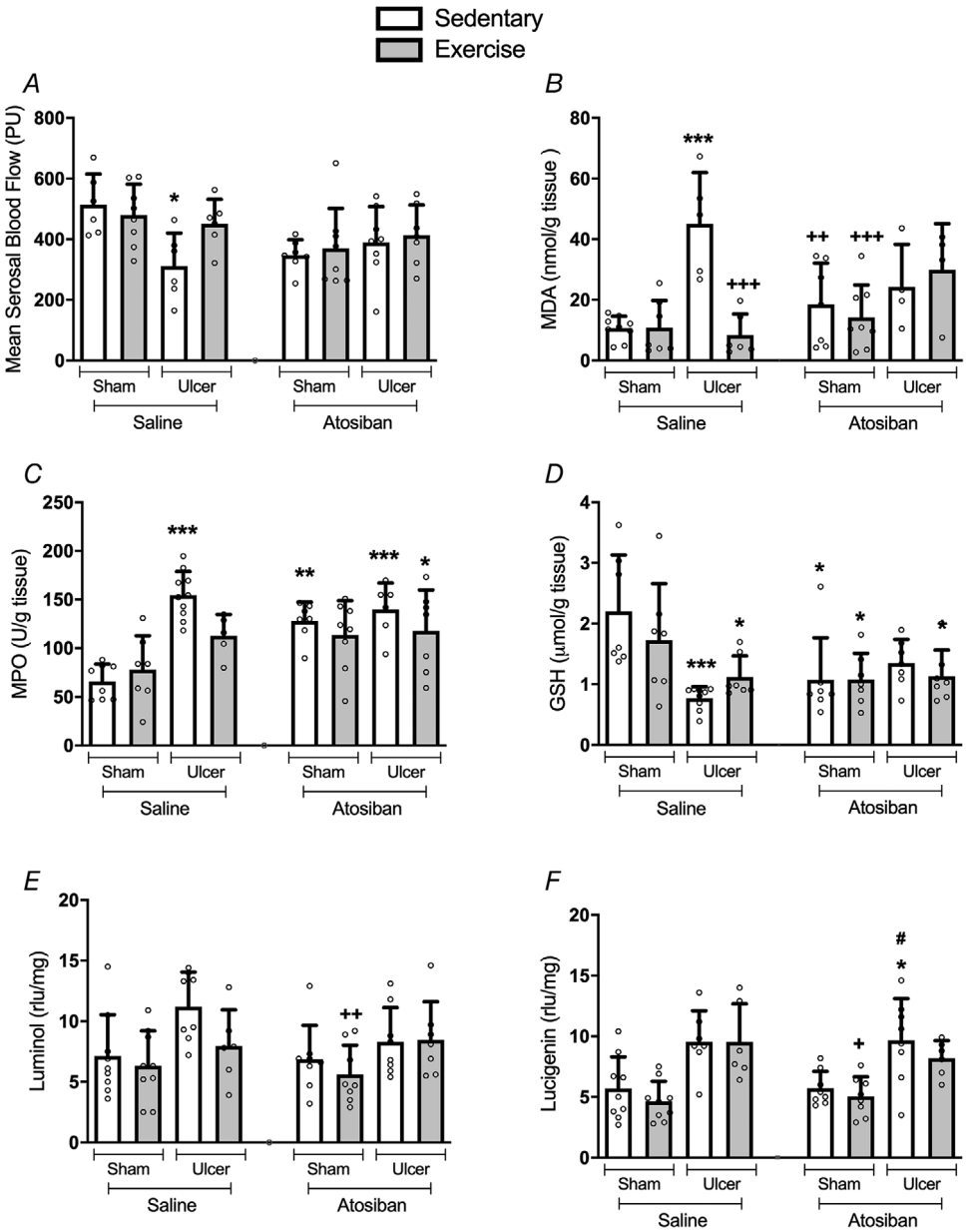

Figure 3. Mean gastric serosal blood flow (*a*) and gastric levels of malondialdehyde (MDA) (*b*), myeloperoxidase (MPO) activity (*c*), glutathione (GSH) levels (*d*) and luminol- and lucigenin-enhanced chemiluminescences (*e*, *f*) in all the experimental groups

n = 6–8, with 3–4 females and 3–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA.) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the saline-treated sedentary sham group; $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$, compared to the saline-treated sedentary ulcer group; $\#P < 0.05$, compared to the atosiban-treated sedentary sham group.

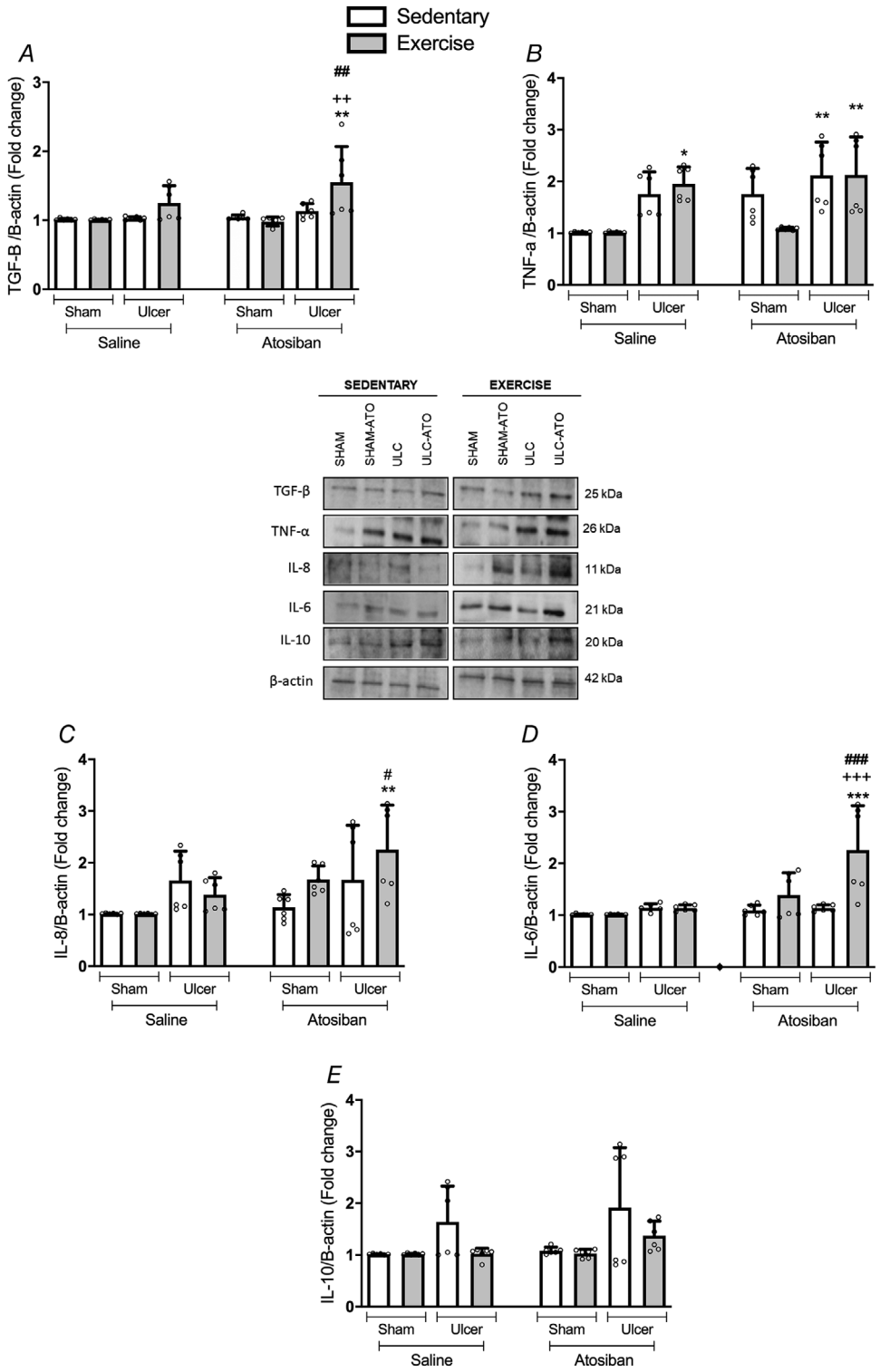

Figure 4. Expressions of transforming growth factor (TGF)-β (*a*), tumour necrosis factor (TNF)-α (*b*), interleukin-8 (IL-8), IL-6, IL-10 (*c*–*e*) in the gastric tissues of all the experimental groups
n = 6–8, with 3–4 females and 3–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. After the proteins were transferred to the membrane, the membrane was cut and exposed to the primary antibody. Since primary antibodies were used more than once, the bands were calculated by overlapping the membranes marked by a marker. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the saline-treated sedentary sham group; $^{++}P < 0.01$, $^{+++}P < 0.001$, compared to the saline-treated sedentary ulcer group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to the atosiban-treated sedentary sham group.

Saline-treated sham groups, both the exercised and sedentary ones, showed mild oxytocin immunoreactivity in their hypothalamus (Fig. 8). Oxytocin immunoreactivity was nearly absent in the ulcer-induced sedentary group, while it was significantly increased in the hypothalamus of the ulcerated rats that had previously exercised ($P < 0.05$). On the other hand, oxytocin immunoreactivity was mild in the atosiban-treated ulcer or sham groups that had exercised or remained sedentary.

Oxytocin receptor immunoreactivity was positive in the gastric myenteric plexus of all rats, and it was detected as mild to moderate intensity in the sham groups and atosiban-treated ulcer groups (Fig. 9). However, oxytocin receptor immunoreactivity in the myenteric plexus of the atosiban-treated sedentary sham group was significantly elevated ($P < 0.05$) with respect to slightly decreased immunoreactivity of the saline-treated sedentary ulcer group.

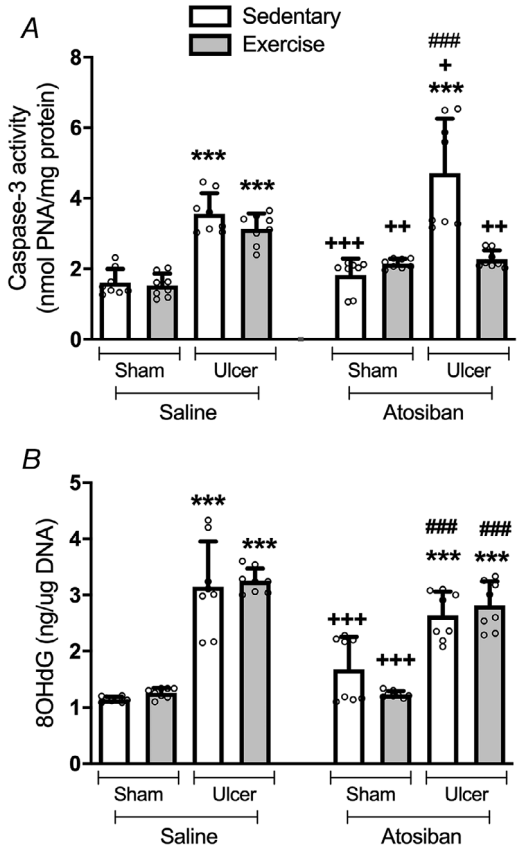

Figure 5. Caspase-3 activity (*a*) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels (*b*) in the gastric tissues of all the experimental groups
n = 6–8, with 3–4 females and 3–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. ***$P < 0.001$, compared to the saline-treated sedentary sham group; +$P < 0.05$, ++$P < 0.01$, +++$P < 0.001$, compared to the saline-treated sedentary ulcer group; ###$P < 0.001$, compared to the atosiban-treated sedentary sham group.

Discussion

The findings demonstrated that induction of ulcers in sedentary rats increased anxiety, reduced gastric blood flow and resulted in apoptosis and oxidative gastric damage. However, when ulcers were induced in pre-exercised rats, histologically assessed damage was alleviated, behavioural and biochemical alterations due to gastric damage were reversed, but apoptosis and DNA damage were sustained. Inhibition of oxytocin receptors by atosiban exaggerated the expressions of pro-inflammatory cytokines in the stomachs of exercised rats with ulcers. Even in the absence of ulcer induction, atosiban increased corticosterone level, resulted in oxidative stress and DNA damage in the stomachs of sedentary rats. Reductions in the immunolabelling of the hypothalamic oxytocin as well as myenteric oxytocin receptors, which were observed in the sedentary rats with gastric ulcers, were abolished when the rats had regularly exercised prior to ulcer induction. Thus, these findings suggest that exercise-induced alleviation of gastric injury involves the reversal of down-regulated expression of hypothalamic oxytocinergic neurons as well as gastric oxytocin receptors, while the inhibition of oxytocin receptor activation partially reverses the gastroprotective effects of exercise.

Physical activity, which reduces the risk for developing chronic diseases (Alessio *et al.* 2005; Beavers *et al.* 2010), is also regarded as a non-pharmacological approach for reducing ulcer incidence in men (Cheng *et al.* 2000*a*; Radak *et al.* 2008). Prolonged moderate-intensity exercise, in contrast to acute and intensive exercise, was proven to reduce gastric mucosal damage and alleviate the stress response with reductions in the circulating levels of cortisol (Cheng *et al.* 2000*b*; Bahadir *et al.* 2016; Shephard, 2017). Although exercise was found to increase serum oxytocin levels (Irianti *et al.* 2017) and peripheral administration of oxytocin was reported to exert an anti-ulcer activity (Asad *et al.* 2001*b*), the role of oxytocin in the protective effect of exercise on gastric ulcer has not yet been evaluated. Our current data revealed that exercise-induced alleviation of gastric damage is partially dependent on the activity of oxytocin receptors. Oxytocin is suggested to be involved in the feedback regulation of the HPA axis, because elevated oxytocin levels accompany high levels of ACTH (Grenbäck *et al.* 2007; Goldman *et al.* 2008). It was shown that peripherally injected oxytocin suppressed HPA axis activity and lowered the corticosterone levels in rats and decreased the expression of hippocampal glucocorticoid receptor mRNA (Petersson *et al.* 1999; Petersson & Uvnäs-Moberg, 2003; Grenbäck *et al.* 2007; Goldman *et al.* 2008). Thus, co-secreted from the same hypothalamic nucleus with vasopressin, oxytocin has a feedback control on the HPA axis activity (Yeğen, 2010). The studies have shown that endogenous glucocorticoids

may facilitate the healing of gastric erosions and injury, while delayed healing occurs at pharmacological doses (Filaretova, 2011). Present findings demonstrated that exercise abolished ulcer-induced anxiety and suppressed corticosterone levels, suggesting the inhibition of the HPA axis by exercise. However, this anxiolytic effect of exercise was not observed when the oxytocin/vasopressin V1 receptors were blocked by atosiban. It was shown that vasopressin is not involved in the regulation of basal HPA axis activity (Makara *et al.* 2004; Roper *et al.* 2011), but blockade of cerebral oxytocin receptors was shown to increase both basal and stress-induced corticosterone secretion in rats (Neumann *et al.* 2000). In primates, when endogenous release of oxytocin was suppressed by social isolation, HPA axis activity and anxiety-like behaviours were increased (Rukstalis & French, 2005). Likewise, enhanced anxiety-like behaviour with higher corticosterone levels was observed in oxytocin-deficient

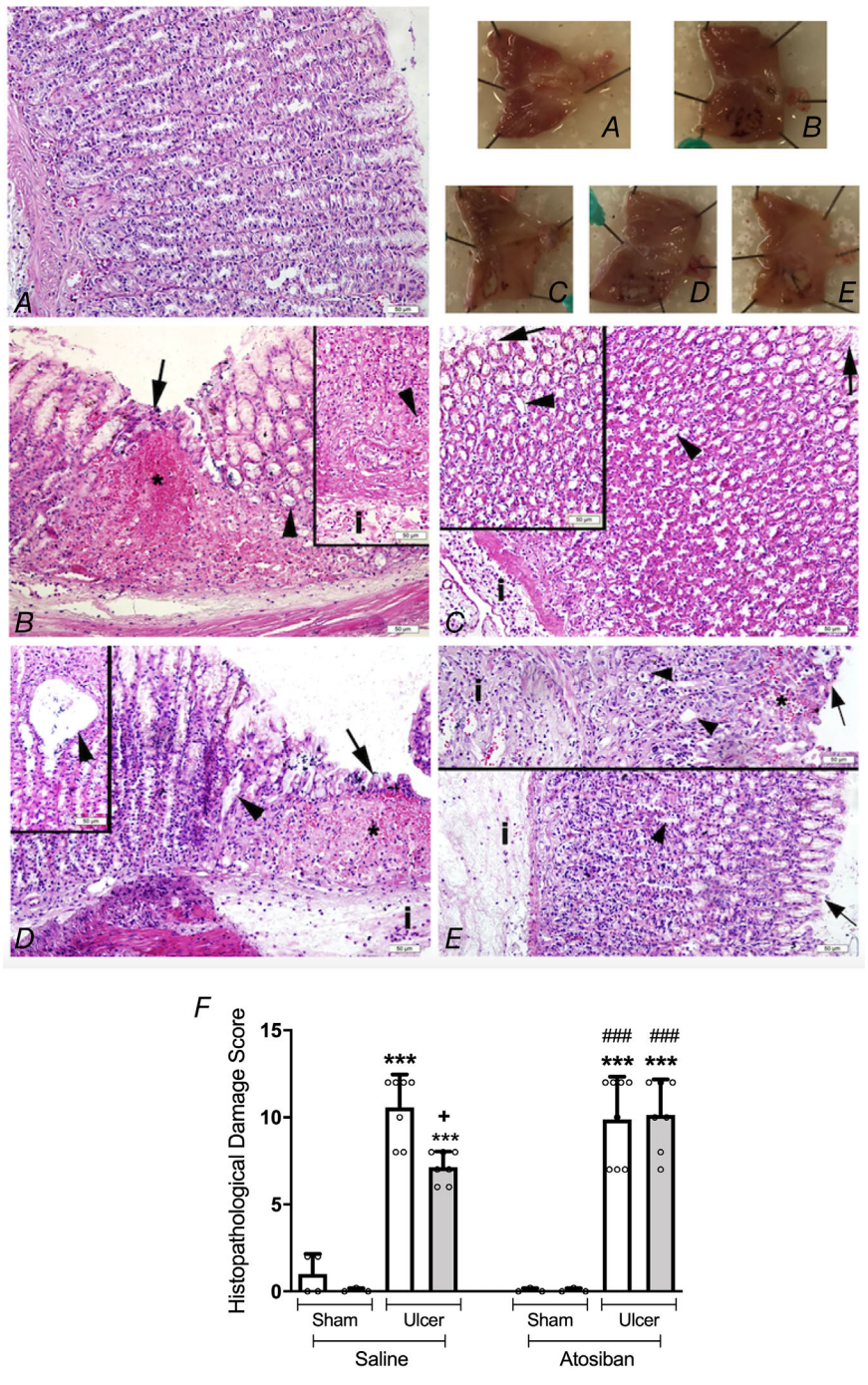

Figure 6. Representative macroscopic and microscopic images of the gastric tissues and histopathological damage scores of all the experimental groups
Surface epithelial damage (arrow), glandular epithalial damage (arrowhead), mucosal bleeding (∗), inflammatory cell infiltration (i) are seen in the sham (*A*: saline sedentary) and ulcer-induced groups (*B*: saline-treated sedentary; *C*: saline-treated pre-exercised; *D*: atosiban-treated sedentary; *E*: atosiban-treated pre-exercised), and the histopathological damage scores of all the experimental groups (*F*). (n = 6–8, with 3–4 females and 3–4 males in each subgroup). Data are the mean ± SD. Data were analysed using a three-way ANOVA. ***$P < 0.001$, compared to the saline-treated sedentary sham group; +$P < 0.05$, compared to the saline-treated sedentary ulcer group; ###$P < 0.001$, compared to the atosiban-treated sedentary sham group. Haematoxylin and eosin staining. [Colour figure can be viewed at wileyonlinelibrary.com]

mice (Amico *et al.* 2004). Thus, it appears that oxytocin receptors may be responsible for the exercise-induced inhibition of the HPA activity. Moreover, down-regulation of hypothalamic oxytocin as well as myenteric oxytocin receptors in response to gastric ulcerogenesis in sedentary rats was not evident in the exercised rats, verifying the role of exercise in up-regulating the oxytocinergic activity.

In the gastrointestinal tract of humans, expression of oxytocin–immunoreactive cell bodies was detected in both the myenteric and the submucous ganglia, which implicates oxytocin in the regulation of secretomotor and vasomotor reflexes (Ohlsson *et al.* 2006). In accordance with that, oxytocin was proven to exert a vasodilatory effect on small and peripheral arteries (Rabow *et al.* 2018). Our current findings showed that induction of

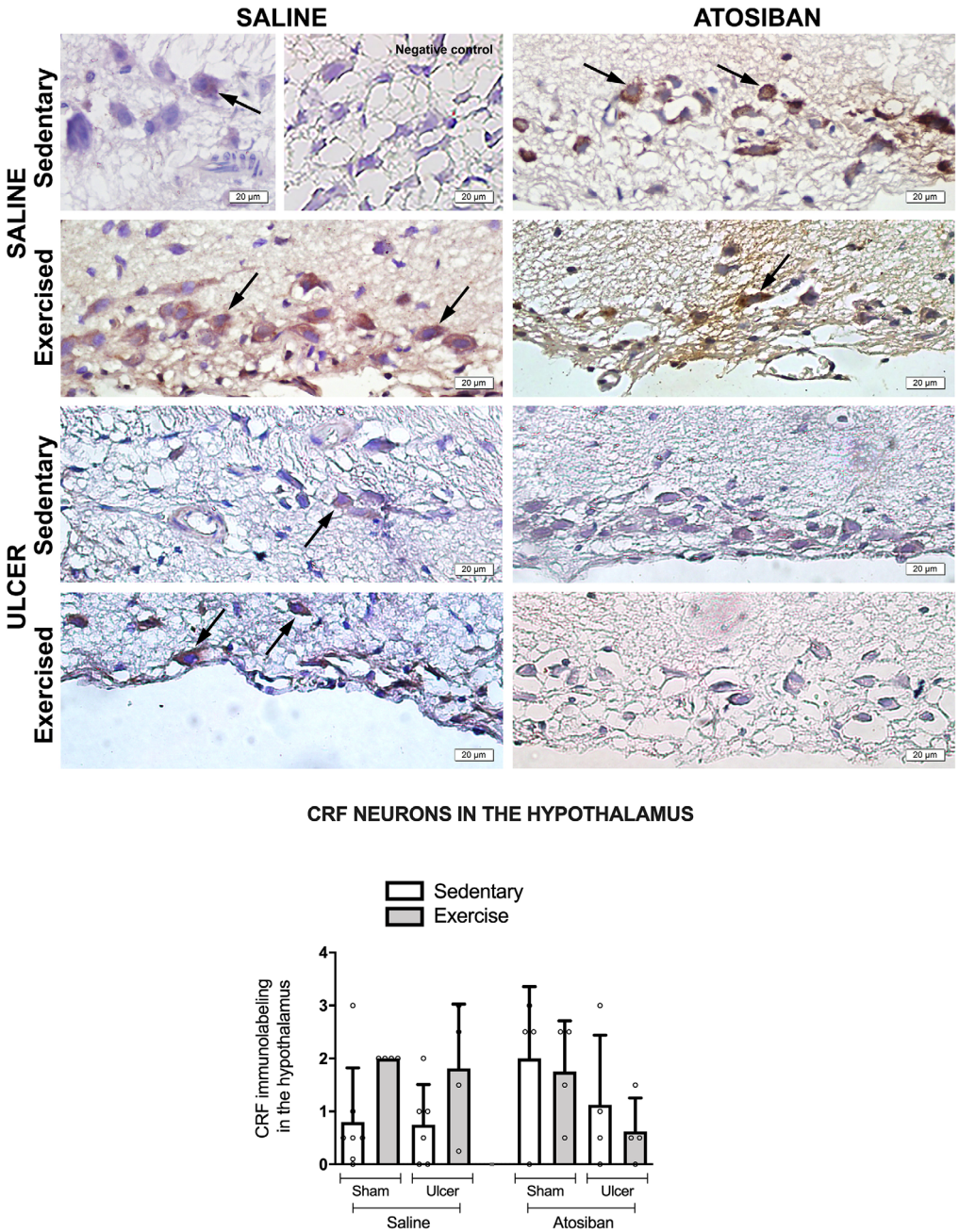

Figure 7. Corticotropin releasing factor (CRF) immunohistochemistry and the immunolabelling scores of all the experimental groups
$n = 4$–8, with 2–4 females and 2–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. Arrows: CRF immunoreactive neurons, compared to negative control. Counterstaining: Mayer's haematoxylin. Bars: 20 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

ulcers was accompanied by suppressed gastric blood flow and down-regulated myenteric oxytocin receptors, while normal blood flow and myenteric oxytocin receptors were maintained when the rats had previously exercised. Since maintenance of the mucosal blood flow is one of the major gastric defence factors against ulcerogenesis (Matsui *et al.* 2011), it can be suggested that the gastroprotective effects of regular exercise may, in part, involve the up-regulation of oxytocin receptors, which may regulate the impaired blood flow in the injured stomach.

Although an acute bout of exercise results in increased cellular metabolism and enhanced need for ATP along with increased generation of ROS (Davies *et al.* 1982), regular exercise performed at a moderate intensity down-regulates

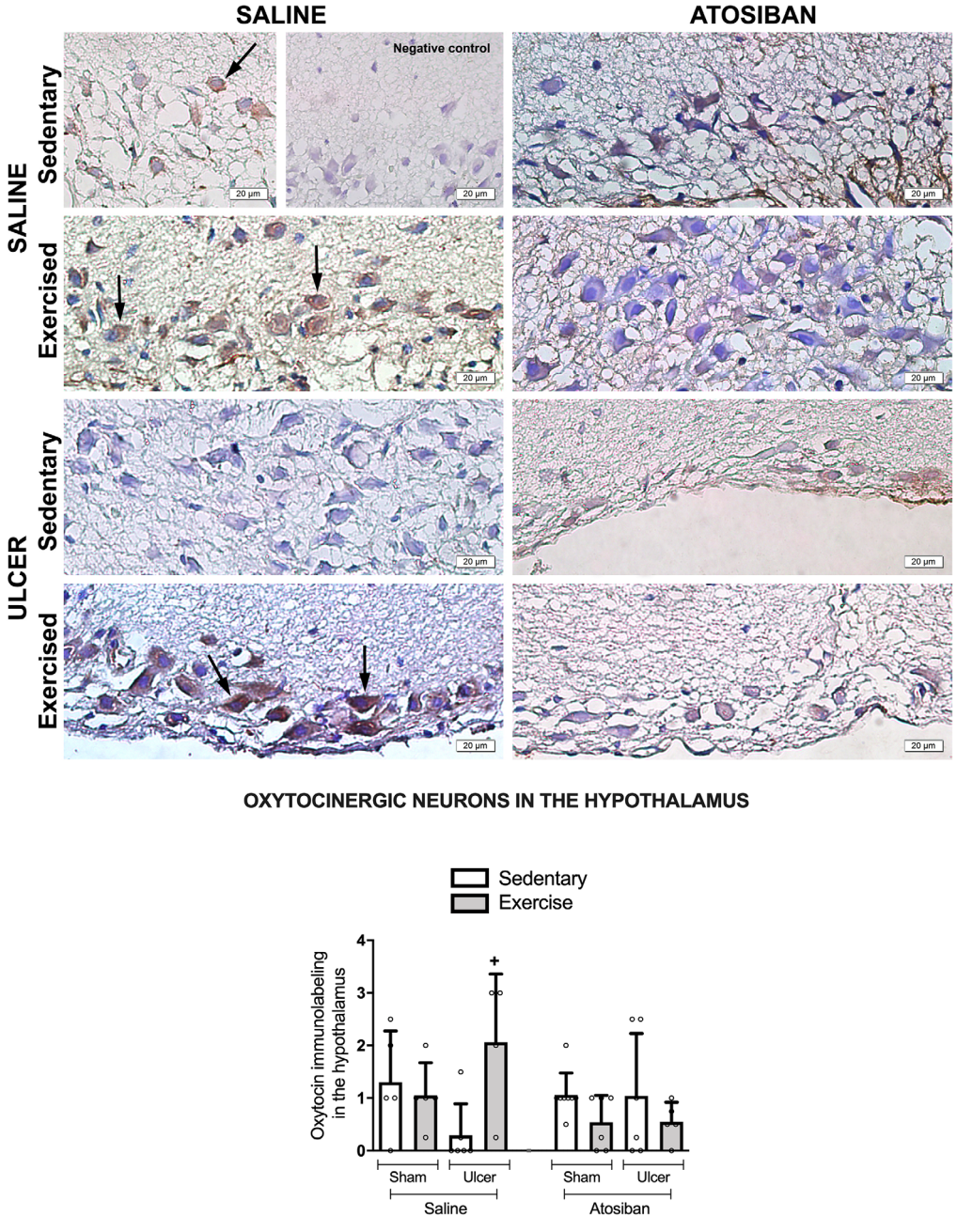

Figure 8. Oxytocin immunohistochemistry in the hypothalamus (supraoptic nucleus) and the immunolabelling scores of all the experimental groups
n = 4–8, with 2–4 females and 2–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. Arrows: Oxytocin–immunoreactive neurons, compared to negative control. Counterstaining: Mayer's haematoxylin. Bars: 20 µm. $+P < 0.05$, compared to the saline-treated sedentary ulcer group. $+P < 0.05$, compared to the saline-treated sedentary ulcer group. [Colour figure can be viewed at wileyonlinelibrary.com]

tissue levels of pro-inflammatory cytokines and elevates the anti-inflammatory IL-10 levels (Agarwal *et al.* 2011; Liu *et al.* 2013*b*). In the present study, prolonged moderate exercise by the control rats had no impact on the generation of either the pro-inflammatory mediators or ROS. Moreover, expressions of the pro-inflammatory cytokines, apoptosis and DNA damage to acute gastric erosion were not significantly altered in the stomachs of the pre-exercised rats. However, having regularly exercised prior to ulcer induction restricted neutrophil infiltration, reduced lipid peroxidation and partially replenished glutathione content of the injured stomach. Thus, it appears that a systemic physiological adaptation occurs as a consequence of exercise-induced oxidative challenge and enhances the resistance to further upcoming oxidative challenges by up-regulating antioxidant and housekeeping enzyme activities, and depressing neutrophil-dependent inflammation (Radak *et al.* 2008). Similarly, peripherally administered oxytocin was shown to enhance wound healing and alleviate inflammation in several tissues (Petersson *et al.* 2001; Işeri *et al.* 2005; Bıyıklı *et al.* 2006; İşeri *et al.* 2008; Çetinel *et al.* 2010). In accordance with that, blockade of oxytocin receptors in the ulcerated rats abolished exercise-induced reduction in gastric lipid peroxidation, while the expressions of pro-inflammatory cytokines were exaggerated in the ulcerated stomachs. Moreover, ulcer-induced reduction in oxytocinergic immunoreactivity in the hypothalamus and the enteric nervous system was abolished when the rats had regularly exercised. Taken with the aforementioned studies, it can be suggested that the beneficial effects of exercise against an acute oxidative challenge to the stomach may involve the activation of oxytocin receptors.

Both animal and human studies suggest that low to moderate levels of physical activity are beneficial, while intensive and prolonged exercise is associated with an increased risk of ulcerogenesis (Shephard, 2017). Earlier, it was reported that a prolonged exhausting running exercise by healthy adults results in high plasma values of oxytocin (Landgraf *et al.* 1982). It was more recently shown that even 10 min moderate running by humans or 10 min forced swimming by rats elevated oxytocin concentrations in the saliva, plasma and hypothalamic paraventricular nuclei (Torner *et al.* 2017). Although physical exercise (Cotman & Berchtold, 2002; Rosenbaum *et al.* 2014) and oxytocin (Uvnas-Moberg & Petersson, 2005) were separately documented to have a valuable impact on brain and body health in both healthy individuals and in patients with several diseases and affective disorders, this is the first study to illuminate the contribution of oxytocin to the beneficial effects of regular physical activity in alleviating gastric injury. Physical exercise, as a non-pharmacological tool known with its health-promoting and anti-stress actions, requires further investigation for its stimulatory effect on oxytocin-mediated anti-inflammatory actions in several other injurious events.

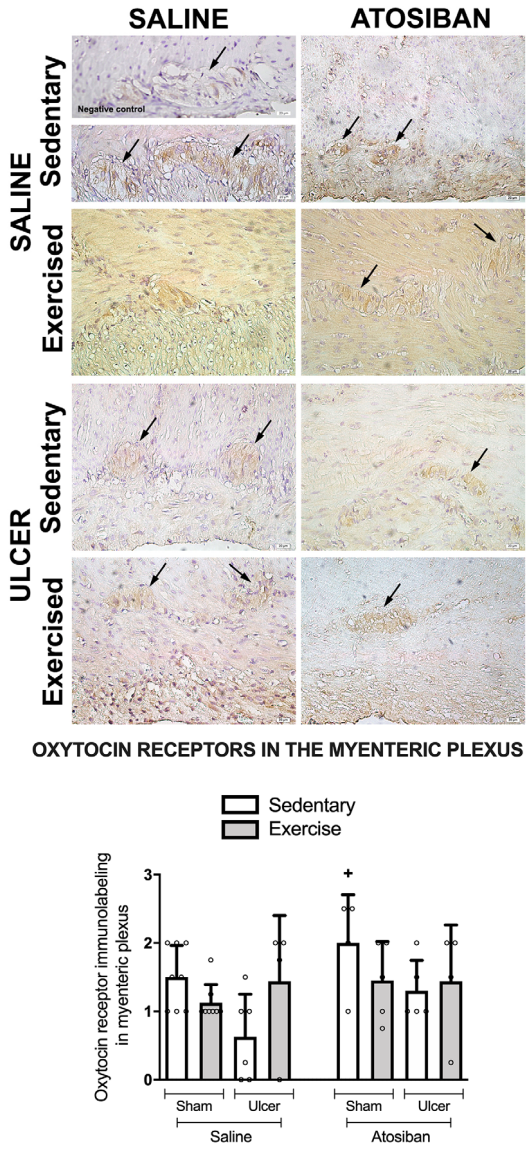

Figure 9. Oxytocin receptor immunohistochemistry in the gastric myenteric plexus and the immunolabelling scores of all the experimental groups
n = 4–8, with 2–4 females and 2–4 males in each subgroup. Data are the mean ± SD. Data were analysed using a three-way ANOVA. Arrows showing myenteric plexus with no immunoreactivity (negative control) or positive oxytocin receptor immunoreactivity. Counterstaining: Mayer's haematoxylin. Bars: 20 μm. $+P < 0.05$, compared to the saline-treated sedentary ulcer group. $+P < 0.05$, compared to the saline-treated sedentary ulcer group. [Colour figure can be viewed at wileyonlinelibrary.com]

References

Agarwal D, Welsch MA, Keller JN & Francis J (2011). Chronic exercise modulates RAS components and improves balance between pro-and anti-inflammatory cytokines in the brain of SHR. *Basic Res Cardiol* **106**, 1069–1085.

Al-Amran FF & Shahkolahi M (2014). Oxytocin ameliorates the immediate myocardial injury in heart transplant through down regulation of the neutrophil dependent myocardial apoptosis. *Heart Views* **15**, 37–45.

Alessio HM, Hagerman AE, Nagy S, Philip B, Byrnes RN, Woodward JL, Callahan P & Wiley RL (2005). Exercise improves biomarkers of health and stress in animals fed ad libitum. *Physiol Behav* **84**, 65–72.

Amico J, Mantella R, Vollmer R & Li X (2004). Anxiety and stress responses in female oxytocin deficient mice. *J Neuroendocrinol* **16**, 319–324.

Arabacı Tamer S, Yıldırım A, Arabacı Ş, Çiftçi S, Akın S, Sarı E, Köroğlu MK, Ercan F, Yüksel M, Çevik Ö, Yeğen BÇ. (2019). Treatment with estrogen receptor agonist ERβ improves torsion-induced oxidative testis injury in rats. *Life Sciences*, **222**, 203–211. https://doi.org/10.1016/j.lfs.2019.02.056.

Aro P, Storskrubb T, Ronkainen J, Bolling-Sternevald E, Engstrand L, Vieth M, Stolte M, Talley NJ & Agréus L (2006). Peptic ulcer disease in a general adult population: the Kalixanda study: a random population-based study. *Am J Epidemiol* **163**, 1025–1034.

Asad M, Shewade DG, Koumaravelou K, Abraham BK, Vasu S & Ramaswamy S (2001*a*). Effect of centrally administered oxytocin on gastric and duodenal ulcers in rats. *Acta Pharmacol Sin* **22**, 488–492.

Asad M, Shewade DG, Koumaravelou K, Abraham BK, Vasu S & Ramaswamy S (2001*b*). Gastric antisecretory and antiulcer activity of oxytocin in rats and guinea pigs. *Life Sci* **70**, 17–24.

Bahadir E, Özbeyli D, Mardinoğlu G, Temiz F, Sağanak M, Şahin H, Yüksel M, Ercan F & Yeğen B (2016). Regular swimming before the induction of ulcer ameliorates oxidative gastric damage in rats: The anxiolytic effect of exercise. *Marmara Med J* **29**, 135–144.

Bahrke MS & Morgan WP (1978). Anxiety reduction following exercise and meditation. *Cognit Ther Res* **2**, 323–333.

Beavers KM, Brinkley TE & Nicklas BJ (2010). Effect of exercise training on chronic inflammation. *Clin Chim Acta* **411**, 785–793.

Bıyıklı NK, Tuğtepe H, Şener G, Velioğlu-Öğünç A, Çetinel Ş, Midillioğlu Ş, Gedik N & Yeğen BÇ (2006). Oxytocin alleviates oxidative renal injury in pyelonephritic rats via a neutrophil-dependent mechanism. *Peptides* **27**, 2249–2257.

Bouzid MA, Filaire E, Matran R, Robin S & Fabre C (2018). Lifelong voluntary exercise modulates age-related changes in oxidative stress. *Int J Sports Med* **40**, 21–28.

Bradley PP, Priebat DA, Christensen RD & Rothstein G (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* **78**, 206–209.

Brown GR & Nemes C (2008). The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? *Behav Processes* **78**, 442–448.

Cheng Y, Macera CA, Davis DR & Blair SN (2000*a*). Does physical activity reduce the risk of developing peptic ulcers? *Br J Sports Med* **34**, 116–121.

Cheng Y, Macera CA, Davis DR & Blair SN (2000*b*). Physical activity and peptic ulcers. Does physical activity reduce the risk of developing peptic ulcers? *West J Med* **173**, 101–107.

Chung KT & Shelat VG (2017). Perforated peptic ulcer-an update. *World J Gastrointest Surg* **9**, 1.

Cotman CW & Berchtold NC (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci* **25**, 295–301.

Çetinel Ş, Hancıoğlu S, Şener E, Üner C, Kılıç M, Şener G & Yeğen BÇ (2010). Oxytocin treatment alleviates stress-aggravated colitis by a receptor-dependent mechanism. *Regul Pept* **160**, 146–152.

Davies KJ, Quintanilha AT, Brooks GA & Packer L (1982). Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* **107**, 1198–1205.

Engert V, Koester AM, Riepenhausen A & Singer T (2016). Boosting recovery rather than buffering reactivity: higher stress-induced oxytocin secretion is associated with increased cortisol reactivity and faster vagal recovery after acute psychosocial stress. *Psychoneuroendocrinology* **74**, 111–120.

Filaretova L (2011). Glucocorticoids are gastroprotective under physiologic conditions. *Ther Adv Chronic Dis* **2**, 333–342.

Goldman M, Marlow-O'Connor M, Torres I & Carter C (2008). Diminished plasma oxytocin in schizophrenic patients with neuroendocrine dysfunction and emotional deficits. *Schizophr Res* **98**, 247–255.

Grassi M & Drago F (1993). Effects of oxytocin on emotional stress and stress-induced gastric lesions. *J Physiol Paris* **87**, 261–264.

Grenbäck E, Hulting A-L, Bucht E & Petersson M (2007). Plasma galanin, vasopressin, and oxytocin in patients with Addison's disease. *Horm Metab Res* **39**, 589–595.

Gutkowska J, Paquette A, Wang D, Lavoie J-M & Jankowski M (2007). Effect of exercise training on cardiac oxytocin and natriuretic peptide systems in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol* **293**, R267-R275.

Haklar U, Yüksel M, Velioglu A, Turkmen M, Haklar G & Yalçin AS (2002). Oxygen radicals and nitric oxide levels in chondral or meniscal lesions or both. *Clin Orthop Relat Res* **403**, 135–142.

Hamer M, Endrighi R & Poole L (2012). Physical activity, stress reduction, and mood: insight into immunological mechanisms. In *Psychoneuroimmunology*, pp. 89–102. Springer.

Heinrichs M, vonDawans B & Domes G (2009). Oxytocin, vasopressin, and human social behavior. *Front Neuroendocrinol* **30**, 548–557.

Irianti S, Ginandjar A, Krisnadi S, Effendi J, Nataprawira D & Gandamihardja S (2017). Aerobic Exercise and Its Effect on Oxytocin Level and Labor Progression. In *IOP Conference Series: Materials Science and Engineering*, pp. 012177. IOP Publishing.

İşeri SÖ, Gedik İE, Erzik C, Uslu B, Arbak S, Gedik N & Yeğen BÇ (2008). Oxytocin ameliorates skin damage and oxidant gastric injury in rats with thermal trauma. *Burns* **34**, 361–369.

Işeri SÖ, Şener G, Sağlam B, Gedik N, Ercan F & Yeğen BÇ (2005). Oxytocin protects against sepsis-induced multiple organ damage: role of neutrophils. *J Surg Res* **126**, 73–81.

Işeri SÖ, Şener G, Sağlam B, Gedik N, Ercan F & Yeğen BÇ (2005). Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. *Peptides* **26**, 483–491.

Ji LL, Gomez-Cabrera MC & Vina J (2006). Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci* **1067**, 425–435.

Konturek PC, Duda A, Brzozowski T, Konturek S, Kwiecien S, Drozdowicz D, Pajdo R, Meixner H & Hahn E (2000). Activation of Genes for Superoxide Dismutase, Interleukin-1ß, Tumor Necrosis Factor-a, and Intercellular Adhesion Molecule-1 during Healing of Ischemia-Reperfusion-Induced Gastric Injury. *Scand J Gastroenterol* **35**, 452–463.

Kwiecien S, Ptak-Belowska A, Krzysiek-Maczka G, Targosz A, Jasnos K, Magierowski M, Szczyrk U, Brzozowski B, Konturek S & Konturek P (2012). Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, interacts with gastric oxidative metabolism and enhances stress-induced gastric lesions. *J Physiol Pharmacol* **63**, 515–524.

Landgraf R, Häcker R & Buhl H (1982). Plasma vasopressin and oxytocin in response to exercise and during a day-night cycle in man. *Endokrinologie* **79**, 281–291.

Liu L, Liu Y, Cui J, Liu H, Liu Y-B, Qiao W-L, Sun H & Yan C-D (2013*a*). Oxidative stress induces gastric submucosal arteriolar dysfunction in the elderly. *World J Gastroenterol* **19**, 9439.

Liu W, Sheng H, Xu Y, Liu Y, Lu J & Ni X (2013*b*). Swimming exercise ameliorates depression-like behavior in chronically stressed rats: relevant to proinflammatory cytokines and IDO activation. *Behav Brain Res* **242**, 110–116.

Makara GB, Mergl Z & Zelena D (2004). The Role of Vasopressin in Hypothalamo-Pituitary-Adrenal Axis Activation during Stress: An Assessment of the Evidence. *Ann N Y Acad Sci* **1018**, 151–161.

Markiewicz K, Cholewa M, Gorski L & Chmura J (1977). Effect of physical exercise on gastric basal secretion in healthy men. *Acta hepato-gastroenterologica* **24**, 377–380.

Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K & Hyodo I (2011). The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. *J Clin Biochem Nutr* **48**, 107–111.

Memi G & Yeğen BÇ (2016). Role of Ovarian Hormones in Psychological Stress-induced Oxidative Organ Damage in Rats. *Clin Exp Health Sci* **6**, 72–79.

Najm WI (2011). Peptic ulcer disease. *Prim Care: Clinics in Office Practice* **38**, 383–394.

Navarro A, Gomez C, López-Cepero JM & Boveris A (2004). Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Am J Physiol Regul Integr Comp Physiol* **286**, R505-R511.

Neumann ID, Wigger A, Torner L, Holsboer F & Landgraf R (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *J Neuroendocrinol* **12**, 235–43.

Ohlsson B, Truedsson M, Djerf P & Sundler F (2006). Oxytocin is expressed throughout the human gastrointestinal tract. *Regul Pept* **135**, 7–11.

Okabe S, Roth JL & Pfeiffer CJ (1971). A method for experimental, penetrating gastric and duodenal ulcers in rats. *Am J Dig Dis* **16**, 277–284.

Peternelj T-T & Coombes JS (2011). Antioxidant supplementation during exercise training. *Sports Med* **41**, 1043–1069.

Petersson M, Hulting A-L & Uvnäs-Moberg K (1999). Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. *Neurosci Lett* **264**, 41–44.

Petersson M & Uvnäs-Moberg K (2003). Systemic oxytocin treatment modulates glucocorticoid and mineralocorticoid receptor mRNA in the rat hippocampus. *Neurosci Lett* **343**, 97–100.

Petersson M, Wiberg U, Lundeberg T & Uvnäs-Moberg K (2001). Oxytocin decreases carrageenan induced inflammation in rats. *Peptides* **22**, 1479–1484.

Pique JM, Whittle BJ & Esplugues JV (1989). The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur J Pharmacol* **174**, 293–296.

Qin J, Feng M, Wang C, Ye Y, Wang P & Liu C (2009). Oxytocin receptor expressed on the smooth muscle mediates the excitatory effect of oxytocin on gastric motility in rats. *Neurogastroenterol Motil* **21**, 430–438.

Rabow S, Hjorth U, Schönbeck S & Olofsson P (2018). Effects of oxytocin and anaesthesia on vascular tone in pregnant women: a randomised double-blind placebo-controlled study using non-invasive pulse wave analysis. *BMC Pregnancy Childbirth* **18**, 453.

Radak Z, Chung HY & Goto S (2008). Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic Biol Med* **44**, 153–159.

Radák Z, Kaneko T, Tahara S, Nakamoto H, Pucsok J, Sasvári M, Nyakas C & Goto S (2001). Regular exercise improves cognitive function and decreases oxidative damage in rat brain. *Neurochem Int* **38**, 17–23.

Roper JA, O'carroll A, Young IIIW & LolaitS (2011). The vasopressin Avpr1b receptor: molecular and pharmacological studies. *Stress* **14**, 98–115.

Rosenbaum S, Tiedemann A & Ward PB (2014). Meta-analysis physical activity interventions for people with mental illness: a systematic review and meta-analysis. *J Clin Psychiatry* **75**, 1–11.

Rukstalis M & French JA (2005). Vocal buffering of the stress response: exposure to conspecific vocalizations moderates urinary cortisol excretion in isolated marmosets. *Horm Behav* **47**, 1–7.

Sarnik S, Hofirek I & Sochor O (2007). Laser Doppler fluxmetry. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **151**, 143–146.

Shephard RJ (2017). Peptic Ulcer and Exercise. *Sports Med* **47**, 33–40.

Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A & Neri LM (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget* **9**, 17181.

Sørbye H & Svanes K (1994). The role of blood flow in gastric mucosal defence, damage and healing. *Dig Dis* **12**, 305–317.

Szabo I & Tarnawski A (2000). Apoptosis in the gastric mucosa: molecular mechanisms, basic and clinical implications. *J Physiol Pharmacol* **51**.

Torner L, Plotsky PM, Neumann ID & deJong TR (2017). Forced swimming-induced oxytocin release into blood and brain: effects of adrenalectomy and corticosterone treatment. *Psychoneuroendocrinology* **77**, 165–174.
Tuğtepe H, Şener G, Bıyıklı NK, Yüksel M, Çetinel Ş, Gedik N & Yeğen BÇ (2007). *Regul Pept* **140**, 101–108.
Uvnas-Moberg K & Petersson M (2005). Oxytocin, a mediator of anti-stress, well-being, social interaction, growth and healing. *Z Psychosom Med Psychother* **51**, 57–80.
Wang JZ, Wu YJ, Rao CM, Gao MT, & Li WG. (1999). Effect of recombinant human basic fibroblast growth factor on stomach ulcers in rats and mice. *Acta pharmacologica Sinica*, **20**(8), 763–768.
Welch MG, Margolis KG, Li Z & Gershon MD (2014). Oxytocin regulates gastrointestinal motility, inflammation, macromolecular permeability, and mucosal maintenance in mice. *Am J Physiol Gastrointest Liver Physiol* **307**, G848-G862.
Yegen BC (2018). Lifestyle and Peptic Ulcer Disease. *Curr Pharm Des* **24**, 2034–2040.
Yeğen B (2010). Oxytocin and hypothalamo-pituitaryadrenal axis. *Marmara Pharm J* **14**, 61–66.
Yüksel O, Ateş M, Kızıldağ S, Yüce Z, Koç B, Kandiş S, Güvendi G, Karakılıç A, Gümüş H & Uysal N (2019). Regular aerobic voluntary exercise increased oxytocin in female mice: the cause of decreased anxiety and increased empathy-like behaviors. *Balkan Med J* **36**, 257.

Additional information

Competing interests

The authors declare no competing interests.

Author contributions

All the experiments were performed at the Physiology (SAT, SÜ, BB, MG, AGK, NY, BÇY) and Histology & Embryology (SŞ, FE) Departments of the School of Medicine, İstanbul; and Biochemistry (ÖÇ) Department of the School of Medicine at Adnan Menderes University, Aydın, Turkey.
All persons (SAT, SÜ, BB, MG, AGK, NY, SŞ, ÖÇ, FE, BÇY) designated as authors qualify for authorship.
All persons who qualify for authorship are listed.
All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
Study conception and design of the work: SAT, BÇY.
Data acquisition: SAT, SÜ, BB, MG, AGK, NY, ÖÇ.
Analysis and data interpretation: SAT, SÜ, BB, MG, AGK, NY, SŞ, ÖÇ, FE, BÇY (all authors).
Drafting of the manuscript: SAT, SÜ, BB, MG, AGK, NY, SŞ, ÖÇ, FE, BÇY (all authors).
Critical revision: SAT, BÇY.
Approval of the final version of the manuscript: SAT, SÜ, BB, MG, AGK, NY, SŞ, ÖÇ, FE, BÇY (all authors).

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Keywords

atosiban, exercise, gastric ulcer, inflammation, oxytocin

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document