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Research Paper

Oxytocin mediates the beneficial effects of the exercise training on breast cancer

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New Findings

- **What is the central question of this study?**
  We hypothesized that potential anti-tumour effects of exercise training might be mediated by oxytocin and explored the underlying mechanisms in a mouse model of breast cancer.
- **What is the main finding and its importance?**
  Interval exercise training, by inducing oxytocin secretion, may reduce the activity of the PI3K/Akt and ERK pathways, and consequently, results in a smaller tumour volume in a mouse model of breast cancer.

Exercise training can affect the growth of breast tumours. We hypothesized that exercise training might reduce breast tumour growth by inducing oxytocin (OT) secretion and its related signalling pathways, such as PI3K/Akt and ERK. Therefore, 56 BALB/c mice were equally divided into seven groups to study the effects of OT and atosiban (an oxytocin receptor antagonist) together with interval exercise training on mammary tumour growth, as well as tumour-related signalling pathways, including PI3K/Akt and ERK. Animal weight, OT plasma concentration, tumour weight and volume were measured at the end of the study. PI3K/Akt and ERK were evaluated by Western blot and qPCR assays. The results showed that OT plasma concentration was significantly increased in trained animals. The volume and weight of tumours were decreased significantly after both exercise training and OT administration. The expression of genes involved in tumour cell proliferation, such as *PI3KR2*, *Akt* and *mTOR*, was notably lower in the exercise-trained and OT-treated groups. Furthermore, the expression of genes involved in cell apoptosis, such as caspase-3 and *Bax*, was significantly increased in the tumour tissues. In addition, Western blot results showed that phosphorylated Akt and ERK were significantly decreased in the exercise training and OT groups compared with the tumour group. Interestingly, atosiban reversed these effects. These results indicated that interval exercise training, acting via OT secretion, may reduce PI3K/Akt and ERK axis activities, and consequently, decrease tumour volume and weight in a mouse model of breast cancer.

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Experimental Physiology

Introduction

Oxytocin (OT) is a neuropeptide that plays a dual role in mammals as a neurotransmitter/neuromodulator and a hormone. Besides the central effects of OT in uterine muscle contraction during childbirth and the milk ejection reflex (Gimpl & Fahrenholz, 2001), it has many peripheral roles, including protective effects against cardiovascular disease and cancer (Alizadeh *et al.* 2011,2012; Alizadeh & Mirzabeglo, 2013; Imanieh *et al.* 2014; Pedersen & Saltin, 2015). In some cancers, such as ovarian and endometrial cancers, OT inhibits cell proliferation (Cassoni *et al.* 2000; Suzuki *et al.* 2003; Morita *et al.* 2004), whereas its role in prostate and breast cancers is still controversial (Cassoni *et al.* 1994; Whittington *et al.* 2007). In this context, exercise training is one of the best approaches to enhance OT secretion via activation of oxytocinergic neurons (Jackson *et al.* 2005; Martins *et al.* 2005; Hew-Butler *et al.* 2008).

Hew-Butler *et al.* (2008) showed that the plasma concentration of OT was increased after exercise training, which guided us to the idea that OT might be a mediator of the positive effects of exercise in various diseases, such as cancer. In this context, breast cancer is one of the cancers known to be affected by exercise, and consequently, can provide a good model for assessment of the mediatory role of OT in the beneficial effects of exercise training, because breast tissues have the highest number of OT receptors (OTRs; Lippert *et al.* 2003). Sala and Freire (1974) suggested modulatory effects of steroid hormones, such as estradiol, on the expression of OT receptors (Sala & Freire, 1974; Soloff, 1979). However, OT can modulate the expression of oestrogen receptor α (ERα) in breast cancer (Cassoni *et al.* 2002). Oxytocin can also inhibit the oestrogen-induced cell growth (Cassoni *et al.* 1994). Thus, it seems that there is a loop between OTR expression and the oestrogen receptor. Moreover, OT can act as a regulator of the PI3K/Akt/mTOR and ERK pathways, which are involved in cell growth, proliferation, motility and survival, as well as protein synthesis, autophagy and transcription (McAuliffe *et al.* 2010; Klein *et al.* 2013).

Knowing these facts, we have recently hypothesized that OT can mediate the protective effects of exercise training on cancer (Imanieh *et al.* 2014); however, the precise mechanisms that underlie these findings remain unknown. Therefore, the main goal of the present study was to test the role of OT in the effects of exercise training on mouse mammary tumours and their related signalling pathways. Initially, we investigated whether interval exercise training can increase the plasma concentration of OT. Then, we explored the effects of exercise training and OT administration on the mammary tumour growth as well as their underlying pathways in a typical animal model of breast cancer.

Methods

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the Tehran University of Medical Sciences research committee and with the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards and followed recommendations provided by the Physiological Society (Grundy, 2015).

Materials

Ketamine (K2753), xylazine (X1126), oxytocin (O4250) and atosiban (A3480) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Total RNA was isolated with a miTotal RNA extraction kit (GRM1002; Viogene, Sunnyvale, CA, USA). Template cDNAs were synthesized with Superscript III (18080-051; Invitrogen, Carlsbad, CA, USA). Quantitative PCR was performed with a quantitative Roter-gene multiplex PCR kit (cat no. 204774; Qiagen, Hilden, Germany).

Animals

Animal studies were conducted according to the relevant national and international guidelines of the Weather all report, and Institutional Animal Care and Use Committee of Tehran University of Medical Sciences (no. 19187). Inbred female BALB/c mice (6–8 weeks old) were purchased from the Pasteur Institute of Iran and were maintained under 12 h–12 h light–dark cycles with free access to food and water.

Tumour transplantation

Mouse mammary adenocarcinoma tumours (derived from MC4-L2 as a stock model; Khori *et al.* 2015) were aseptically separated from the mammary-cancer-bearing BALB/c mice, cut into pieces of less than 0.2−0.3 cm^3, and subcutaneously transplanted into the animals′ right flank under ketamine (100 mg kg^{-1}, I.P) and xylazine (10 mg kg^{-1}, I.P) anaesthesia (Farhangi *et al.* 2015).

Study design

Fifty-six mice were randomized to seven groups ($n = 8$ per group) by blinded draw: (i) control (C); healthy animals with neither tumour nor exercise training protocol; (ii) tumour (T); animals with mammary tumour; (iii) tumour plus exercise training (T + E), in which animals with a mammary tumour underwent 6 weeks of the interval exercise training protocol; (iv) tumour plus OT (T + OT), in which OT was administered (0.03 μg kg day^{-1}, I.P) for 2 weeks after tumour establishment (Faghihi

Table 1. PCR primer sequences

Gene	Forward sequence	Reverse sequence	NCBI
Bax	5′-GATGATTGCTGACGTGGAC-3′	5′-ACGGAGGAAGTCCAGTGTC-3′	NM_007527
Bcl2	5′-GAGCCTGTGAGAGACGTGG-3′	5′-CGAGTCTGTGTATAGCAATCCCA-3′	NM_007546
Caspase-3	5′-ATGGGAGCAAGTCAGTGGACTC-3′	5′-GTCTCTCTGAGGTTGGCTGC-3′	NM_021284
mTOR	5′-TGTGCCAGTGGGTGCTGAAGTG-3′	5′-GAAGCGCTGCTCGGATGATGTC-3′	NM_031248
ER	5′-TGATCATGGAGTCTGCCAAG-3′	5′-AACTCTTCCTCCGGTTCTTG-3′	NM_001039128
OTR	5′-CACGGGTCAGTAGTGTCAAG-3′	5′-GGTTGCAGCAGCTGTTGAG-3′	NM_020564
Akt	5′-CCCTGCTCCTAGTCCACCA-3′	5′-TGTCTCTGTTTCAGTGGGCTC-3′	NM_026270
PI3K2R	5′-GGACAGTGAATGCTACAGTAAGC-3′	5′-CCTGCAACCTCTCGAAGTG-3′	NM_001145881
P53	5′-CACCTGCACAAGCGCCTCTCC-3′	5′-CTGCTGTCTCCAGACTCCTCTGTAGC-3′	NM_013843
VEGF	5′-TGAACTTTCTGCTCTCTTGGG-3′	5′-GGTTCGCTGGTAGACATCG-3′	NM_009506
CS	5′-GGACAATTTTCCAACCAATCTGC-3′	5′-AGTCAATGGCTCCGATACTGC-3′	NM_026609
GAPDH	5′-TCAACAGCAACTCCCACTCTTCC-3′	5′-ACCCTGTTGCTGTAGCCGTATTC-3′	NM_008084

et al. 2012); (v) tumour plus OT plus interval exercise training (T + E + OT); (vi) tumour plus atosiban (T + ATO), in which OT receptor antagonist atosiban was administered (1.5 μg kg day^{-1}, I.P) for 2 weeks after tumour establishment (Alizadeh *et al.* 2011); and (vii) tumour plus ATO plus interval exercise training (T + E + ATO). At the end of the study, all animals were killed 48 h after the last session of the interval exercise training.

Interval exercise training protocol

Before the initiation of exercise training, the animals were assigned to the treadmill for 5 days. Acclimation entailed running done at the end of the dark cycle (07.00 h) at gradually increasing speeds (10, 12, 16 and 18 m min^{-1} and 0% gradient). After acclimation, the interval exercise training protocol commenced at 16–18 m min^{-1}, 0% gradient, for 10−14 min, 5 days each week for 6 weeks, and the exercise intensity was gradually increased each week (Isanejad *et al.* 2016). All animals in the exercise groups ran at the same intensity and duration in each session. No electrical stimulation was used, and the mice were encouraged to run by a gentle tap on their tails or hindquarters. The untrained animals were put on the switched-off treadmill during 6 weeks of the protocol. The exercise training was stopped 48 h before the animals were killed.

Measurement of body weight and tumour volume

Animals were weighed weekly and regularly monitored for abnormal sequels. Tumour volume was measured with digital vernier callipers (Digimatic, model no. CD-S6″ CP; Mitutoyo Corp., Japan) on a weekly basis, and reported as cubic millimetres using the following formula (Mohsenikia *et al.* 2016): $V = 1/6(\pi LWD)$, where V is volume, L length, W width and D depth.

Blood and tissue sampling

To avoid an acute exercise response, all animals were killed 48 h after the last exercise session. Under general anaesthesia, blood was withdrawn from the heart (1.5 ml), and the animals were killed by cervical dislocation. The tumour tissues were harvested and stored at −80°C for analysis (Isanejad *et al.* 2016).

Plasma OT assay

Blood samples were centrifuged at 2800*g* at 4°C for 10 min in tubes containing EDTA (10% solution, 10 μl EDTA per 100 μl blood), aprotinin (a protease inhibitor; 10 μl per tube) and phenyl methyl sulfonyl fluoride (PMSF; 5 μl per tube). The plasma aliquots were frozen at −80°C until assay. The plasma concentration of OT was measured in duplicate using an enzyme immunoassay (EIA)-based kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). The sensitivity of the assay was 0.01 ng ml^{-1} (range; 0–100 ng ml^{-1}). The intra-assay coefficients of variation were 6.8%.

RT-PCR and real-time PCR assays

Tumours and soleus muscle were harvested 48 h after the last training session. A total of 50–100 mg of tumour tissue and soleus muscles were immediately stored in liquid nitrogen and kept frozen at −80°C until assay. Total RNA was extracted in accordance with the instructions provided with the miTotal RNA extraction kit (GRM1002; Viogene). The optical density 260/280 ratio was measured using a spectrophotometer to determine the quantity and purity of the RNA. The RNA was reverse transcribed into cDNA in the following reaction conditions: 37°C for 15 min and 85°C for 5 s. Quantitative fluorescence PCR was performed to amplify the *PI3K2R*, *Akt*, *Bax*, *Bcl2*, caspase-3, *ER*, *mTOR*, *OTR*, *P53*, *VEGF* and citrate synthase (*CS*) gene

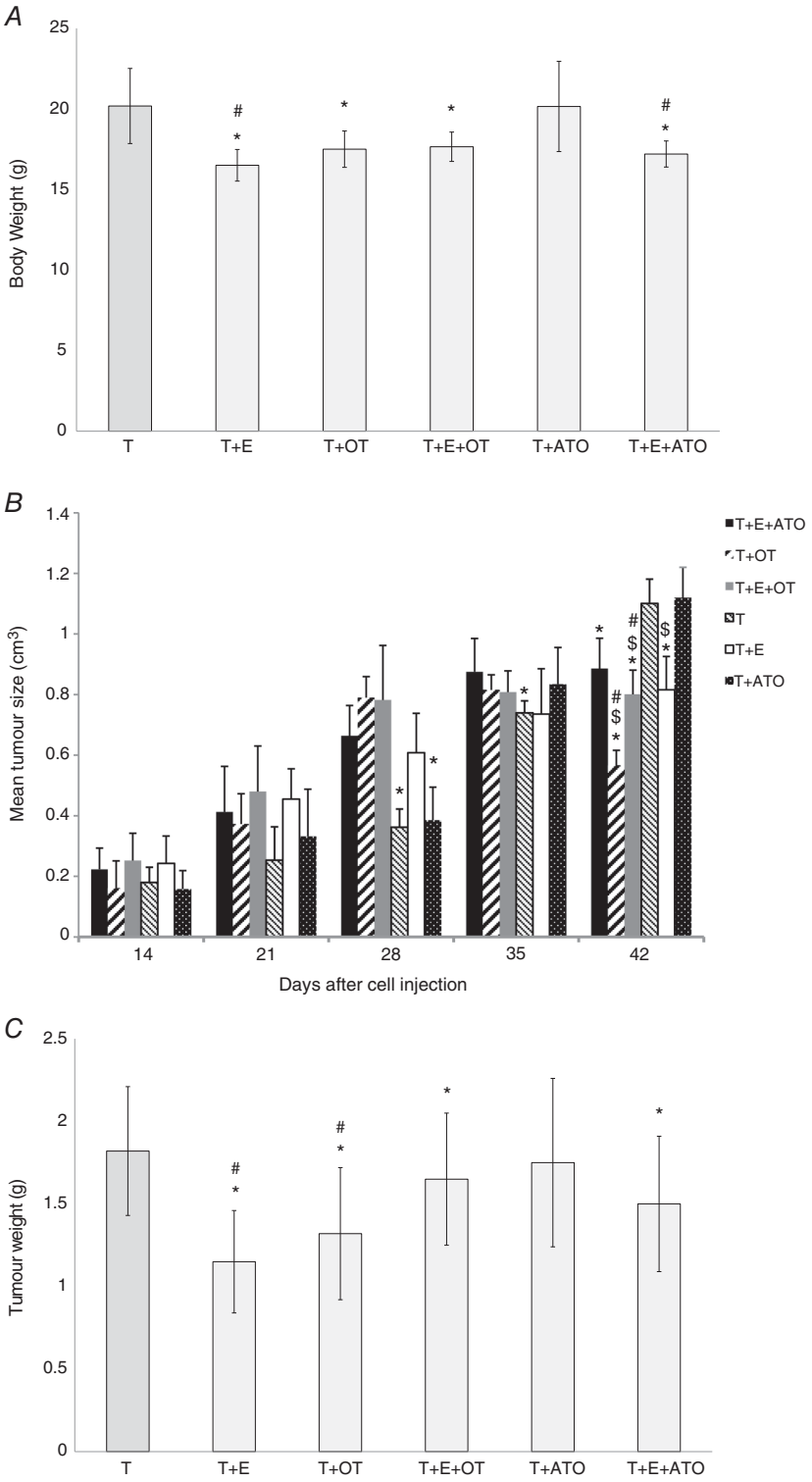

Figure 1. Effects of exercise training together with oxytocin (OT) on the body weight (*A*), tumour volume (*B*) and tumour weight (*C*) in a mouse model of breast cancer
Data are means ± SD. $^{*}P < 0.05$ compared with T group, $^{\$}P < 0.05$ compared with T + E + ATO group and $^{\#}P < 0.05$ compared with T + ATO group. Abbreviations: ATO, atosiban; C, control group; OT, oxytocin; T, tumour; T + ATO = tumour plus atosiban; T + E, tumour plus exercise training; T + E + ATO, tumour plus exercise training and atosiban; T + E + OT, tumour plus OT and exercise training; and T + OT, tumour plus oxytocin.

products in a 25 μl reaction system, using SYBR-Green mix (12.5 μl), upstream and downstream primers (1 μl), cDNA (2 μl) and RNase-free H_2O (8.5 μl). The reaction conditions were as follows: 94°C for 5 min, 94°C for 15 s, 72°C for 15 s, 40 cycles; the detection of the dissolution curve was carried out at 55–94°C. For primer design, the gene sequences were extracted from NCBI gene bank (www.NCBI.nlm.nih.gov). We used Primer 3 software to design PCR primers, and then the sequence analysis of primers was performed by using BLAST databases for alignment. The primers were designed for amplification of 88–120 bp on a real-time PCR device (Rotor-Gene Q; Qiagen, Valencia, CA, USA). The lyophilized primers were synthesized by CinnaGen Co. (Iran). PCR primer sequences used for the amplification of the protein-coding genes are shown in Table 1. The experiments were run in triplicate. *GAPDH* was used as the reference gene to determine a normalized arbitrary value for each gene. Relative expression was calculated according to $2^{-\Delta\Delta C_T}$ (Isanejad *et al.* 2016).

Evaluation of Akt and ERK proteins by Western blot

Frozen tissues were homogenized in SDS lysis buffer (25 mM Tris–HCl pH 6.8, 0.8% SDS, 4% glycerol, 2% 2-mercaptoethanol and 0.008% Bromophenol Blue). SDS-PAGE (10%) was prepared according to the standard procedures. Proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane (Roche, USA). Blocking of residual protein-binding sites on the membrane was performed with 5% non-fat dry milk in Phosphate-Buffered Saline with Tween 20 (TBST) (50 mmol l^{-1} Tris–HCl, pH 7.5, 150 mmol l^{-1} NaCl and 0.1% Tween-20) for 1 h. The membrane was then rinsed three times with TBST and incubated overnight at 4°C in TBST with d-Akt (dephosphorylated Akt), P-Akt (phosphorylated Akt), d-ERK (dephosphorylated ERK) or P-ERK (phosphorylated ERK) antibody (Santa Cruz, USA), at 1:1000 dilution. After overnight incubation, the membrane was washed several times for 5 min with TBST and incubated with the secondary antibody (peroxidase-conjugated IgG-HRP; sc-2004; Santa Cruz, USA) at 1:1000 dilution for 1 h at room temperature. After another three washes for 5 min with TBST, the blots were developed by ECL with the use of Kodak BioMax Light film and digitalized with a GS-700 Imaging Densitometer (Bio-Rad, Hercules, CA, USA). Relative pixel density was calculated for each band following background subtraction (Khori *et al.* 2015).

Statistical analysis

Depending on the number of groups compared within each trial and the *P* value of the Kolmogorov–Smirnov test of normality, Student′s unpaired *t* test, one-way ANOVA was used for parametric analyses. If significant effects were found, the Bonferroni *post hoc* test was used to determine the source of the difference. The values are represented as means ± SD. A value of $P < 0.05$ was considered to be statistically significant. Statistical analysis was done using SPSS statistical software, version 14.0 (version 14.0; SPSS Inc., Chicago, IL, USA).

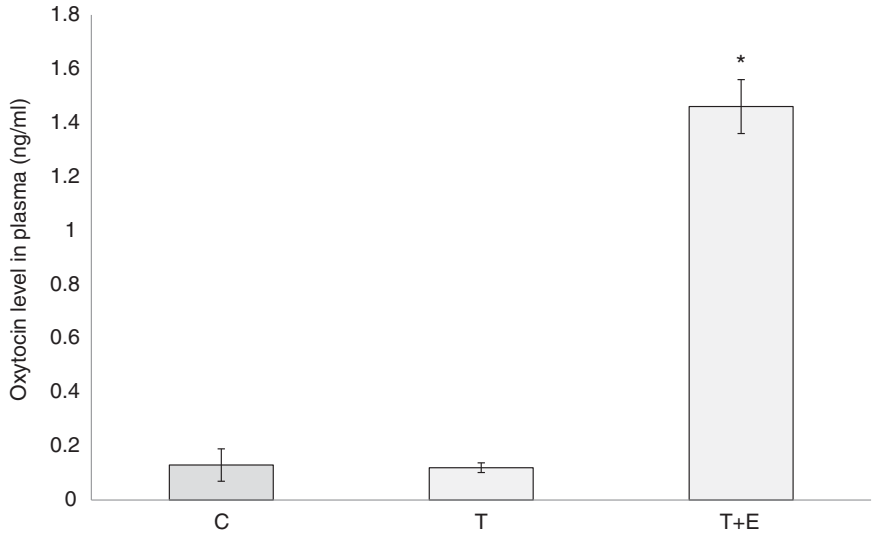

Figure 2. Effects of exercise training on the plasma concentration of oxytocin in a mouse model of breast cancer
Data are means ± SD. $^*P < 0.05$ compared with T group. Abbreviations: C, healthy animals; T, tumour group; and T + E, tumour plus exercise training.

Results

Clinical observations, body weight, tumour weight and tumour volume

There were no behavioural changes in animals during the study. Body weight was measured weekly during the treatment. The body weight of mice in the exercise training and OT-treated groups was lower than that of mice in the control group at the end of the study ($P < 0.05$; Fig. 1*A*). The average tumour volume was less in the T + E + ATO, T + E + OT, T + OT and T + E groups than in the T group at 28, 35 and 42 days after tumour transplantation ($P = 0.05$). The final mean tumour volume was 1.10 ± 0.10, 0.88 ± 0.03, 0.80 ± 0.06, 1.12 ± 0.10, 0.56 ± 0.10 and 0.81 ± 0.11 cm^3 in T, T + E + ATO, T + E + OT, T + ATO, T + OT and T + E groups, respectively ($P = 0.05$; Fig. 1*B*). Furthermore, exercise training decreased the tumour weight in T + E *versus* T group ($P < 0.05$; Fig. 1*C*). There were no signs of the tumour metastasis in the main organs of both the treatment and tumour groups.

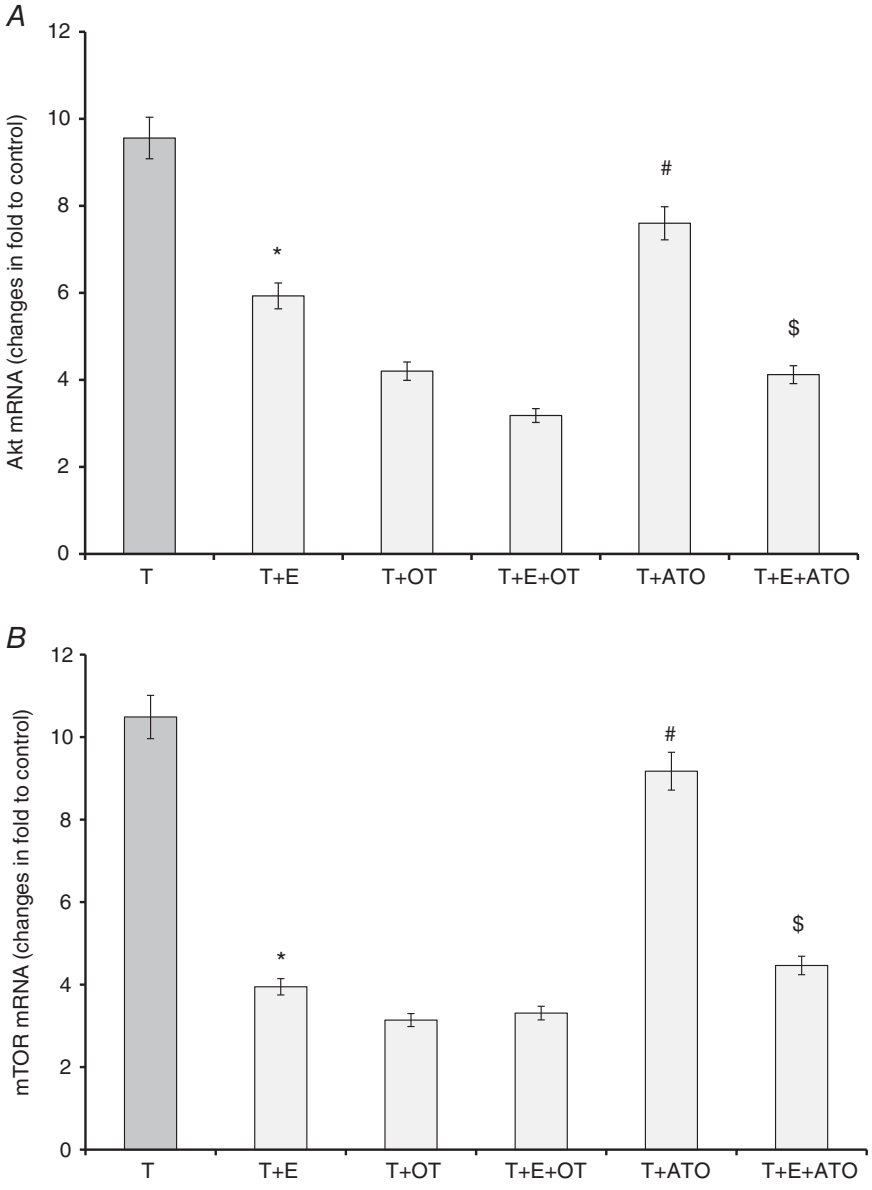

Figure 3. Effects of exercise training and OT on the gene expression of *Akt* (*A*) and *mTOR* (*B*) in mouse mammary tumour cells
Data are means ± SD. $^{*}P < 0.05$ compared with T group, $^{\$}P < 0.05$ compared with T + E + OT group and $^{\#}P < 0.05$ compared with T + OT group. Groups are as in Fig. 1.

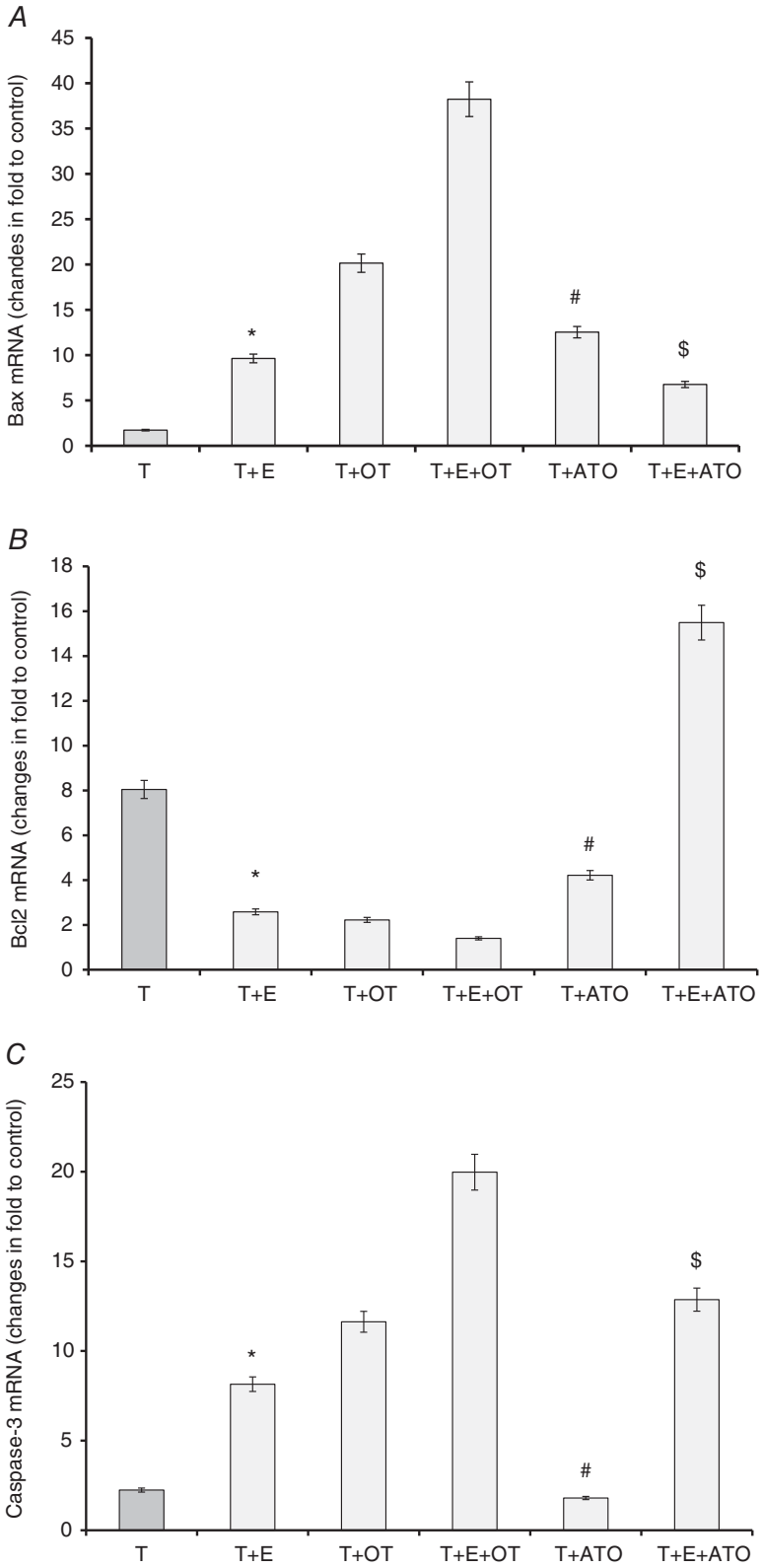

Figure 4. Effects of exercise training together with OT on the gene expression of *Bax (A)* and *Bcl2 (B)* and caspase-3 (*C*) in mouse mammary tumour cells
Data are means ± SD. $^{*}P < 0.05$ compared with T group, $^{\$}P < 0.05$ compared with T + E + OT group and $^{\#}P < 0.05$ compared with T + OT group. Groups are as in Fig. 1.

Plasma concentration of OT

The exercise training increased plasma oxytocin concentrations in the T + E group compared with the control group ($P < 0.05$). Plasma concentrations of oxytocin were 0.13 ± 0.06, 0.12 ± 0.01 and 1.46 ± 0.05 ng ml^{-1} in C, T and T + E groups, respectively (Fig. 2).

Messenger RNA expression of *Akt* and *mTOR*

One-way ANOVA results showed that *Akt* mRNA expression in tumour tissue was decreased in the T + E group in comparison with the T group. It also was increased in the T + E + ATO compared with the T + E + OT group and in the T + ATO group compared with the T + OT group ($P < 0.05$; Fig. 3*A*).

The *mTOR* mRNA expression in tumour tissue was reduced in the T + E group in comparison with the T group. It was increased in the T + E + ATO group *versus* the T + E + OT group and in the T + ATO group compared with the T + OT group ($P < 0.05$; Fig. 3*B*).

Messenger RNA expression of *Bax*, *Bcl2* and caspase-3

Bcl2 mRNA expression in tumour tissue was lower in the T + E group than in the T group. It was increased in the T + E + ATO group compared with the T + E + OT group. It was increased in the T + ATO and T + E + ATO groups compared with the T + OT group ($P < 0.05$; Fig. 4*B*).

Bax mRNA expression in tumour tissue was increased in the T + E group compared with the T group. It was

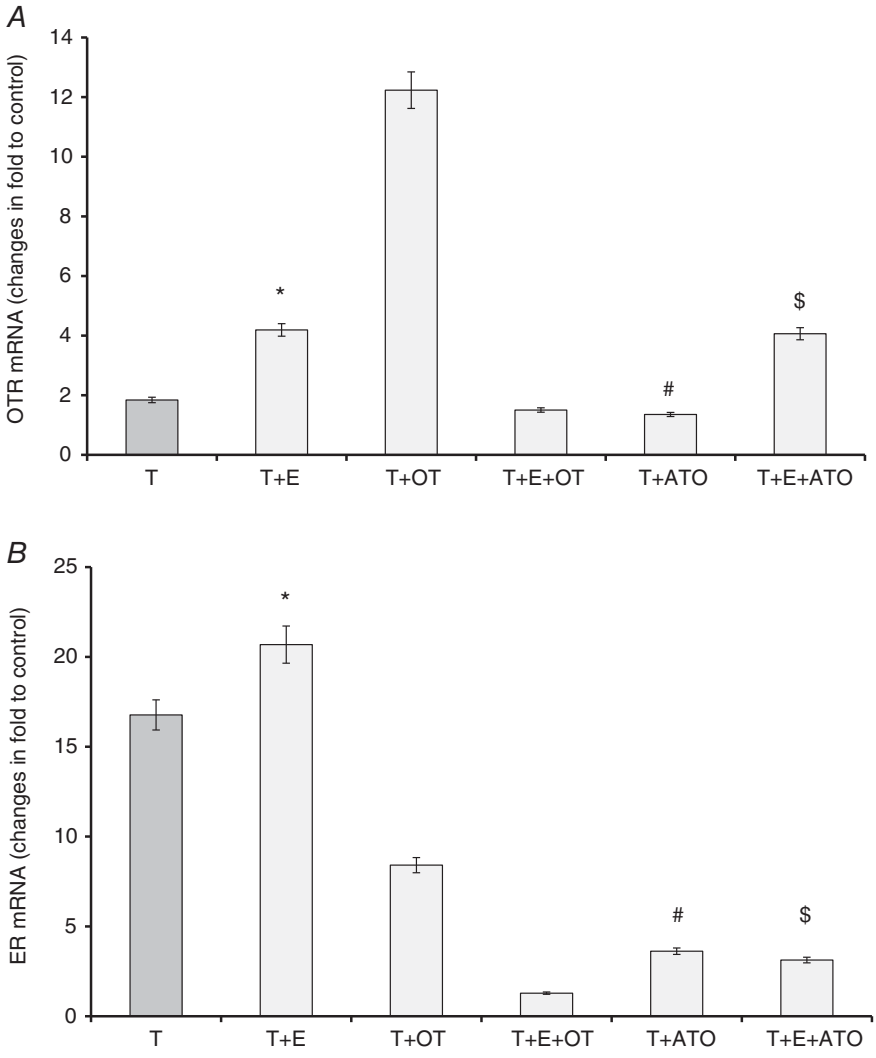

Figure 5. Effects of exercise training together with OT on the gene expression of *OTR* (*A*) and *ER* (*B*) in the mammary tumour cells in a mouse model of breast cancer
Data are means ± SD. $^{*}P < 0.05$ compared with T group, $^{\$}P < 0.05$ compared with T + E + OT group and $^{\#}P < 0.05$ compared with T + OT group. Groups are as in Fig. 1.

lower in the T + E + ATO group than in the T + E + OT group. In addition, it was decreased in the T + ATO group compared with the T + OT group ($P < 0.05$; Fig. 4*A*).

Caspase-3 mRNA expression in tumour tissue was increased in the T + E group compared with the T group. It was lower in the T + E + ATO group than in the T + E + OT group. It was decreased in the T + ATO group compared with the T + OT group ($P < 0.05$; Fig. 4*C*).

Messenger RNA expression of *OTR* and *ERα*

OTR mRNA expression in tumour tissue was increased in the T + E group compared with the T group ($P < 0.05$). It was also higher in the T + E + ATO group than in the T + E + OT group. It was decreased in the T + ATO group *versus* the T + OT group ($P < 0.05$; Fig. 5*A*).

ERα mRNA expression in tumour tissue was increased in the T + E group compared with the T group. It was lower in the T + AOT group than in the T + OT group and in the T + E + ATO group compared with the T + E + OT group ($P < 0.05$; Fig. 5*B*).

Messenger RNA expression of *P53*

P53 mRNA expression in tumour tissue was increased in T + E group compared with the T group. It was lower in the T + ATO group than in the T + OT group. It was decreased in the T + E + ATO group compared with the T + E + OT group ($P < 0.05$; Fig. 6).

Messenger RNA expression of citrate synthase, *PI3K2R* and *VEGF* in soleus muscle

Our results indicated that exercise training induced the adaptation in soleus muscle by increasing citrate synthase (*CS*), *PI3K2R* and *VEGF* expressions. The mRNA expressions of *CS* (2.24 ± 1.49-fold), *VEGF* (0.3 ± 0.79-fold) and *PI3K2R* (2.9 ± 1.11-fold) were increased in soleus muscle in the T + E group compared with the E group 6 weeks after beginning the interval exercise training ($P < 0.05$; Fig. 7).

Expression of Akt and ERK proteins by Western blot

Western blot analysis showed that phosphorylated Akt (p-Akt) and ERK (p-ERK) were significantly decreased in the exercise training and OT groups compared with the tumour group. In contrast, the dephosphorylated Akt (d-Akt) and ERK (d-ERK) were significantly increased in the exercise training and OT groups compared with the tumour group. Interestingly, atosiban reversed the effects of OT on Akt and ERK protein expression (Fig. 8).

Discussion

The exercise training and OT administration resulted in the inhibition of tumour growth in the present study. Our results showed that increased level of plasma concentration of OT after the exercise training was paralleled to lower tumour volume.

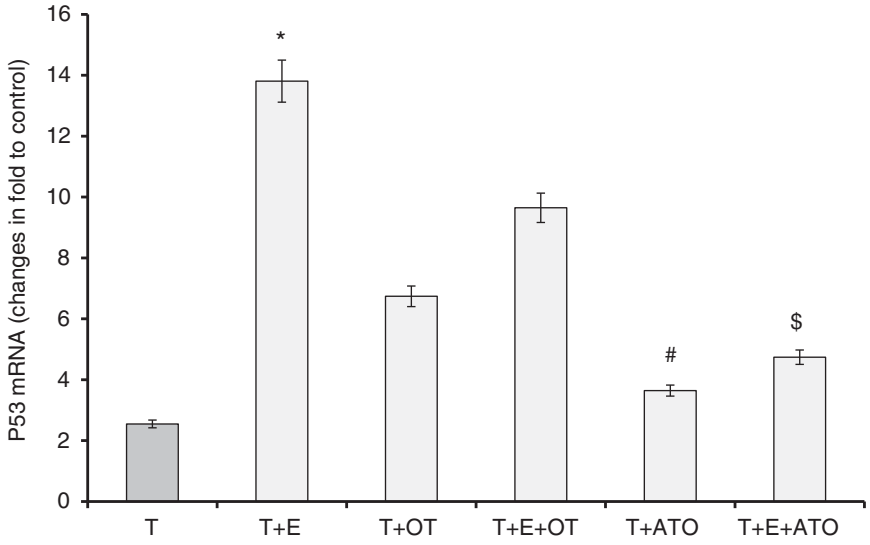

Figure 6. Effects of exercise training together with OT on *P53* expression in mouse mammary tumour cells
Data are means ± SD. $^{*}P < 0.05$ compared with T group, $^{\$}P < 0.05$ compared with T + E + OT group and $^{\#}P < 0.05$ compared with T + OT group. Groups are as in Fig. 1.

PI3K, Akt, mTOR and ERK can play an important role in cell proliferation and breast tumour growth (McAuliffe *et al.* 2010). In this context, the OT receptor can be a regulator of these pathways (Klein *et al.* 2013). It belongs to the G-protein-coupled receptor family, specifically Gq, and its activity is mediated by G-proteins that activate several different second messenger systems (Gimpl & Fahrenholz, 2001). The OT stimulatory effect is mostly mediated by increasing cytosolic Ca^{2+} (Cassoni *et al.* 2001, 2004; Péqueux *et al.* 2002, 2004), whereas its inhibitory effect is mediated by increasing cAMP and PKA (Cassoni *et al.* 1997; Péqueux *et al.* 2004). It has been also reported that cAMP inhibits the cell proliferation stimulated by G-protein-coupled receptors, such as the OTR (Magnaldo *et al.* 1989). This effect is mediated by the inhibitory action of cAMP on the PI3K/Akt and ERK signalling pathways (Lou *et al.* 2002; Nakada *et al.* 2011). PI3K can also be affected by exercise training and may reduce cell proliferation and enhance apoptosis (Xie *et al.* 2007).

In the present study, OT treatment decreased the expression of Akt and ERK as well as the effects of exercise training. On the contrary, the OT antagonist atosiban reversed the regulation of these genes. We have reported that exercise training and OT decreased the levels of both the phosphorylated Akt and ERK. Phosphorylated Akt plays key roles in cell proliferation and survival and in the formation of new blood vessels that is associated with tumour development. However, the innovative finding of the present study is that exercise training contributes to inhibition of Akt and its downstream effectors, such as mTOR and ERK. In this respect, activated Akt leads to the activation of mTOR (McAuliffe *et al.* 2010) and thus, the inhibition of Akt and ERK by the exercise training could be responsible for the inhibition of mTOR in our study. These data indicate that changes in these factors by exercise and OT may be responsible for their anticancer activities. Thus, we reported, probably for the first time, that OT secretion during interval exercise training may reduce PI3K/Akt and ERK axis activities, which resulted in less proliferation of mammary tumour cells. Moreover, our study may confirm the other findings on the role of OT in inducing apoptosis in prostate cancer (Sheina *et al.* 2007).

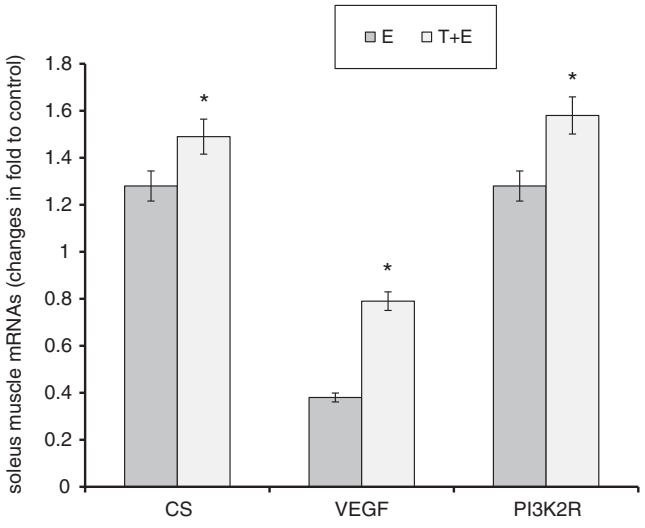

Figure 7. Effects of exercise training on the gene expression of *CS*, *VEGF* and *PI3KR* in mouse soleus muscle cells
Data are means ± SD. $^*P < 0.05$ compared with T group. Abbreviations: *CS*, citrate synthase; E, exercise training; and T + E, tumour plus exercise training.

As shown in Fig. 4*C*, caspase-3 expression was significantly increased in the OT-treated and exercise training groups. In addition, the mRNA expression patterns of *Bcl2* and *Bax*, two important proteins involved in apoptosis, were changed after OT treatment and exercise training. Physical activities can increase the Bax level compared with Bcl2 (Campbell *et al.* 2007). If the expression of Bax becomes greater than that of Bcl2, cell apoptosis is induced; otherwise, the cell survives (Oltval *et al.* 1993; Barnard *et al.* 2007). Therefore, a therapeutic approach is to inhibit Bcl2 family proteins, and the targeting of anti-apoptotic Bcl2 proteins can promote cell death (Farsinejad *et al.* 2015). In our experiment, the groups that showed a greater ratio of *Bax* to *Bcl2* may indicate more caspase-3 expression and, consequently, cell apoptosis. In addition, P53 is a regulator of *Bcl2* and *Bax* gene expression (Miyashita *et al.* 1994; Miyashita & Reed, 1995). In accordance with the apoptotic role of P53 through regulation of *Bax* and *Bcl2*, we showed that P53 it was increased by exercise training. Therefore, the effects of exercise training on tumour growth may be mediated, in part, by P53 and the induction of apoptosis.

Oxytocin may affect tumour through the ER. In ER^+ breast tumours, which are mostly drug resistant, OT treatment may be helpful by inhibiting ER expression. In the groups undergoing OT treatment and exercise training, *ER* gene expression was significantly reduced. In this respect, there is some cross-talk between the PI3K/Akt pathway and ER expression (Gil, 2014), which can be suggested as a mediatory role of ER in PI3K/Akt/mTOR and ERK suppression by OT. Bailey *et al.* (2012) showed that the ER may prevent P53-dependent apoptosis in breast cancer (Bailey *et al.* 2012). Moreover, the Bax/Bcl2 ratio is regulated by the ER (Zhang *et al.* 2012). Therefore, it seems that the ER can mediate some the beneficial effects of OT in breast cancer. The results of our experiments revealed that exercise training associated with the enhancement of plasma concentrations of OT and OT–OTR interactions might relate to tumour risk reduction.

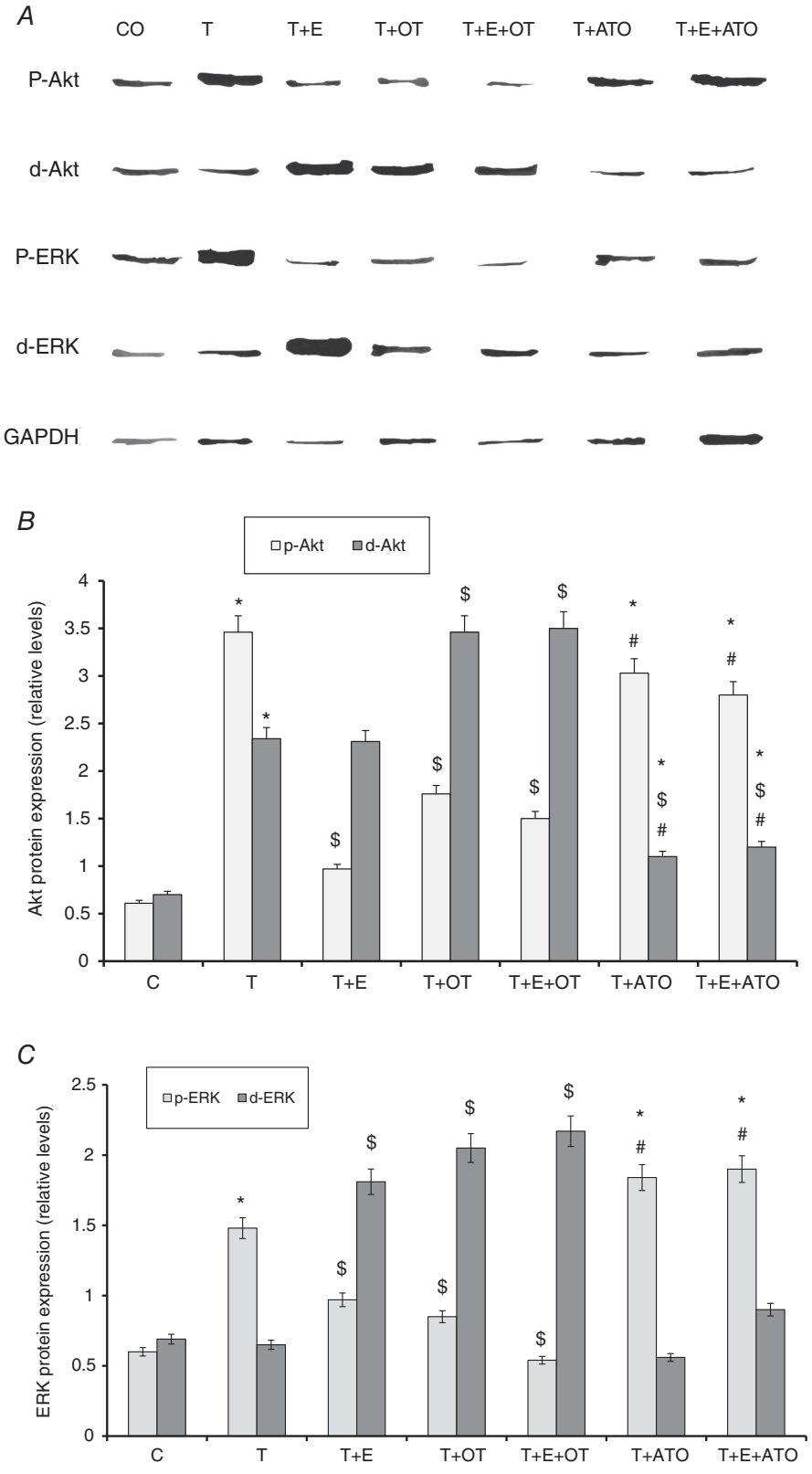

Figure 8. Effects of exercise training together with OT on the expression of Akt and ERK proteins in a mouse model of breast cancer

Akt (*B*) and ERK (*C*) proteins were extracted and resolved by Western blotting and antibodies for expression of d-Akt (dephosphorylated Akt) and d-ERK (dephosphorylated ERK) P-Akt (phosphorylated Akt) and P-ERK (phosphorylated ERK). GAPDH served as the loading control. *A*, the intensities of Akt and ERK bands were quantified with a GS-700 Imaging Densitomer. *B* and *C*, relative pixel density was calculated for each band following background subtraction. Data are means ± SD. $^{*}P < 0.05$ compared with C group, $^{\$}P < 0.05$ compared with T group and $^{\#}P < 0.05$ compared with T + E, T + OT and T + E + OT groups. Groups are as in Fig. 1.

Conclusions

Exercise training and OT administration can reduce tumour growth. Exercise training, associated with OT–OTR interaction, can reduce the expression of proteins of the signalling pathways involved in cell proliferation and cancer induction, such as Akt and ERK. Exercise training also enhances apoptosis through various pathways, including Bax, Bcl2 and caspase-3. ER^{+} breast cancer can also be controlled by exercise training via inhibition of ER expression. Exercise training, by inducing OT secretion, could be a useful tool in cancer treatment or prevention. Moreover, for those patients with acute conditions, we might enhance plasma OT concentrations by exogenous OT besides the exercise training, which can have a stronger effect on controlling the disease. Overall, our findings provided new insights into the potential anticancer role of exercise. According to these results, further investigation is required to determine the factors induced by the exercise training and OT, and responsible for the observed anticancer effects of exercise in clinical settings. It is also suggested that OT mediatory effects on tumour level should be investigated in OT knockout mice.

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Additional information

Competing interests

None declared.

Author contributions

A.M.A., A.I. and Z.H. conceived and designed the research. S.A., Y.H., M.R., B.B., H.S. and S.K. performed the experiments.

A.M.A., A.I. and S.K. analysed the data, prepared the figures and drafted the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript and 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. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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