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## **Title**

Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation

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### **Conflict of interest**

The authors of this study declare they have no conflict of interest

### **List of abbreviations**

BM	Body mass
CT	Cycle threshold
HA	Heat acclimation
HR	Heart rate
Hsp72 mRNA	Heat shock protein 72 messenger ribonucleic acid
HSP	Heat shock protein
HSR	Heat shock response
RH	Relative humidity
RT- QPCR	Reverse transcription polymerase chain reaction
T <sub>re</sub>	Rectal temperature

## Abstract

**Purpose:** Thermotolerance is an acquired state of increased cytoprotection achieved following single or repeated exposures to heat stress, in part characterised by changes in the intracellular 72kda heat shock protein (HSP72; HSPA1A). Females have demonstrated reduced exercise induced HSP72 in comparison to males. This study examined sex differences in heat shock protein 72 messenger ribonucleic acid (Hsp72 mRNA) transcription during heat acclimation (HA) to identify whether sex differences were a result of differential gene transcription.

**Methods:** Ten participants (5M, 5F) performed ten, 90 min controlled hyperthermia [rectal temperature ( $T_{re}$ )  $\geq 38.5^{\circ}\text{C}$ ] HA sessions over 12 d. Leukocyte Hsp72 mRNA was measured pre and post D1, D5, and D10, via Reverse transcription polymerase chain reaction (RT-QPCR).

**Results:** HA was evidenced by a reduction in resting  $T_{re}$  ( $-0.4 \pm 0.5^{\circ}\text{C}$ ) and resting heart rate [(HR);  $-13 \pm 7 \text{ beats}\cdot\text{min}^{-1}$ ] following HA ( $p \leq 0.05$ ). During HA no difference ( $p > 0.05$ ) was observed in  $\Delta T_{re}$  between males (D1 =  $1.5 \pm 0.2^{\circ}\text{C}$ ; D5 =  $1.6 \pm 0.4^{\circ}\text{C}$ ; D10 =  $1.8 \pm 0.3^{\circ}\text{C}$ ) and females (D1 =  $1.5 \pm 0.5^{\circ}\text{C}$ ; D5 =  $1.4 \pm 0.2^{\circ}\text{C}$ ; D10 =  $1.8 \pm 0.3^{\circ}\text{C}$ ). This was also true of mean  $T_{re}$  demonstrating equality of thermal stimuli for mRNA transcription and HA. There were no differences ( $p > 0.05$ ) in Hsp72 mRNA expression between HA sessions or between males (D1 =  $+1.8 \pm 1.5$  fold; D5 =  $+2.0 \pm 1.0$  fold; D10 =  $+1.1 \pm 0.4$  fold) and females (D1 =  $+2.6 \pm 1.8$  fold; D5 =  $+1.8 \pm 1.4$  fold; D10 =  $+0.9 \pm 1.9$  fold).

**Conclusions:** This experiment demonstrates that there is no difference in Hsp72 mRNA increases during HA between sexes when controlled hyperthermia HA is utilised. Gender specific differences in exercise-induced HSP72 reported elsewhere likely result from post-transcriptional events.

### Key words:

Controlled hyperthermia; males; females; thermotolerance; heat shock protein.

## Introduction

Repeated exposure to stressful thermal environments initiates heat adaptation in humans [1]. Heat adaptation incorporates the interrelated acclimation and thermotolerance [2,3]. A heat acclimated phenotype describes enhanced heat loss effector responses and hypervolemia which mitigate physiological, perceptual, and functional detriments to heat exposure [1,4]. Thermotolerance or acquired cellular thermotolerance is the nomenclature used to describe cellular adaptations caused by a single, or repeated severe, but non-lethal heat exposure [e.g. heat acclimation (HA)] [5].

HA repeatedly initiates the heat shock response (HSR), typically increasing various basal heat shock proteins (HSP), including HSP72 (HSP72). In response to 10 d HA, baseline intracellular HSP72 has been shown to increase by 18% [6] whilst heat shock protein 72 messenger ribonucleic acid (Hsp72 mRNA) demonstrates a pattern whereby transcription occurs within each HA session (+195%) before returning to baseline 24 hr later [7,8]. These transient HA mediated cellular adaptations to iHSP72 can confer cytoprotection to subsequent thermal [6] and non-thermal [8] stressors *in vitro* [6] and *in vivo* [9]. Eloquent in-vitro data demonstrates that cytoprotection to stress (thermal or otherwise) is abolished when HSP72 is knocked out [10–12] or blocked [13]. HA mediated in-vivo cytoprotection is dependent upon sufficient Hsp72 mRNA transcription [14] and subsequent HSP72 protein translation [15].

Controlled hyperthermia HA results in a greater Hsp72 mRNA compared with matched training in cool conditions as a result of greater endogenous stimuli for transcription [8]. Due to the consistent endogenous thermal stimulus there is an equality of Hsp72 mRNA

transcription during 10 d controlled hyperthermia HA [7]. Thus, controlled hyperthermia is a preferred HA method compared with traditional exogenously prescribed HA since it induces robust Hsp72 mRNA responses, ensuring sufficient and consistent increases in endogenous stimuli throughout an *in vivo* chronic intervention, particularly when comparing independent groups.

Morton and colleagues (2009) reported a sex specific HSP adaptation in human skeletal muscle following six weeks of continuous and interval training. Specifically, HSP70 increased by  $38 \pm 41\%$  and  $23 \pm 36\%$  following continuous and interval training respectively in males ( $n = 5$ ); however females ( $n = 5$ ) had no changes ( $3 \pm 37\%$  and  $4 \pm 14\%$  increase respectively), despite similar training status, training prescription and training adaptations ( $\dot{V}O_2\text{max}$ ) [16]. Differential sex responses reported by Morton et al. (2009) may be attributed to cytoprotective effects of oestrogen. Elevated oestrogen has been shown to afford cellular protection [17], accordingly increased oestrogen in females versus males may provide a mechanism for inhibited changes in HSP72 expression [18]. Oestrogen binds to the oestrogen receptor, which is a member of the steroid family of nuclear receptors and is the oestrogen response element in target genes, leading to the transcriptional regulation of many genes [19]. Gillum et al. (2013) reported higher intracellular HSP72 concentrations following a single bout of exercise in the heat in males compared with females (in both the follicular and luteal phase of the menstrual cycle), despite similar baseline values and identical endogenous stimuli for Hsp72 mRNA transcription [20]. Differential sex responses were also suggested to be a result of oestrogen providing cellular protection and thus, decreasing the necessity for translation of HSP72 in females. Although, stress-mediated sex specific differences in the HSP72 have been seen [16,20], they have not been examined at an mRNA level across the course of controlled hyperthermia HA.

Determination of Hsp72 mRNA transcription in females would facilitate identification of whether the inhibited HSP72 response resulted from absent gene signalling, or mitigated protein translation, potentially due to elevated oestrogen [16,20]. Absence of data in female populations could be problematic for practitioners who may adopt HA protocols that are informed by mechanistic cellular adaptations from male only cohorts [7,21]. This may reduce the magnitude to which females are protected against heat injury [14].

The aim of the current study was to determine whether the Hsp72 mRNA response during controlled hyperthermia HA, differed between males and females. It was hypothesised that the Hsp72 mRNA response would be attenuated in females compared to males across the course of controlled hyperthermia HA.

## **Materials and methods**

### **Participants**

Based on *a priori* power analyses using previous experimental data with identical methods [7,8], four participants in each group would result in 95% probability of detecting a difference in Hsp72 mRNA across the course of controlled hyperthermia HA. In line with power analysis, and previous work in the area [16], five males and five females (table 1) provided written informed consent to participate in the current study. All procedures were performed in accordance with the ethical standards of the institute and with the 1964 Declaration of Helsinki, as revised in 2013. Experimental trials were performed between 07:00 and 10:00 h to control for the time of day effects [7,22]. Confounding variables of smoking, caffeine, glutamine, alcohol, generic supplementation, and prior thermal, hypoxic, and hyperbaric exposures were all controlled in line with previous work in the field [23]. To control for hormonal fluctuations associated with the menstrual cycle, female participants began testing during the early follicular phase (3 d after the onset of menstruation) of their

self-reported menstrual cycle; where oestrogen ( $\sim 30 \text{ pg.ml}^{-1}$ ) and progesterone ( $\sim 1 \text{ ng.ml}^{-1}$ ) value are expected to be stable.

**Table 1** Participant characteristics. Mean  $\pm$  SD.

	<b>Age</b>	<b>Height</b>	<b>BM</b>	<b><math>\dot{V}O_2</math> peak</b>	
	<b>(years)</b>	<b>(cm)</b>	<b>(kg)</b>	<b>(L.min<sup>-1</sup>)</b>	<b>(mL.kg<sup>-1</sup>.min<sup>-1</sup>)</b>
<b>Males (N = 5)</b>	24 $\pm$ 7	175 $\pm$ 3	70.1 $\pm$ 5.1	2.64 $\pm$ 0.34	45.7 $\pm$ 4.4
<b>Females (N = 5)</b>	20 $\pm$ 1	163 $\pm$ 9	57.1 $\pm$ 4.9	3.22 $\pm$ 0.50	46.2 $\pm$ 4.1

**Notes:** BM, body mass;  $\dot{V}O_2$  peak, peak oxygen uptake

### **Preliminary testing**

2 hr prior to arrival participants consumed 3-5 mL.kg<sup>-1</sup> of water. On arrival to the laboratory for all experimental sessions, participants voided their bladder to provide a mid-flow urine sample. When two out of the following three criteria were achieved, adequate hydration to perform the trial was assumed based upon an osmolality value of  $\leq 700 \text{ mOsm.kg}^{-1}$ , a urine specific gravity value of  $\leq 1.020$  or body mass within 1% of daily average [24]. These experimental controls were not violated for any participant for any of the preliminary or experimental procedures. Height was measured using a fixed stadiometer recorded to 1 cm (Detecto Physicians Scales; Cranlea & Co., Birmingham, UK), and nude body mass (BM) recorded to 0.01 kg from digital scales (ADAM GFK 150, USA). A graded exercise test was performed in temperate laboratory conditions [20°C, 40% relative humidity (RH)] to determine  $\dot{V}O_2$  peak using a cycle ergometer (Monark e724, Vansbro, Sweden). The cycling intensity was set to 80 W and resistance was applied to the flywheel to elicit a 16 to 24 W.min<sup>-1</sup> increase (selected depending on the BM of the participant). Expired air was



measured using online gas analysis (Metalyzer Sport, Cortex, Germany). Peak  $\dot{V}O_2$  was considered the highest  $\dot{V}O_2$  obtained in any 30 s period.

### **Experimental design**

Two, 5 d consecutive blocks (10 d total) of controlled hyperthermia HA were completed separated by 48 hrs. Immediately prior to each HA session participants inserted a rectal thermometer (Henley, Reading, UK) 10 cm past the anal sphincter and affixed a heart rate (HR) monitor (Polar Electro Oy, Kempele, Finland). After a 20 min seated stabilisation period, resting measures were recorded and participants entered the environmental chamber (TISS, Hampshire, UK). The daily sessions consisted of a 90 min exposure to 40°C, 40% RH. Exercise intensity was set at 65%  $\dot{V}O_2$  peak from the outset and adjusted with work: rest intervals to maintain a rectal temperature ( $T_{re}$ )  $\sim$  38.5°C [25,26].  $T_{re}$  and HR were recorded at 5 min intervals. Fluid intake was restricted during the 90 min HA session.

### **Blood sampling, RNA extraction, and one-step reverse transcription quantitative polymerase chain reaction (RT-qPCR)**

Venous blood samples were taken immediately before and immediately after exercise heat exposure on D1, D5, and D10 of controlled hyperthermia HA. All blood samples were drawn from the antecubital vein into 6 mL EDTA Vacuette tubes (Grenier BIO-One, Stonehouse, UK). 1 mL of venous blood was pipetted into 10 mL of 1 in 10 red blood cell lysis solution (10X red blood Cell Lysis Solution; Miltenyi Biotech, Bisley, UK). Samples were incubated for 15 min at room temperature then isolated via centrifugation at 5°C and 400 *g* for 5 min and washed twice in 2 mL phosphate-buffered saline at 400 *g* for 5 min to isolate all leukocytes. RNA was then extracted via the previously validated acid guanidium thiocyanate–phenol–chloroform extraction method [27]. Quantity was determined at an optical density of 260

nm while quality was determined via the 260/280 and 260/230 ratios using a nanodrop spectrophotometer (NanoDrop 2000c; Thermo Scientific, Waltham, MA, USA).

Hsp72-relative mRNA expression (Hsp72 mRNA) was quantified using Reverse transcription polymerase chain reaction (RT-QPCR). Primers (table 2) were designed using primer design software (Primer Quest and Oligoanalyzer; Integrated DNA Technologies, Coralville, IA, USA). 20  $\mu$ L reactions containing 10  $\mu$ L SYBR-Green RT-PCR Mastermix (Quantifast SYBRgreen Kit; Qiagen, Manchester, UK), 0.15  $\mu$ L forward primer, 0.15  $\mu$ L reverse primer, 0.2  $\mu$ L reverse transcription mix (Quantifast RT Mix; Qiagen) and 9.5  $\mu$ L sample (70 ng RNA/ $\mu$ L) were prepared in separate tubes. Each PCR reaction (Rotorgene Q; Qiagen) was then performed as follows: 10 min, 50°C (reverse transcription), 5 min 95°C (transcriptase inactivation and initial denaturation); followed by: 10 s, 95°C (denaturation), 30 s, 60°C (annealing and extension) for 40 cycles. Fluorescence was measured following each cycle as a result of the incorporation of SYBR green dye into the amplified PCR product. Melt curves (50 to 95°C; ramp protocol 5-s stages) were analysed for each reaction to ensure only the single gene of interest was amplified. The relative quantification of mRNA expression for each sample was assessed by determining the ratio between the cycle threshold (CT) value of the target mRNA and the CT values for  $\beta$ 2-microglobulin. Fold change in relative mRNA expression was calculated using the  $2^{-\Delta\Delta CT}$  method [28].

**Table 2** Hsp72 mRNA primer sequences.

Gene	NCBI Accession #	Primer	Sequence (5'→3')	Amplitude length
<b>B2- Microglobulin</b> <b>(<math>\beta</math>2-M)</b>	NM_004048	Forward	CCGTGTGAACCATGTGACT	91
		Reverse	TGCGGCATCTTCAAACCT	
<b>Hsp72</b>	NM_005345	Forward	CGCAACGTGCTCATCTTTGA	198

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**Notes:** NCBI National Centre for Biotechnology Information

### **Statistical analysis**

All data were first checked for normality using the Shapiro-Wilk method and corrected for sphericity using the Greenhouse Geisser method. A two way mixed design analysis of variance (ANOVA) was performed to determine differences between the physiological and performance characteristics between D1, D5, and D10 in males and females. A three-way mixed design ANOVA was performed to identify differences between the Hsp72 mRNA, pre and post, on D1, D5, and D10 of controlled hyperthermia HA between males and females. When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison. Effect sizes [partial eta squared ( $np^2$ )] were calculated to analyse the magnitude of trends associated with controlled hyperthermia HA. All data were analysed using a standard statistical package (SPSS version 20.0, IBM, Armonk, New York, USA) and reported as mean  $\pm$  SD. Statistical significance was accepted at the level of  $p \leq 0.05$ .

## **Results**

### **Evidence of heat acclimation**

Figure 1 presents the resting  $T_{re}$  and resting HR data for D1, D5, and D10 of controlled hyperthermia HA. There was a main effect of day on  $T_{re}$  rest ( $F_{(2, 16)} = 11.219$ ,  $p \leq 0.001$ ,  $np^2 = 0.584$ ). No differences were observed from D1 to D5 ( $p = 0.563$ ). However,  $T_{re}$  rest reduced from D1 to D10 ( $p = 0.027$ ) and from D5 to D10 ( $p = 0.003$ ). There was no interaction effect between day and sex on  $T_{re}$  rest ( $F_{(2, 16)} = 3.287$ ,  $p = 0.064$ ,  $np^2 = 0.291$ ), however this was approaching significance with a moderate effect. The mean reduction in  $T_{re}$  rest from D1 to

D5 was  $-0.3 \pm 0.2^{\circ}\text{C}$  in males, whereas in females there were no changes ( $+0.1 \pm 0.2^{\circ}\text{C}$ ). The mean reduction in  $T_{re}$  rest from D5 to D10 was greater in females ( $-0.4 \pm 0.2^{\circ}\text{C}$ ) compared with males ( $-0.1 \pm 0.1^{\circ}\text{C}$ ).

There was a main effect of day on HR rest ( $F_{(2,16)} = 15.227$ ,  $p \leq 0.001$ ,  $np^2 = 0.656$ ). HR rest reduced from D1 to D5 ( $p = 0.040$ ), from D1 to D10 ( $p = 0.008$ ), and from D5 to D10 ( $p = 0.008$ ). There was no interaction effect between day and sex on HR rest ( $F_{(2,16)} = 0.383$ ,  $p = 0.688$ ,  $np^2 = 0.046$ ).

### **Hsp 72 mRNA responses to heat acclimation between sexes**

Figure 2 presents the means  $\pm$  SD for Hsp72 mRNA, pre and post on D1, D5, and D10 of controlled hyperthermia HA between males and females. Figure 2 also presents individual participants percentage change relative to D1. There was a main effect of time on Hsp72 mRNA response ( $F_{(1,8)} = 32.998$ ,  $p \leq 0.001$ ,  $np^2 = 0.805$ ). Hsp 72 mRNA increased pre to post on D1 ( $1.7 \pm 0.8$  fold,  $3.9 \pm 1.8$  fold;  $p = 0.003$ ), D5 ( $1.6 \pm 0.8$  fold,  $3.5 \pm 1.7$  fold;  $p \leq 0.001$ ), and D10 ( $2.0 \pm 0.7$  fold,  $3.0 \pm 1.4$  fold;  $p = 0.050$ ). There was no interaction effect between time and sex ( $F_{(2,16)} = 1.027$ ,  $p = 0.381$ ,  $np^2 = 0.114$ ). The increase in Hsp72 mRNA from pre to post was similar between males (D1 =  $1.8 \pm 1.5$  fold; D5 =  $2.0 \pm 1.0$  fold; D10 =  $1.1 \pm 0.4$  fold) and females (D1 =  $2.6 \pm 1.8$  fold; D5 =  $1.8 \pm 1.4$  fold; D10 =  $0.9 \pm 1.9$  fold).

There was no main effect of day on Hsp72 mRNA ( $F_{(1,8)} = 0.052$ ,  $p = 0.826$ ,  $np^2 = 0.006$ ).

There was no interaction effect between day and sex on Hsp72 mRNA ( $F_{(2,16)} = 1.027$ ,  $p = 0.381$ ,  $np^2 = 0.114$ ). There was no interaction effect between time, day and sex on Hsp72 mRNA ( $F_{(2,16)} = 0.479$ ,  $p = 0.628$ ,  $np^2 = 0.057$ ).

### **Comparable heat acclimation sessions**

Table 3 presents the mean  $\pm$  SD for performance and physiological variables during D1, D5, and D10 of controlled hyperthermia HA for males and females. There was no main effect of day ( $F_{(2, 16)} = 3.042, p = 0.076, np^2 = 0.275$ ) or interaction effect between day and sex ( $F_{(2, 16)} = 0.234, p = 0.794, np^2 = 0.028$ ) on  $\Delta T_{re}$ . There was no main effect of day ( $F_{(2, 16)} = 2.536, p = 0.143, np^2 = 0.241$ ) or interaction effect between day and sex ( $F_{(2, 16)} = 1.880, p = 0.185, np^2 = 0.190$ ) on mean  $T_{re}$ . There was no main effect for day ( $F_{(2, 16)} = 0.488, p = 0.623, np^2 = 0.057$ ) or interaction effect between day and sex ( $F_{(2, 16)} = 0.242, p = 0.788, np^2 = 0.029$ ) on mean HR.

There was a main effect of day on relative exercise intensity ( $F_{(2, 16)} = 21.593, p \leq 0.001, np^2 = 0.730$ ). Relative exercise intensity was higher on D5 ( $p \leq 0.001$ ) and D10 ( $p \leq 0.001$ ) compared to D1. There were no differences between D5 and D10 ( $p = 0.221$ ). There was no interaction effect between day and sex on relative exercise intensity ( $F_{(2, 16)} = 0.1034, p = 0.378, np^2 = 0.114$ ).

**Table 3** Physiological and performance measures on D1, D5, and D10 of controlled hyperthermia heat acclimation. Mean  $\pm$  SD.

	D1		D5		D10	
	Males	Female	Males	Females	Males	Females
<b>T<sub>re</sub> change (°C)</b>	1.5 $\pm$ 0.2	1.5 $\pm$ 0.5	1.6 $\pm$ 0.4	1.40 $\pm$ 0.22	1.8 $\pm$ 0.3	1.8 $\pm$ 0.3
<b>Mean T<sub>re</sub> (°C)</b>	38.2 $\pm$ 0.2	38.3 $\pm$ 0.2	38.1 $\pm$ 0.18	38.1 $\pm$ 0.1	38.2 $\pm$ 0.1	38.3 $\pm$ 0.9
<b>Mean HR (beats.min<sup>-1</sup>)</b>	146 $\pm$ 14	152 $\pm$ 9	145 $\pm$ 8	146 $\pm$ 10	143 $\pm$ 12	148 $\pm$ 9
<b>Intensity (% <math>\dot{V}O_2</math> peak)</b>	38 $\pm$ 5	35 $\pm$ 8	50 $\pm$ 6	44 $\pm$ 7	52 $\pm$ 11	53 $\pm$ 14

**Notes:** HR, heart rate; T<sub>re</sub>, rectal temperature;  $\dot{V}O_2$  peak, peak oxygen uptake.

## Discussion

This is the first study to compare Hsp72 mRNA expression in males and females across the course of controlled hyperthermia HA. In contrast to our hypothesis, this experiment demonstrates that the Hsp72 mRNA response is similar between males and females on D1, D5, and D10 of a controlled hyperthermia HA. This suggests that sex differences in HSP following acute [20] and chronic [16] *in vivo* exercise bouts are due to post transcriptional events. Controlled hyperthermia HA resulted in typical phenotypic adaptations, evidenced by reduction in resting  $T_{re}$  and HR across the course of controlled hyperthermia HA. Males and females demonstrated equal physiological responses ( $\Delta T_{re}$ , mean  $T_{re}$  and HR) to each HA session where Hsp72 mRNA was measured. Accordingly, equality of these endogenous stimuli, both between groups, and throughout HA elicited equal increases in Hsp72 mRNA transcription. Comparable transcription of Hsp72 mRNA between males and females, suggests endogenous stimuli which induce the HSR are the most important criteria for increasing Hsp72 mRNA [7], with no sex dependent inhibition or amplification in transcription.

In the current study, HA produced a significant increase in Hsp72 mRNA providing further evidence that the controlled hyperthermia HA method presents a sufficient endogenous stress to surpass the minimum requirement to elicit increased transcription of Hsp72 mRNA, in both males and females, at the onset and culmination of discrete and repeated bouts of exercise-heat stress. Gibson et al. [7] reported an increase in Hsp72 mRNA pre to post on D1 ( $1.9 \pm 0.6$  fold,  $4.9 \pm 1.1$  fold), D5 ( $2.3 \pm 0.8$  fold,  $5.3 \pm 2.5$  fold) and D10 ( $2.1 \pm 0.7$  fold,  $4.3 \pm 1.3$  fold) during a 10 d controlled hyperthermia HA protocol in male participants. The data in the current study demonstrates females have a comparable magnitude of response to males. Accordingly, this data provides mechanistic support for practitioners prescribing controlled hyperthermia HA for female athletes. Sustained increases in Hsp72 mRNA throughout the HA, further demonstrates the continued stimulation of the pathways responsible for thermotolerance, i.e. the equality of HSR, in both males and females.

Previously, a greater HSP72 increase has been reported in males compared to females [16,20]; however, these studies measured the protein (HSP) whereas the current study measured the gene (Hsp mRNA). Interestingly, the current data contradicts Paroo et al. [29] findings, who reported a sex specific HSR at the level of protein and mRNA; with male rats having a significantly higher HSP70 (200% of control) and Hsp70 mRNA (+900% of control) response following 60 min of exercise at 70%  $\dot{V}O_2$  max when compared with females (HSP70 = 100% of control; Hsp70 mRNA = 450% of control). Paroo et al. [29] did however provide mechanistic evidence for an interaction between HSP70, Hsp70 mRNA and oestrogen. Ovariectomized female animals treated with a placebo demonstrated equivalent increases in HSP72 (+150% of control) and Hsp72 mRNA (+1,200% of control) to males, whilst endogenous oestrogen returned the typical inhibited female HSR response (HSP70 100% of control; Hsp70 mRNA 300% of control). Methodologically, Paroo et al. [29] implemented the northern blotting technique which is less sensitive than the RT- QPCR technique used in the current study, potentially explaining the non-significantly increased Hsp72 mRNA.

Elevated oestrogen affords cellular protection [17] and thus, this cytoprotective pathway may inhibit changes in HSP72 translation [18]. It is likely, that oestrogen most greatly mediates post Hsp72 mRNA transcriptional changes which inhibit the translation of HSP72. The mechanism by which oestrogen attenuates HSR may be mediated through its indirect antioxidant properties by stabilising cellular membranes and attenuating oxidative stress; such an effect could protect thermal sensitive cells against exercise-induced damage, and thereby result in a blunted HSP72 response [29]. The lack of observed difference between males and females in the current study, may be a result of low oestrogen concentrations, which may not have been sufficient to exert an antioxidant effect.

## **Limitations**

Future work should involve the measurement of HSP72 protein alongside Hsp72 mRNA across the course of controlled hyperthermia HA in males and females, to help underpin the true effect of sex



on the HSR; the absence of which is a limitation of the present study. Furthermore, oestrogen is reported to have a dose dependent inhibition of HSP72 expression at the transcription level [17]. Future work should investigate the HSR, and subsequent HSP72 and Hsp72 mRNA response to discrete and repeated bouts of exercise-heat stress in high and low oestrogen conditions and in post-menopausal women, who naturally have lower oestrogen concentrations. This information would help practitioners implement controlled hyperthermia HA strategies that ensure an optimal stimulus for cellular adaptation.

## **Conclusion**

Males and females have equal Hsp72 mRNA expression throughout 10 d of controlled hyperthermia HA. This suggests that there are no differences in the endogenous criteria to transcribe Hsp72 mRNA via the HSR between males and females. Differences in basal HSP72 observed elsewhere are therefore likely to result from inhibited protein translation, potentially due to the influence of oestrogen.

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