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SYSTEMATIC REVIEW

Plasma heat shock protein-70 response to acute prolonged exercise: a systematic review, meta-analysis, and meta-regression

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Abstract

Extracellular heat shock protein 70 (HSP70) acts as a damage-associated molecular pattern, or "danger signal" for the immune system. Acute prolonged exercise evokes various physiological stresses that can stimulate the release of extracellular HSP70. However, exercise-induced extracellular HSP70 responses are inconsistent in human studies. Therefore, the purpose of this meta-analysis and meta-regression was to systematically evaluate the effect of exercise on plasma HSP70 expression and to determine the exercise-associated factors contributing to plasma HSP70 response. Data were extracted from 26 experimental trials from 13 studies, including 154 participants, in which plasma HSP70 was measured before and after prolonged, continuous running or cycling exercise at a fixed intensity relative to $\dot{V}O_{2max}$. Meta-analysis was performed to determine the raw mean difference (MD) between post- and pre-exercise HSP70 concentration. Meta-regression was performed to establish the moderating effects of $\dot{V}O_{2max}$, exercise intensity, duration, modality, environmental temperature, humidity, and hypoxia on the plasma HSP70 response. There was a significant effect of exercise on plasma HSP70 concentration (MD = 0.73 ng·mL^{-1}, 95% CI [0.13, 1.34], $P = 0.02$). Meta-regression explained ~57.1% of variation in exercise-induced change in plasma HSP70 concentration (marginal $R^2 = 0.571$). The $\dot{V}O_{2max}$ ($\beta = 0.51$, 95% CI [0.03, 1.00]), exercise duration ($\beta = 0.43$, 95% CI [0.21, 0.65]), intensity ($\beta = 0.40$, 95% CI [0.08, 0.73]), and environmental temperature ($\beta = 0.27$, 95% CI [0.10, 0.43]) explained variation in the plasma HSP70 response. These data contribute to our understanding of the factors that modulate the plasma HSP70 response to acute prolonged exercise.

exercise; heat shock protein 70; stress response

INTRODUCTION

Heat shock proteins (HSPs) are stress-responsive proteins synthesized to defend cellular integrity and homeostasis (1– 5). The heat shock protein-70 (HSP70) family is the most abundant of human HSP (6). The two major isoforms of the HSP70 family are: *1*) HSP73, a constitutively expressed protein thought to contribute to most chaperoning activity under resting circumstances; and *2*) HSP72, a highly inducible isoform that can be rapidly synthesized in the cytoplasm to respond to stressors (7). The synthesis of HSP72 is activated by the transcription factor heat shock factor-1 (HSF-1), which is translocated to the nucleus upon activation and binds to heat shock elements, resulting in HSP72 transcription (8). In human studies, it has been reported that acute prolonged exercise can increase the intracellular synthesis of HSP70 in various tissues and organs [e.g., peripheral blood mononuclear cells (PBMCs) (9, 10), skeletal muscle (11, 12), hepatosplanchnic tissue (13), the brain (14)], and the extracellular release of HSP70 into the circulation as evidenced in serum (12) and plasma (15, 16).

Intracellular HSP70 acts as a molecular chaperone that accompanies misfolded and denatured proteins to maintain cellular homeostasis (17). Stress-induced intracellular HSP70 expression inhibits the activation of nuclear factor κB (NF-κB), which has profound implications for immunity, inflammation, cell survival, and apoptosis (18–20). During "homeostasis-threatening" circumstances, HSP70 can be released from cells into the extracellular milieu and circulation through a necrosis release or receptor-mediated exocytosis pathway (21). Specifically, HSP70 release may be mediated by the hypothalamic-pituitary-adrenal or sympathoadrenal medullary axis, through the α_1-adrenoceptor pathway (17, 22).

Extracellular HSP70 acts as a DAMP, or "danger signal" for the immune system (23). Extracellular HSP70 stimulates innate immune responses by binding to toll-like receptors (TLR2 and TLR4), which in turn activate NF-κB and mitogen-activated protein kinases (MAPK), and therefore release

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of pro-inflammatory cytokines (24, 25). Extracellular HSP70 may serve a cytoprotective function in response to low doses of stress, but potentiates a dysregulated inflammatory response at higher stress levels, or above a critical threshold (26).

Acute prolonged exercise imposes a multitude of physiological stresses, such as elevated core and/or contracting skeletal muscle temperature (5, 15), reduced pH (27), increased Ca^{2+} concentration (28), decreased carbohydrate availability (29), and production of reactive oxygen species (ROS) (30). These physiological alterations can trigger exercise-induced increases in circulating HSP70 (5, 16), although this is not always the case (15, 31, 32). The varied exercise-induced extracellular HSP70 responses in the literature could plausibly be related to exercise factors such as intensity, duration, or environmental conditions, but the moderating effect of these variables has not been systematically explored.

Accordingly, the primary purpose of this meta-analysis and meta-regression was to systematically evaluate the effect of acute prolonged exercise on plasma HSP70 expression. We also sought to establish the moderating effect of selected individual attributes, exercise characteristics, and environmental conditions on the variability in exercise-induced plasma HSP70 expression.

METHODS

Study Design

We systematically reviewed the literature and critically appraised relevant research investigating the effect of acute, constant-load, prolonged exercise on exercise-induced ethylenediaminetetraacetic acid (EDTA)-treated plasma HSP70 concentration. A meta-analysis was then performed to statistically assess and summarize the data derived from the systematic review on exercise-induced EDTA-treated plasma HSP70 responses. Subsequently, a meta-regression was performed to identify the moderating effect of selected variables on the effect of acute, constant-load, prolonged exercise on EDTA-treated plasma HSP70 concentration. In this article, data extracted from each selected study were at the trial level, and each experimental trial was individually screened against our selection criteria. Therefore, some experimental trials of the selected studies were excluded because they did not meet the inclusion criteria, and multiple experimental trials from a study could be included in the analyses if they satisfied all the selection criteria. The study protocol was registered in the Prospective Register of Systematic Reviews (CRD42024558901).

Literature Search

All studies that investigated the effect of acute prolonged exercise on extracellular HSP70 expression were identified according to the PRISMA guidelines (33), with a predetermined search strategy. A comprehensive literature search was conducted using the following Boolean search terms: ("heat shock protein 70" OR "heat shock protein 72" OR hsp70 OR hsp72) AND (exercise). Searches were performed in six electronic databases: MEDLINE/EBSCO, PubMed, Scopus, SPORTDiscus/EBSCO, ScienceDirect, and CINAHL. The searches were conducted on November 1, 2023. Thereafter, My NCBI was registered to set up automatic searches to obtain and assess the potential studies published between the completion of database searches and the submission of this review article. Duplicates were removed using a reference management software (EndNote X9, Clarivate Analytics), and the list of potential studies was then screened using the inclusion and exclusion criteria.

Selection Criteria

Inclusion criteria.

Papers were only selected for quantitative synthesis if they satisfied the following criteria: *1*) the full text was written in English and published in a peer-reviewed scientific journal; *2*) a crossover or parallel experimental study design was used; *3*) participants were healthy adults (18–60-yr old) of any training background and sex; *4*) the measurement of peak (or maximal) oxygen uptake ($\dot{V}O_{2peak}$ or $\dot{V}O_{2max}$) was performed and reported; *5*) the experimental trials involved acute cycling or running exercise; *6*) exercise was performed at a constant work rate; *7*) exercise intensity was programmed according to $\%\dot{V}O_{2peak}$ or $\%\dot{V}O_{2max}$; *8*) exercise duration was fixed and $\geq$20 min; and *9*) EDTA-treated venous blood samples were obtained pre- and immediately postexercise for analysis of plasma HSP70 concentration.

Regarding the inclusion *criterion 4*, some included studies reported $\dot{V}O_{2max}$, whereas others reported $\dot{V}O_{2peak}$ depending on their testing protocols. The attainment of $\dot{V}O_{2max}$ versus $\dot{V}O_{2peak}$ could influence the absolute work rate where $\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$ is observed, and therefore the absolute, external workload prescribed to individual participants within each investigation. However, based on the findings from Ref. 34 reporting that $\dot{V}O_{2peak}$ attained on a maximum-effort incremental test is statistically similar to $\dot{V}O_{2max}$, the difference in these values is assumed to be negligible.

Exclusion criteria.

Studies (i.e., individual experimental trials), were excluded if they involved participants with any medical condition [e.g., diabetes (35, 36), polycystic ovary syndrome (37), premenstrual dysphoric disorder (38)] or experienced any thermoregulatory dysfunction [e.g., exertional heat illness (39, 40), heat stroke (41)] that might moderate extracellular HSP70 responses to acute prolonged exercise. Studies of children and teenagers were excluded due to differences in metabolic responses to exercise versus adults (42). Similarly, studies of older adults (>60 yr old) were excluded since previous research has observed a decrease in HSP70 expression with aging (43, 44).

Studies of unilateral prolonged exercise (e.g., single-legged cycling) and exercise modalities other than cycling and running were excluded to maximize generalizability and practical application. Studies using downhill running were excluded because it is not clear if downhill running exercise would initiate the synthesis of HSP70 in skeletal muscle due to its eccentric muscle-damaging nature (45, 46) and differences in physiological responses [e.g., changes in energy cost (47)] compared with level running. Studies using deep-water exercise were excluded due to differences in physiological responses to exercise [e.g., increased cardiac output (48), decreased expiratory reserve volume

(49), reduced $\dot{V}O_{2max}$ (50, 51)] compared with whole-body exercise in controlled laboratory conditions.

Interval exercise trials were excluded due to the fluctuation in work rates. However, if there was a change in exercise intensity (and a blood sample was obtained at the end of the first intensity), the first bout of exercise was included, but the subsequent bout was excluded (52). Exercise of nonfixed duration (e.g., race and time-trial) was excluded due to the variability of pacing and intensity, and time-to-exhaustion exercise was excluded due to between-individual differences in exercise duration. Studies programming exercise intensity using an approach other than the absolute work rate corresponding to $\%\dot{V}O_{2peak}$ or $\%\dot{V}O_{2max}$ were excluded to maintain comparability as it is less clear if the approach used for programming exercise intensity would mediate extracellular HSP70 responses. During the process of full-text screening, only one study was excluded due this criterion (31).

Exercise trials with any intervention strategy [e.g., precooling (53), layered clothing (54)], or any supplementation [e.g., caffeine (22, 52), blackcurrant extract (55)] administered to either enhance or impede HSP70 responses before or during exercise were excluded. Exercise trials conducted before and after the period of endurance training in a normoxic, temperate environment were included in the quantitative synthesis (meta-analysis). However, only exercise trials performed before heat- or hypoxic-acclimation were included to avoid the possible influence of repeated exposure to environmental heat/hypoxic stress on extracellular HSP70 responses (10).

Studies that collected serum-derived, and heparinized plasma samples were excluded. This exclusion criterion is necessary due to differences in extracellular HSP70 concentration in comparison with EDTA-treated samples (56).

Text Screening

Article titles and abstracts were independently screened by the primary researcher (T.C.). The full text of each paper was then assessed against the a priori inclusion and exclusion criteria by two individuals (T.C. and A.M.S.B.). Discrepancies were resolved by consensus with a third reviewer (E.M.). After a consensus on article selection was reached, each experimental condition of the selected studies was independently assessed, in accordance with the predetermined inclusion and exclusion criteria, through discussions between two researchers (T.C. and E.M.). Only experimental trials that satisfied all selection criteria were included in the quantitative synthesis. Figure 1 illustrates the stages of selection criteria, according to the PRISMA guidelines for systematic review and meta-analysis (57).

Quality of Evidence Assessment

Study quality was independently assessed by two reviewers (T.C. and A.M.S.B.) and verified by a third reviewer (E.M.). The initial appraisal tool used was based on the quality assessment tool for before-after (pre-post) studies with no control group, developed by the National Heart, Lung, and Blood Institute (NHLBI) (58). To enhance specificity in research contexts and improve applicability to this study design, we modified the NHLBI quality assessment tool for evaluation of the studies included in this review (see Supplemental Table S1).

The modified NHLBI quality assessment tool comprises 10 items, from six domains (of the twelve domains from the NHLBI tool): *1*) study question; *2*) study population; *3*) sample size; *4*) intervention; *5*) outcome measure; and *6*) statistical analysis. Each of the reviewers selected "yes," "no," or "cannot determine/not reported/not applicable" in response to each item on the tool. Where "yes" was selected, the item was awarded one point. One exception is *question 6* assessing the description of pretesting controls, two points were given if all relevant factors were standardized, whereas one point was given if some relevant factors were standardized (see Supplemental Table S1). Where "no" or "cannot determine/not reported/not applicable" was selected, the item was noted as the potential risk of bias that could be introduced by flaws in the study design or implementation (59); and therefore, was awarded zero points.

The results of the modified NHLBI quality assessment tool were used to assign an a priori quality rating to each study, using a strategy based on the recommendations of the Grading of Recommendations Assessment Development and Evaluation (GRADE) working group (60). A maximum attainable score of 11 points could be awarded, whereby a priori study quality was categorized as follows: "high" (9–11); "moderate" (6–8); "low" (4–5); or "very low" (0–3).

This a priori rating was then either maintained or downgraded by one level, based on the response to a specific question that was considered key to the precision of the primary outcome measures obtained from each study: Qa. Was plasma HSP70 concentration reported in a numeric format [means ± standard deviation (or standard error)]? If pre- and postexercise plasma HSP70 concentration was reported as means ± standard deviation (or standard error), the a priori quality rating was maintained, and if not, the study was downgraded by one level (e.g., from "high" to "moderate"). The inclusion of a specific question and reassessment of quality rating were based on the methodological strategies from the GRADE approach (60), which have been adopted in previous systematic reviews (61, 62).

Overall, this procedure allowed the final quality of evidence of each included study to be categorized as either "high," "moderate," "low," or "very low." This quality appraisal was not used to exclude any study.

Data Extraction

The following data were extracted: *1*) study characteristics (authors, year of publication, country of origin, and study design); *2*) participant characteristics [*n*, sex, training background, age (years), height (m), body mass (kg), $\dot{V}O_{2max/peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)]; *3*) exercise prescription [duration (min), modality (running/cycling), intensity ($\%\dot{V}O_{2max/peak}$), specific intervention (if applicable)]; *4*) environmental conditions [environmental temperature (°C), humidity (% relative humidity), the fraction of inspired oxygen ($\%FI_{O_2}$)]; and *5*) biochemical analysis [HSP measurement (HSP70/HSP72), blood collection tube, ELISA manufacturer, intra-assay variability (%)]; and *6*) HSP70 concentration [pre- and postexercise concentration (nanograms per milliliter, $ng \cdot mL^{-1}$)]. When numerical values were not stated in the text and only presented in figures, available software (DigitizeIt, Brunswick, Germany) was

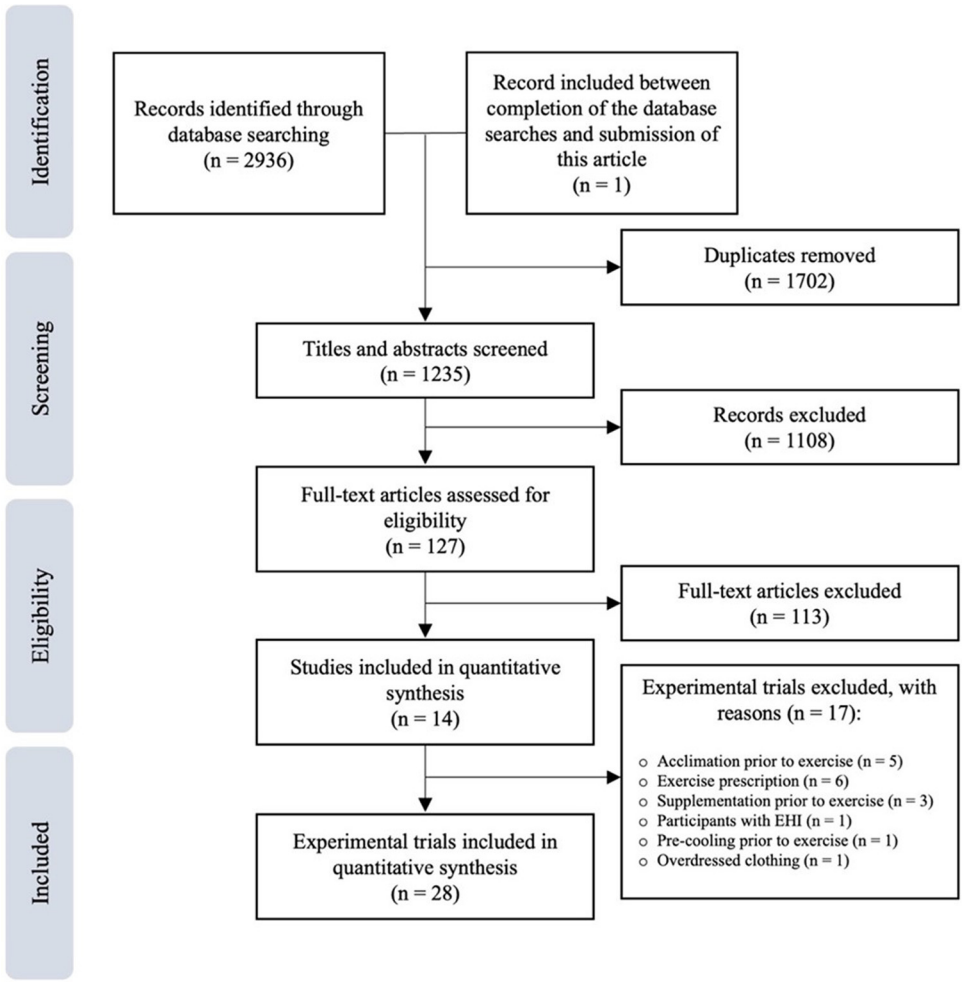

Figure 1. Schematic flow diagram of selection processes for the systematic review, meta-analysis, and meta-regression. EHI, exertional heat illness.

used for calculating mean and standard deviation values by measuring pixel lengths. All information was collated in a spreadsheet (Microsoft Excel; Microsoft Corporation, Washington, DC).

The candidate moderators were selected based on their theorized influence on HSP70 responses to exercise. It is acknowledged that elevations in core and contracting muscle temperatures might be an initiating factor in HSP70 activation during prolonged exercise (8); however, core or local temperature was not included as a moderator in meta-regression models due to insufficient data. It is also worth mentioning that variation in the sensitivity and detection capacities of the ELISA kits used for quantifying HSP70 concentrations may also contribute to between-study variability in EDTA-treated plasma HSP70 levels. The precision in determination of resting EDTA-treated plasma HSP70 concentration can vary between ELISA kits, even if they were supplied from the same manufacturer (EKS-715 HSP70 high-sensitivity ELISA vs. ENZ-KIT-101-001 HSP70 AMP'D ELISA, Enzo Life Sciences, Lausen, Switzerland) (63). Unfortunately, the influence of the ELISA kit used on EDTA-treated plasma HSP70 concentration could not be statistically assessed in the present research, owing to the incomplete reporting or the lack of information on the ELISA kits used in the included studies.

Statistical Analysis

Statistical analyses were performed in R version 4.3.2 (The R Foundation for Statistical Computing, Vienna, Austria), using the "metafor" (64) and the "meta" (65) packages for meta-analysis, and the "lme4" (66) and the "stats" (67) packages for meta-regression. Random-effects models were preferred for all analyses in anticipation of between-experimental trial heterogeneity (68). Outliers (experimental trials with 95% confidence intervals [CIs, lower bound; upper bound] that lie outside the 95% CI of the pooled effect) (69) were detected during the initial analysis and then removed from further analyses. Descriptive data are presented as weighted (based on sample size) means ± standard deviation (SD), unless otherwise stated.

To determine the raw, unstandardized difference in means between post- and pre-exercise plasma HSP70 concentration, a three-level random-effects (three levels of variance in effect sizes were specified: *1*) random-sampling variance, *2*) within-study variance, and *3*) between-study variance) and a traditional inverse-variance (without adding "study" as a random effect) models were constructed, and compared to determine if a random intercept is needed, using the likelihood ratio and Wald-type tests. The restricted maximum-likelihood method was used for estimating the parameters of the meta-analysis models. The model that has a better fit was preferred and

proceeded with, but the other can be found in Supplemental Fig. S1. The effect of acute prolonged exercise on post-exercise plasma HSP70 concentration (vs. pre-exercise) was reported as raw mean difference with 95% confidence intervals and 95% prediction intervals (MD, 95% CIs, 95% PIs [lower bound; upper bound]), and comparison of model performance indices between the two meta-analysis models is presented in Supplemental Table S2. Moreover, subgroup analysis was performed based on exercise modality (running and cycling interventions).

To examine which moderators explained variability in the MD of plasma HSP70 concentration from pre- to postexercise, a multilevel linear mixed-effects regression (with "study" specified as a random intercept) and a simple linear regression (without adding "study" as a random intercept) models were constructed and compared using information-theoretic approaches (see Supplemental Table S3). The model which had the lowest Akaike information criterion (AIC) was selected for presentation, with the others available in Supplemental Table S4 and Supplemental Fig. S2. The MD effect size was specified as the dependent variable, whereas the potential moderators were specified as fixed effects. The moderators considered for inclusion in the meta-regression were: *1*) participant's $\dot{V}O_{2max/peak}$, *2*) ambient temperature, *3*) ambient humidity, *4*) the inspired oxygen concentration, *5*) exercise modality, *6*) exercise intensity, and *7*) exercise duration. The restricted maximum-likelihood method was used for estimating the parameters of the meta-regression models. For the multilevel linear mixed-effects model, the study ID was specified as a random intercept given the two-level structure of the data: individuals (*level 1*) nested within an experimental study (*level 2*). Model fit was reported as marginal R^2, which describes the proportion of variance explained by the fixed effects, and conditional R^2, which describes the proportion of variance explained by both the fixed effects and the random effects (70). For the simple linear regression model, adjusted R^2 was used as a summary statistic (70). Statistical significance was inferred when $P \leq 0.05$.

The selection of a three-level random-effects model and a multilevel linear mixed-effects regression, with the study ID specified as a random intercept was made to account for covariance among multiple effect sizes per study and/or cohort. Dependencies between-experimental trial sampling errors and/or outcomes may exist within a study (e.g., where a study had multiple experimental trials including in meta-analysis, within-study sampling errors and/or outcomes might be on the same construct, and more similar to each other than the sampling errors and/or outcomes derived from studies that were conducted in different laboratories, and/or assayed plasma samples using different ELISAs, etc.) (71).

RESULTS

A total of 2,936 records were identified through the initial search, of which 1,702 duplicates were removed, and one further paper (55) was identified through the automatic searches (via My NCBI registration). Article titles and abstracts were then screened, and 1,108 studies were eliminated. Consequently, 127 full-text articles were assessed, and 14 studies met the inclusion criteria, all of which had multiple experimental trials that were independently assessed against the a priori criteria. Following the exclusion of 17 individual experimental trials, 28 exercise trials met all the inclusion criteria and were included in quantitative synthesis (Fig. 1). However, two experimental trials [from the same study (69)] were identified as outliers through an initial multilevel meta-analysis and subsequently removed, thus 26 experimental trials from 13 articles were included in the main analyses.

Study Characteristics

A total of 154 participants (134 males, 6 females, 14 unidentified) were evaluated from 13 selected studies, and all studies included small samples (≤13 participants). Weighted mean and standard deviation (based on sample size) for age (24.0 ± 3.5 yr), height (1.77 ± 0.03 m, $n = 144$), body mass (72.1 ± 4.6 kg), and $\dot{V}O_{2max/peak}$ (54.9 ± 6.2 $mL \cdot kg^{-1} \cdot min^{-1}$) were extracted from the included studies.

Of the 13 studies, seven used a parallel study design, and six used a randomized, crossover study design. The 13 studies were conducted in six different countries (United Kingdom, $n = 8$; Australia, $n = 1$; Brazil, $n = 1$; Germany, $n = 1$; Japan, $n = 1$; and United States, $n = 1$). The selected studies measured pre- and postexercise HSP70 concentration using enzyme-linked immunosorbent assays (ELISAs) from five different suppliers (Enzo Life Sciences, $n = 5$: Stressgen, $n = 5$; Assay Designs, $n = 1$; Proteintech, $n = 1$; and R&D Systems, $n = 1$). Table 1 provides an overview of the selected studies, including study description, participant characteristics, exercise prescription, environmental conditions, and biochemical analysis.

Quality Assessment of Included Studies

Quality assessment of included studies ($n = 13$) and the GRADE classification are presented in Table 2 and summarized in Fig. 2. The quality of the evidence from the 13 studies included in this review was primarily classified as "moderate" in quality (15% "high"; 46% "moderate"; 15% "low"; 23% "very low"; Fig. 2) (60). In particular, 62% of the a priori study ratings were downgraded one grade following the application of the data presentation question.

Effect of Acute Prolonged Exercise on Plasma Heat Shock Protein-70

The findings of the three-level random-effects meta-analysis of plasma HSP70 responses to acute prolonged exercise are demonstrated in Fig. 3. There was a statistically significant increase in plasma HSP70 concentration from pre- to postacute prolonged exercise (MD = 0.73 $ng \cdot mL^{-1}$, 95% CI [0.13, 1.34], 95% PI [−1.36, 2.83], $P = 0.02$). The I^2 statistic demonstrated 78.7% heterogeneity. Estimates of within-study ($\tau^2 = 0.08$, 95% CI [0.02, 0.31]) and between-study ($\tau^2 = 0.86$, 95% CI [0.11, 3.05]) variances were identified. Before outlier removal, the raw mean difference was much larger, with wider confidence intervals (MD = 2.18 $ng \cdot mL^{-1}$, 95% CI [−0.39, 4.74], 95% PI [−8.38, 12.73], $P = 0.09$, $I^2 = 98.7\%$).

In addition, subgroup analysis revealed that the magnitude of the acute prolonged exercise-induced change in plasma HSP70 concentration was not influenced by the

Table 1. *Study characteristics for included acute prolonged exercise trials with plasma heat shock protein 70/72 measurement before and immediately after exercise*

Study	Location	Study Design	Participants (*n*, Sex, Training Background, Age, Height, Body Mass, $\dot{V}O_{2peak/max}$)	Experiment No.	Exercise Prescription (Duration, Modality, Intensity, Specific Intervention—If Applicable)	Environmental Conditions (Temperature, Humidity, Hypoxia)	Biochemical Analysis (HSP Measurement, Blood Collection Tube, ELISA Manufacturer, Intra-Assay Variability)
Conrad et al. (55)	Chichester, UK	Double-blind, placebo-controlled, randomized crossover design (blackcurrant extract vs. placebo)	10, M, recreationally active individuals, 29 ± 2 yr, 1.82 ± 0.02 m, 80.3 ± 2.7 kg, 56 ± 2 mL·kg^{-1}·min^{-1}	EXPT01	60 min, treadmill running at 65% $\dot{V}O_{2max}$, placebo	34°C, 40% rH	Plasma HSP70, EDTA, Proteintech, unknown
Ely et al. (54)	Oregon	Randomized, counterbalanced, crossover design (activewear clothing in HEAT vs. overdressed clothing in TEMP)	13, M (7) and F (6), well-trained athletes, 24 ± 6 yr, 1.72 ± 0.1 m, 62.4 ± 8.7 kg, 58.7 ± 10.7 mL·kg^{-1}·min^{-1}	EXPT02	60 min, treadmill running, 55% $\dot{V}O_{2max}$, activewear clothing	40°C, 30% rH	Plasma HSP72, EDTA, Enzo Life Sciences, 4.6%
Fehrenbach et al. (16)	Münster, Germany	Parallel design	7, unclear, endurance-trained athletes, 29.9 ± 3.8 yr, 1.8 ± 0.06 m, 74 ± 6.4 kg, 66.0 ± 5 mL·kg^{-1}·min^{-1}	EXPT03	60 min, treadmill running, 75% $\dot{V}O_{2max}$	18°C, 50% rH	Plasma HSP72, EDTA, Stressgen, unknown
			7, unclear, endurance-trained athletes, 31.6 ± 7.5 yr, 1.75 ± 0.07 m, 66.2 ± 7.9 kg, 64.6 ± 8 mL·kg^{-1}·min^{-1}	EXPT04	120 min, treadmill running, 60% $\dot{V}O_{2max}$	18°C, 50% rH[a]	
Fortes and Whitham (52)	Bangor, UK	Single-blind, placebo-controlled, randomized crossover design (caffeine vs. placebo)	6, M, endurance-trained athletes, 21.8 ± 1.9 yr, 1.79 ± 0.04 m, 71.4 ± 3.1 kg, 60.7 ± 2.8 mL·kg^{-1}·min^{-1}	EXPT05	30 min, treadmill running, 50% $\dot{V}O_{2max}$, placebo (bout one)	30°C, 40% rH	Plasma HSP72, EDTA, Assay Designs, 2.7%
Gibson et al. (15)	Eastbourne, UK	Crossover design (20°C vs. 30°C vs. 40°C)	10, M, healthy individuals, 21.0 ± 0.5 yr, 1.72 ± 0.1 m, 71.1 ± 8 kg, 53.6 mL·kg^{-1}·min^{-1}	EXPT06	90 min, cycling, 50% $\dot{V}O_{2max}$	20°C, 52% rH	Plasma HSP72, EDTA, Enzo Life Sciences, 10.5%
				EXPT07	90 min, cycling, 50% $\dot{V}O_{2max}$	30°C, 53% rH	
				EXPT08	90 min, cycling, 50% $\dot{V}O_{2max}$	40°C, 39% rH	
Lee and Thake (10)	Coventry, UK	Parallel design	7, M, unclear, 22 ± 3 yr, 1.74 ± 0.08 m, 72.5 ± 11.4 kg, 51.4 ± 10.0 mL·kg^{-1}·min^{-1}	EXPT09	60 min, cycling, 50% $\dot{V}O_{2peak}$, D1-TRG	18°C, 35% rH	Plasma HSP72, EDTA, Enzo Life Sciences, unknown
				EXPT10	60 min, cycling, 50% $\dot{V}O_{2peak}$, D10-TRG	18°C, 35% rH	
			7, M, unclear, 22 ± 5 yr, 1.75 ± 0.06 m, 71.2 ± 2.8 kg, 52.3 ± 7.1 mL·kg^{-1}·min^{-1}	EXPT11	60 min, cycling, 50% $\dot{V}O_{2peak}$, D1-HYPA	18°C, 35% rH, 14% FI_{O_2}	

Continued

Table 1.— *Continued*

Study	Location	Study Design	Participants (*n*, Sex, Training Background, Age, Height, Body Mass, $\dot{V}O_{2peak/max}$)	Experiment No.	Exercise Prescription (Duration, Modality, Intensity, Specific Intervention—If Applicable)	Environmental Conditions (Temperature, Humidity, Hypoxia)	Biochemical Analysis (HSP Measurement, Blood Collection Tube, ELISA Manufacturer, Intra-Assay Variability)
			7, M, unclear, 22 ± 6 yr, 1.78 ± 0.08 m, 71.7 ± 9.2 kg, 50.7 ± 4.7 mL·kg^{-1}·min^{-1}	EXPT12	60 min, cycling, 50% $\dot{V}O_{2peak}$, D1-HA	40°C, 25% rH	
Lee et al. (53)	Coventry, UK	Randomized, counter-balanced, crossover design (resting in TEMP environment vs. precooling in water)	8, M, recreational runners, 28 ± 6 yr, 1.76 ± 0.08 m, 72.6 ± 12.5 kg, 53 ± 6 mL·kg^{-1}·min^{-1}	EXPT13	90 min, treadmill running, 65% $\dot{V}O_{2max}$, preceded by 60-min resting in 20°C, 60% rH	32°C, 47% rH	Plasma HSP72, EDTA, Enzo Life Sciences, 1.8%
Lee et al. (32)	Coventry, UK	Parallel design	8, M, physically active individuals, 20 ± 1.3 yr, 1.8 ± 0.1 m, 76 ± 10 kg, 46.3 ± 8 mL·kg^{-1}·min^{-1}	EXPT14	60 min, cycling, 50% $\dot{V}O_{2max}$, before TRG	18°C, 20% rH, 14% FI_{O_2} [b]	Plasma HSP72, EDTA, Stressgen, <10%
				EXPT15	60 min, cycling, 50% $\dot{V}O_{2max}$, D1-TRG	18°C, 20% rH	
				EXPT16	60 min, cycling, 50% $\dot{V}O_{2max}$, D3-TRG	18°C, 20% rH	
				EXPT27	60 min, cycling, 50% $\dot{V}O_{2max}$, after TRG	18°C, 20% rH, 14% FI_{O_2} [b]	
			8, M, physically active individuals, 21 ± 2.7 yr, 1.8 ± 0.1 m, 75.7 ± 8.2 kg, 46.2 ± 10 mL·kg^{-1}·min^{-1}	EXPT18	60 min, cycling, 50% $\dot{V}O_{2max}$, before HA	18°C, 20% rH, 14% FI_{O_2} [b]	
				EXPT19	60 min, cycling, 50% $\dot{V}O_{2max}$, D1-HA	40°C, 20% rH	
Lee et al. (63)	Coventry, UK	Parallel design	6, M, recreationally active individuals, 20 ± 2 yr, 1.79 ± 0.04 m, 71.8 ± 2.7 kg, 57.9 ± 9.7 mL·kg^{-1}·min^{-1}	EXPT20	60 min, treadmill running, 70% $\dot{V}O_{2max}$	33°C, 29% rH	Plasma HSP70, EDTA, Enzo Life Sciences, 4.1%
			7, M, recreationally active individuals, 22 ± 5 yr, 1.76 ± 0.05 m, 70.9 ± 5.7 kg, 54.9 ± 3.2 mL·kg^{-1}·min^{-1}	EXPT21	60 min, cycling, 50% $\dot{V}O_{2max}$	41°C, 25% rH	
			8, M, recreationally active individuals, 23.4 ± 4 yr, 1.8 ± 0.08 m, 70 ± 9.1 kg, 52.2 ± 3.3 mL·kg^{-1}·min^{-1}	EXPT22	60 min, cycling, 50% $\dot{V}O_{2max}$	21°C, 35% rH, 14% FI_{O_2}	
Magalhães et al. (72)	Minas Gerais, Brazil	Parallel design	9, M, healthy individuals, 25 ± 2.1 yr, 1.79 ± 0.05 m, 80.5 ± 5.7 kg, 50.5 ± 3 mL·kg^{-1}·min^{-1}	EXPT23	90 min, treadmill running, 50% $\dot{V}O_{2peak}$, before HA	40°C, 45% rH	Plasma HSP72, EDTA, Stressgen, unknown

Continued

Table 1.— *Continued*

Study	Location	Study Design	Participants (*n*, Sex, Training Background, Age, Height, Body Mass, $\dot{V}O_{2peak/max}$)	Experiment No.	Exercise Prescription (Duration, Modality, Intensity, Specific Intervention—If Applicable)	Environmental Conditions (Temperature, Humidity, Hypoxia)	Biochemical Analysis (HSP Measurement, Blood Collection Tube, ELISA Manufacturer, Intra-Assay Variability)
Ogawa et al. (73)	Tokyo, Japan	Parallel design	9, M, healthy individuals, 22.3 ± 0.9 yr, 1.71 ± 0.02 m, 68.8 ± 8.8 kg, 44.1 ± 10.8 $mL \cdot kg^{-1} \cdot min^{-1}$	EXPT24	60 min, cycling, 70% $\dot{V}O_{2max}$	25°C, 45% rH	Plasma HSP72, EDTA, Stressgen, unknown
Peake et al. (69)	Brisbane, Australia	Randomized, crossover design (MOD run vs. HEAVY run vs. MOD downhill run)	9, M, well-trained runners/triathletes, 28 ± 3 yr, 1.78 ± 0.05 m, 75 ± 7 kg, 61 ± 3 $mL \cdot kg^{-1} \cdot min^{-1}$	OUTLIER1	60 min, running, 60% $\dot{V}O_{2max}$, 0% gradient	Unclear, unclear	Plasma HSP70, EDTA, Stressgen, <7%
				OUTLIER2	60 min, running, 85% $\dot{V}O_{2max}$, 0% gradient	Unclear, unclear	
Ruell et al. (39)	Sydney, Australia	Parallel design	7, M, healthy individuals, 29.7 ± 3.2 yr, 1.79 ± 0.1 m, 73.8 ± 9 kg, 65.7 ± 5.3 $mL \cdot kg^{-1} \cdot min^{-1}$	EXPT25	60 min, treadmill running, 72% $\dot{V}O_{2max}$	30°C, 40% rH	Plasma HSP72, EDTA, R&D System, 5%
Whitham et al. (22)	Bangor, UK	Randomized, counterbalanced, crossover design (caffeine vs. placebo)	10, M, endurance-trained athletes, 21 ± 3.2 yr, unclear, 71.9 ± 7.3 kg, 61 ± 5.4 $mL \cdot kg^{-1} \cdot min^{-1}$	EXPT26	90 min, cycling, 70% $\dot{V}O_{2max}$, placebo	21°C, 52% rH	Plasma HSP72, EDTA, Stressgen, 6.2%

Data are represented in means ± standard deviation (SD). Some articles are included more than once in meta-analysis and meta-regression, depending on the study design and exercise prescription. $\%FI_{O_2}$, percentage of fraction of inspired oxygen; %rH, percentage of relative humidity; D, day; EXPT, experiment; F, female; HA, heat acclimation; HEAT, heat condition; HEAVY, heavy intensity; HYP, hypoxic condition; HYPA, hypoxia acclimation; M, male; MOD, moderate intensity; TEMP, temperate condition; TRG, training intervention (in temperate condition); $\dot{V}O_{2peak/max}$, peak or maximum oxygen uptake. Environmental conditions in temperate conditions were not reported, and therefore assumed by matching the conditions of separate experimental trials from the same study as follows: [a]18°C, 50% rH; or [b]18°C, 20% rH.

Table 2. *Quality assessment of included studies*

Study	Experiment No.	Modified NHLBI Quality Assessment Tool (Maximum Score = 11)						GRADE A Priori Quality (Score)	Data Presentation	GRADE Final Quality Rating
		Study Question	Study Population[a]	Sample Size[b]	Intervention[c]	Outcome Measure[d]	Statistical Analysis			
Conrad et al. (55)	EXPT01	N	Y/Y	Y/Y	YY/Y	Y/N	Y	High (9)	N	Moderate
Ely et al. (54)	EXPT02	Y	Y/Y	Y/N	Y/Y	Y/Y	Y	High (9)	Y	High
Fehrenbach et al. (16)	EXPT03, EXPT04	Y	N/N	N/CD	Y/N	Y/N	Y	Low (4)	N	Very low
Fortes and Whitham (52)	EXPT05	Y	Y/Y	N/Y	YY/Y	Y/Y	Y	High (10)	N	Moderate
Gibson et al. (15)	EXPT06, EXPT07, EXPT08	Y	Y/Y	N/N	Y/Y	Y/Y	Y	Moderate (8)	Y	Moderate
Lee and Thake (10)	EXPT09, EXPT10, EXPT11, EXPT12	Y	N/N	N/N	Y/Y	Y/N	Y	Low (5)	N	Very low
Lee et al. (53)	EXPT13	Y	Y/N	N/N	YY/Y	Y/Y	Y	Moderate (8)	Y	Moderate
Lee et al. (32)	EXPT14, EXPT15, EXPT16, EXPT17, EXPT18, EXPT19	Y	Y/Y	N/CD	YY/N	Y/Y	Y	Moderate (8)	Y	Moderate
Lee et al. (63)	EXPT20, EXPT21, EXPT22	Y	Y/Y	N/N	YY/Y	Y/Y	Y	High (9)	Y	High
Magalhães et al. (72)	EXPT23	Y	Y/Y	Y/CD	Y/Y	Y/N	Y	Moderate (8)	N	Low
Ogawa et al. (73)	EXPT24	Y	Y/N	N/CD	Y/Y	Y/N	N	Low (5)	N	Very low
Peake et al. (69)	OUTLIER1, OUTLIER2	Y	Y/N	N/Y	CD/N	Y/Y	Y	Moderate (6)	Y	Moderate
Ruell et al. (39)	EXPT25	Y	Y/Y	N/CD	CD/Y	Y/N	Y	Moderate (6)	N	Low
Whitham et al. (22)	EXPT26	Y	Y/N	Y/CD	YY/Y	Y/Y	Y	High (9)	N	Moderate

For the modified NHLBI quality assessment tool: Y, N, CD, NA, and NR. For the GRADE a priori quality rating: high, 9–11 pt; moderate, 6–8 pt; low, 4 or 5 pt; very low, 0–3 pt. For the GRADE final quality rating: high, moderate, low, and very low. The a priori rating is maintained if plasma HSP70 concentration is reported in a numeric format [means ± SD (or SE)]. If not, the study is downgraded a level. CD, cannot determine; EXPT, experiment; HSP70, heat shock protein-70; N, no; NA, not applicable; NHLBI, National Heart, Lung, and Blood Institute; NR, not reported; Y, yes; YY, yes with two points awarded (intervention). The concept that covers two assessment items: [a]study population: *1*) the specification of the cohort of participants, and *2*) the description of the eligibility criteria for participant recruitment; [b]sample size: *1*) the calculation of statistical power to determine the sample size, and *2*) the sufficiency of plasma HSP70 samples included in statistical analyses; [c]intervention: *1*) the description of pretesting controls (2 points—if all relevant factors were standardized), and *2*) the information on environmental conditions by which exercise was performed; [d]outcome measure: *1*) the specification of HSP70 ELISA assay, and *2*) the availability of the coefficient of variation for intra-assay variability data.

modality of exercise ($F_{1,24} = 1.61$, $P = 0.22$). Numerically, running interventions elicited a higher MD than cycling interventions; however, this was not statistically significant ($MD_{running} = 1.07$ ng·mL^{-1}, 95% CI [−0.44, 1.83], $P = 0.22$ vs. $MD_{cycling} = 0.38$ ng·mL^{-1}, 95% CI [−0.45, 1.21], $P = 0.36$, running vs. cycling intervention, respectively).

Effect of Moderator Variables on Plasma Heat Shock Protein-70 Responses to Acute Prolonged Exercise

The findings for the multilevel linear mixed-effects meta-regression model of acute prolonged exercise-induced plasma HSP70 responses are reported in Table 3 and Fig. 4. The mixed-effects model containing all selected moderators (participants' $\dot{V}O_{2max/peak}$, exercise intensity, exercise duration, exercise modality, environmental temperature, relative humidity, and the fraction of inspired oxygen) explained ~57.1% of variation in acute prolonged exercise-induced change in plasma HSP70 concentration (marginal R^2 = 0.571, conditional R^2 = 0.995, AIC = 51.4; Table 3). Standardized coefficients are provided to allow a better comparison of the relative influence of each moderator (Table 3 and Fig. 4).

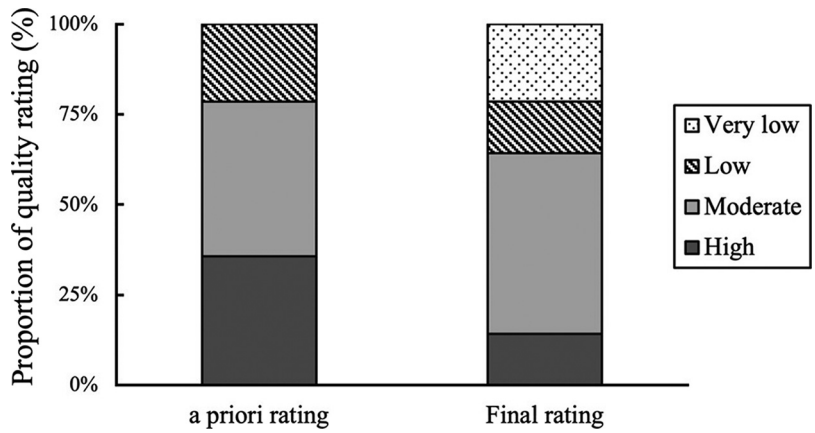

Figure 2. Quality rating of outcomes from all included studies ($n = 13$). Each bar represents the proportion of studies assigned a "high," "moderate," "low," or "very low" quality rating. The *x*-axis represents the different stages of the quality appraisal process, including *1*) a priori quality rating, and *2*) final quality rating following the reassessment of study quality, with an additional question to determine the reporting format of plasma HSP70 concentration. HSP70, heat shock protein-70.

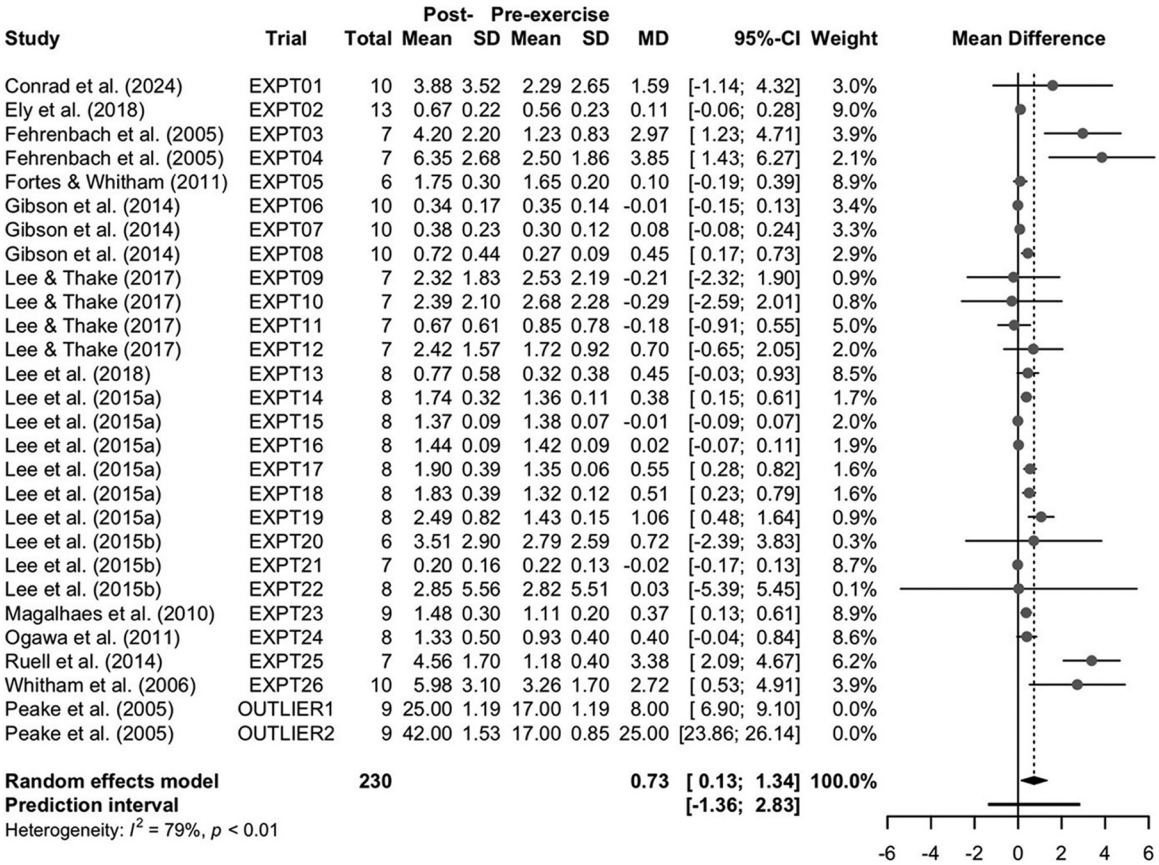

Figure 3. Forest plot of the three-level hierarchical meta-analysis of pre- to postacute prolonged exercise change in plasma heat shock protein-70 (HSP70) concentration, expressed in nanograms per milliliter (ng·mL^{-1}). CI, confidence interval; MD, mean difference; SD, standard deviation; total, sample size.

DISCUSSION

The primary purposes of this systematic review, meta-analysis, and meta-regression were to quantify the plasma HSP70 response to acute prolonged exercise and to investigate the moderating effect of selected variables on the magnitude of exercise-induced plasma HSP70 expression. The main findings were that: *1*) acute prolonged exercise increases plasma HSP70 concentration and *2*) a considerable degree of variation in exercise-induced plasma HSP70 expression (~57%) could be explained by $\dot{V}O_{2max/peak}$, exercise intensity, exercise duration, exercise modality, environmental temperature, relative humidity, and the fraction of inspired oxygen. These findings contribute to our understanding of how acute prolonged exercise affects plasma HSP70 concentration and provide insights into the influence of various exercise factors on the plasma HSP70 response.

Effect of Acute Prolonged Exercise on Plasma Heat Shock Protein-70

Our meta-analysis indicates that plasma HSP70 concentration increases following a single bout of prolonged cycling or running (Fig. 3). The exercise-induced increase in plasma HSP70 was consistent across most included experimental trials. As the exercise-induced increase in plasma HSP70 may contribute to an exercise-related inflammatory response (74), exert a cytoprotective function in response to low doses of stress, and potentiate a dysregulated inflammatory response at higher levels of stress (26), these data support the use of extracellular HSP70 response as a marker of exercise-induced stress. Nevertheless, it should be noted that although extracellular HSP70 can provide valuable insight into systemic cellular stress and "danger signal" for the immune system, it does not capture all dimensions of the physiological stress accumulated during exercise. Consequently, the measurement of extracellular HSP70 is likely to be most informative when interpreted alongside complementary markers that reflect other aspects of the exercise-induced stress responses.

The exercise-induced increase in plasma HSP70 concentration mirrors studies reporting increased intracellular HSP70 following acute exercise [e.g., monocyte HSP72 protein content (75, 76), leukocyte HSP72 protein content (72), peripheral blood mononuclear cell (PBMC) HSP72 mRNA (77), skeletal muscle HSP72 mRNA (12)]. Therefore, the exercise-induced increase in plasma HSP70 may be mediated by the release of newly synthesized intracellular HSP70, possibly via exosomes (78). Exosomes are released in a calcium-dependent fashion (79), and activation of α_1-adrenergic receptors results in cellular calcium influx (28). Therefore, exosome release is one potential mechanism via which exercise-induced increases in circulating catecholamines may trigger increased plasma HSP70 (22).

Although it is well established that acute prolonged exercise per se is sufficient to increase adrenaline and noradrenaline

Table 3. *Summary of the multilevel linear mixed-effects meta-regression (with "study" specified as a random intercept) model of acute prolonged exercise-induced plasma heat shock protein-70 concentration*

					Random-Effects				Fixed-Effects						
Model	AIC	RMSD	R^2 (Marg.)	R^2 (Cond.)	Group	Variance	SD	Input Variable	Estimate	SE	df	*t* Value	*Pr*(>|*t*|)	Std. Coef.	95% CI
Mixed-effects	51.4	0.147	0.571	0.995	(intercept)	0.75	0.86	(intercept)	−8.90	2.51	9.14	−3.54	0.006	0.08	[−0.49, 0.66]
					Residual	0.01	0.09	$\dot{V}O_{2max}$	0.09	0.04	10.66	2.24	0.047	0.51	[0.03, 1.00]
								Modality, running	−0.55	0.44	11.27	−1.25	0.238	−0.46	[−1.24, 0.32]
								Duration	0.03	0.01	17.99	4.19	<0.001	0.43	[0.21, 0.65]
								Intensity	0.05	0.02	10.40	2.61	0.025	0.40	[0.08, 0.73]
								Temperature	0.04	0.01	9.39	3.37	0.007	0.27	[0.10, 0.43]
								Humidity	<0.01	0.02	15.94	0.04	0.973	<0.001	[−0.38, 0.39]
								FI_{O_2}	−5.24	2.43	5.72	−2.16	0.076	−0.12	[−0.25, 0.00]

Mixed-effects model: the multilevel linear mixed-effects meta-regression (with "study" specified as a random intercept). Random intercept: study ID; independent variables: participant's $\dot{V}O_{2max/peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), exercise modality (running or cycling), exercise duration (min), exercise intensity (relative to participant's $\dot{V}O_{2max/peak}$, %), environmental temperature (°C), environmental humidity (relative humidity, %), the fraction of inspired oxygen (FI_{O_2}, %). 95% CI, 95% confidence intervals [lower bound, upper bound]; AIC, Akaike information criterion; df, degrees of freedom; R^2 (cond.), conditional *R*-squared; R^2 (marg.), marginal *R*-squared; RMSD, root mean square deviation; SD, standard deviation; SE, standard error; Std. Coef., standardized coefficients. *Pr*(>|*t*|) represents the *P* value associated with the value in *t* value. HSP70 units are $ng \cdot mL^{-1}$.

concentrations (22, 80–82), less is known about the involvement of catecholamines in the exercise-induced increase in HSP70 concentrations, particularly in humans. Whitham et al. (22) stimulated sympathetic activity with caffeine supplementation to examine extracellular HSP72 responses in exercising humans. A single bout of 90-min cycling at 70% $\dot{V}O_{2max}$ combined with caffeine supplementation resulted in larger increases in plasma HSP72 concentrations than equivalent exercise with placebo treatment (post-exercise HSP72 concentration, 8.6 ± 4.1 vs. 5.9 ± 2.8 $ng \cdot mL^{-1}$, caffeine vs. placebo treatments, $n = 10$, $P < 0.05$) (22). Indeed, this greater extracellular HSP72 response was associated with a greater adrenaline response to exercise combined with caffeine supplementation ($P < 0.05$), suggesting that catecholamines may be an important mediator of the exercise-induced extracellular HSP72 response in humans (22). Therefore, the increase in extracellular HSP70 concentration in response to acute prolonged exercise identified in this meta-analysis may be mediated by exercise-induced increases in catecholamine concentrations.

Effect of Moderator Variables on the Plasma Heat Shock Protein-70 Response to Acute Prolonged Exercise

Our meta-regression reveals that a considerable proportion (~57%) of the variation in the exercise-induced change in plasma HSP70 concentration can be explained by $\dot{V}O_{2max/peak}$, exercise characteristics (intensity, duration, and modality), and environmental conditions (temperature, relative humidity, and the fraction of inspired oxygen; Table 3). The difference (0.424) between marginal and conditional R^2 suggests that study-level differences (random effects) explain a substantial portion of the variation in

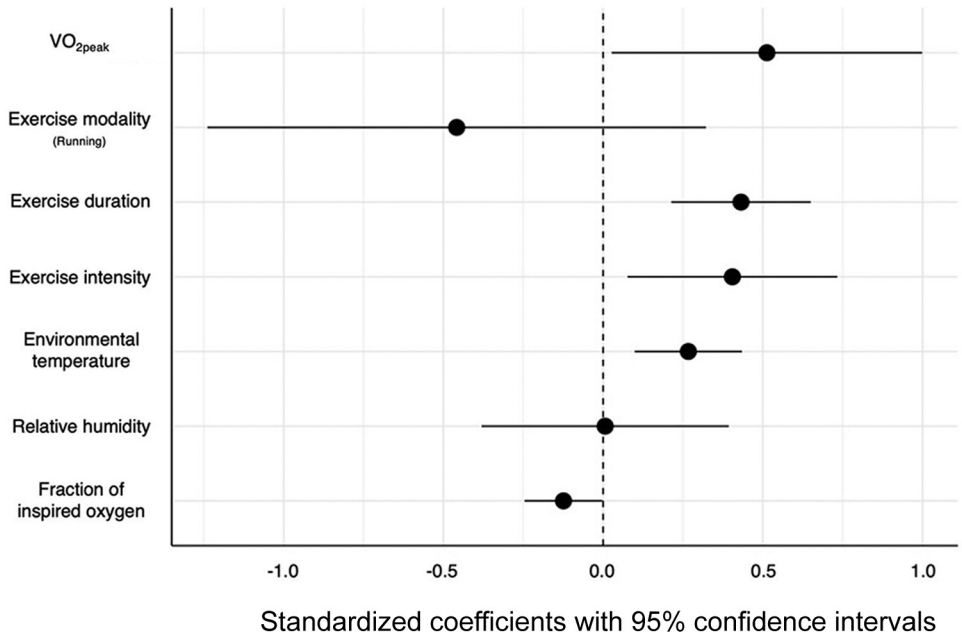

Figure 4. Plot of standardized coefficients, with 95% confidence interval for the moderators in the multilevel linear mixed-effects meta-regression (with "study" specified as a random intercept) model of acute prolonged exercise-induced plasma heat shock protein-70 concentration ($ng \cdot mL^{-1}$).

acute prolonged exercise-induced change in plasma HSP70 concentration, which is likely due to various factors related to biochemical analyte (e.g., blood sample handling, ELISAs used for quantifying HSP70 concentrations, etc.) or exercise per se (e.g., progressive dehydration and glycemic status) that are not included as fixed effects.

Individual peak (or maximal) oxygen uptake.

Differences in training history and physical activity levels might have an influence on the magnitude of exercise-associated plasma HSP70 responses. Here, it appears that individuals with higher $\dot{V}O_{2max/peak}$ had larger increases in plasma HSP70 concentration in response to acute prolonged exercise ($\beta = 0.51$, 95% CI [0.03, 1.00], $P = 0.047$; Table 3), although this is not always observed (83). First, endurance-trained athletes with higher $\dot{V}O_{2max}$ exercise at higher absolute external workloads at a given percentage of $\dot{V}O_{2max}$, and therefore at greater metabolic work rates. This is likely to result in higher carbohydrate oxidation rates. Previous research demonstrated that carbohydrate availability has been associated with activation of HSP70 synthesis during exercise (29, 84–86). Febbraio et al. (84) found that both HSP72 mRNA and HSP72 protein content only increased in the vastus lateralis of the leg that had previously performed glycogen-depleting exercise. Furthermore, Febbraio et al. (86) demonstrated that glucose ingestion attenuated hepatosplanchnic serum HSP72 release ($P < 0.05$) during 120-min semi-recumbent cycling at ~65% $\dot{V}O_{2peak}$. Therefore, it is possible that the larger plasma HSP70 responses of athletes with higher $\dot{V}O_{2max}$ may be related to greater rates of energy expenditure and carbohydrate oxidation at given relative intensities.

Second, this might be related to the magnitude of sympathoadrenal responses to acute prolonged exercise that (as previously discussed) have been suggested to play a stress signal role for the extracellular release of HSP70 (4, 22, 52, 78). As exercise-induced adrenaline and noradrenaline responses likely occur in an intensity-dependent fashion, it seems logical that the release of extracellular HSP70 in response to exercise might be correspondingly stimulated and, therefore, associated with greater exercise-induced release of HSP70 concentration observed from those individuals with higher $\dot{V}O_{2max/peak}$ exercising at higher absolute workloads.

Third, endurance-trained individuals might respond to exercise-associated stressors with a faster and larger production of intracellular HSP70 and have higher baseline intracellular HSP70 content available for release into the circulation (27, 87). Fehrenbach et al. (88) demonstrated that endurance-trained male runners had higher baseline HSP70 mRNA expression in leukocytes, and a larger increase in HSP70 mRNA expression in leukocytes following in vitro heat shock compared with sedentary untrained men. Similarly, Liu et al. (27) reported increased resting intramuscular HSP70 expression in highly trained rowers during four weeks of rowing training. Therefore, regular endurance training may potentiate the HSP70 response to acute exercise (88), as a useful mechanism for managing exercise-associated stress (89).

Exercise intensity.

We observed that the plasma HSP70 response increases with the intensity of exercise ($\beta = 0.40$, 95% CI [0.08, 0.73], $P = 0.025$; Table 3). This is likely related to the magnitude of sympathoadrenal responses to acute prolonged exercise that stimulate HSP70 release (4, 22, 52, 78). In support, previous research has demonstrated that extracellular HSP70 responses were correlated with circulating adrenaline and noradrenaline responses when exercise was performed at a constant workload corresponding to ~60%–70% $\dot{V}O_{2max}$ (5, 22).

It is likely that exercise intensity needs to be above a threshold to induce sufficient activation of α_1-adrenergic receptors to elicit the release of HSP70 into the circulation (83). Existing evidence demonstrated that moderate-intensity prolonged exercise (~60% $\dot{V}O_{2max}$) is sufficient to increase plasma HSP70 expression (5, 16, 69); however, this is not always the case (15, 31, 54). One of the possible reasons for this inconsistency may be the different approaches to programming exercise intensity. The most common approach for prescribing exercise intensity is the absolute work rate corresponding to a given percentage of $\dot{V}O_{2max}$ (15, 16, 22, 63, 69). However, this approach is problematic, as a given percentage of $\dot{V}O_{2max}$ may be above or below metabolic thresholds such as the lactate threshold, or boundary between the moderate and heavy intensity domains (90). Exercise in the heavy-intensity domain produces physiological responses that are distinct from the moderate domain, such as greater muscle glycogen utilization (91), whole-body carbohydrate metabolism, and plasma adrenaline concentrations (80). Based on the known robust exercise-induced increase in HSP70 expression associated with decreased carbohydrate availability (84, 85) and increased circulating adrenaline concentrations (22), it is plausible that extracellular HSP70 responses to exercise are specific to the intensity domains. However, this warrants specific investigation to provide more clarity on the exercise intensity required to stimulate HSP70 accumulation. Specifically, we recommend that future studies explore the plasma HSP70 response to exercise in the moderate, heavy, and severe intensity domains.

Exercise duration.

Exercise duration also moderates the effect of prolonged exercise on plasma HSP70 concentration ($\beta = 0.43$, 95% CI [0.21, 0.65], $P < 0.001$; Table 3). This aligns with the results of individual studies comparing extracellular HSP70 responses with exercise at the same intensity with different durations (12, 16, 92). Although the precise mechanism for how exercise duration plays a role in stimulating extracellular HSP70 response is not yet clarified, it is possible that the progressive accumulation of physiological strain as exercise progresses, such as rising core and/or local temperatures (11, 93), increased sympathetic nervous stimulation (94), and decreased carbohydrate availability (84, 85), can individually and collectively influence the magnitude of the exercise-induced extracellular HSP70 response (16, 95).

A newly emerging concept in the exercise physiology literature is “durability.” Durability refers to the resilience of an individual’s intensity domain transitions during prolonged exercise, whereby an individual with poor durability sees a large reduction in work rate at the intensity domain transitions as exercise extends (96). As there is evidence of substantial inter-individual variability in durability (97–102), it is plausible that the effect of exercise duration on stress responses such as plasma HSP70 expression is at least partially mediated by durability. Accordingly, we recommend that

future studies exploring the effect of prolonged exercise on plasma HSP70 expression consider the effects of durability.

Exercise modality.

Exercise modality may also have an influence on the exercise-induced change in plasma HSP70 concentration. Given that running can lead to substantially more muscle damage (as evidenced by increases in blood markers (103–106)) in comparison with cycling, we hypothesized that running would be associated with a larger exercise-induced increase in plasma HSP70. However, we did not observe a moderating influence of exercise modality (cycling vs. running) on the exercise-induced change in plasma HSP70 concentration ($\beta = -0.46$, 95% CI [−1.24, 0.32], $P = 0.238$; Table 3). This suggests that exercise-induced muscle damage is unlikely a potent stimulus for the systemic release of HSP70, which may be explained by the fact that the contracting skeletal muscle is not the primary source of HSP70 secretion during exercise (12, 84). Nevertheless, it is worth mentioning that there were only nine running trials included in this meta-regression [EXP01 (55); EXP02 (54); EXP03, EXP04 (16); EXP05 (52); EXP13 (53); EXP20 (63); EXP23 (72), EXP25 (39)] whereas the other 17 were cycling trials. Therefore, these data should be interpreted with caution as a small number of included running trials may limit the ability of the regression model to detect the moderating effect of exercise modality on plasma HSP70 expression.

Environmental temperature.

Environmental heat stress has long been considered as a primary stimulus for activating the synthesis of intracellular HSP70 (11, 107) and the release of extracellular HSP70 into the circulation during exercise (5). Accordingly, our meta-regression demonstrates that environmental heat stress has a moderating effect on the plasma HSP70 response to exercise ($\beta = 0.27$, 95% CI [0.10, 0.43], $P = 0.007$; Table 3). Gibson et al. (15) reported that the rate of rectal temperature increase ($^{\circ}C \cdot h^{-1}$) and absolute change in rectal temperature ($^{\circ}C$) were predictors of the increase in plasma HSP72 concentration, suggesting that the greater rise in core temperature during prolonged exercise performed under environmental heat stress promotes the plasma HSP70 response. Interestingly, Whitham et al. (5) reported that although 120-min deep-water running at ~60% $\dot{V}O_{2max}$, which did not elicit a rise in core temperature, was sufficient to increase plasma HSP72 concentration, the equivalent exercise with a change in core temperature ($+2.2^{\circ}C$) resulted in a significantly greater increase in extracellular HSP72 concentration ($P < 0.05$). These data suggest that the rise in core temperature during exercise is a contributing, but not the sole, factor responsible for the exercise-induced increase in plasma HSP70 (5).

Furthermore, the heat stress-induced reductions in external work rates associated with the intensity-domain transitions (108) and the peak oxygen consumption (109) might also be a potential factor contributing to the greater plasma HSP70 response to exercise performed under heat stress. In our meta-regression, six of the 12 experimental trials conducted in 30–41°C investigated exercise-induced plasma HSP70 responses during exercise at the absolute, external workload corresponding to thermoneutral $\dot{V}O_{2max}$ [EXPT07, EXPT08 (15); EXPT12 (10); EXPT20, EXPT22 (63); EXPT25 (39)]. Accordingly, the heat stress-induced increase in plasma HSP70 concentrations in our meta-regression may be influenced by participants exercising at the same absolute, but higher physiological, work rates under hot conditions versus comparator thermoneutral trials.

Relative humidity.

Relative humidity could potentially influence the exercise-induced plasma HSP70 response, given the heat storage increases as relative humidity increases (above ~70% relative humidity), especially when combined with hot temperatures (110, 111). During exercise in high relative humidity, sweat evaporation is compromised, as evidenced by greater increases in core temperature and heat storage (110, 111). Due to the known influence of elevated core temperature in upregulating HSP70 responses (5, 11, 15), high relative humidity may therefore promote the plasma HSP70 response to exercise. However, we did not observe a moderating effect of relative humidity on the plasma HSP70 response to exercise in this meta-regression ($\beta < 0.001$, 95% CI [−0.38, 0.39], $P = 0.973$; Table 3). This may be an artifact of the spread of studies included in this meta-regression, as relative humidity has been matched (10, 32) or varied in relation to the different ambient temperatures (15). An experimental study involving exercise in at least two humidity conditions is therefore required to allow a better understanding of the direct link between relative humidity (where temperature and fraction of inspired oxygen are matched) and the plasma HSP70 response to acute prolonged exercise.

Hypoxia.

Our meta-regression reveals that the fraction of inspired oxygen did not moderate the plasma HSP70 response to acute prolonged exercise ($\beta = -0.12$, 95% CI [−0.25, 0.00], $P = 0.076$; Table 3). This is an interesting finding as a stimulated increase in intracellular HSP70 has been previously documented as an acute response to exercise combined with hypoxic exposure (75, 76). Hypoxic exposure (112) and exercise (113) can individually and collectively disturb redox balance. The disturbances to redox balance are postulated as a potent stimulus for increases in HSP70 responses (114, 115), and the induction of intracellular HSP70 expression in response to hypoxic stress might provide protection against the disturbances to redox balance during prolonged exercise (116). Conversely, the extracellular HSP70 measure is likely unaffected by hypoxic exercise-regulated stress (10, 32, 63). These data suggest that hypoxic stress, in addition to exercise, may only be sufficient to upregulate the synthesis of HSP70 at the cellular level, but inadequate for stimulating the release of HSP70 into the circulation. Nevertheless, it is worth mentioning that there were only five experimental trials conducted in an FI_{O_2} of 14% included in this meta-regression [EXPT11 (10); EXPT14, EXPT17, EXPT18 (32); EXPT22 (63)] whereas the other 21 trials were conducted in normoxia (21% FI_{O_2}). Therefore, these findings should be interpreted cautiously.

LIMITATIONS

Our analysis has noteworthy limitations. One important limitation may relate to the fact that the scope of this review is limited to plasma HSP70 responses to acute prolonged

exercise, whereas intracellular HSP70 measures have not been covered in the present review due to the limited sample sizes of each specific cell type. Therefore, we were unable to examine the mechanistic relationships between exercise-induced intracellular and extracellular HSP70 responses. Study participants were nearly all male (~96%), reflecting the under-representation (or exclusion) of female participants in applied physiology research (117). Of the 13 selected studies evaluated in this present research, only one (54) clearly included female endurance-trained participants, but did not report plasma HSP70 concentrations separately by biological sex. Therefore, we were not able to evaluate sex differences in plasma HSP70 responses to prolonged exercise. Furthermore, some of the findings from our analyses may be susceptible to the relatively modest size of the dataset. For instance, we did not observe a moderating effect of hypoxia on exercise-induced changes in plasma HSP70 concentration (Table 3), but only five experimental trials were performed in hypoxia. In addition, a methodological consideration is that, to the best of our knowledge, no experimental study has investigated the reliability of extracellular HSP70 responses to repeated, identical exercise protocols, which limits the interpretation of between-trial variability.

CONCLUSIONS

Acute prolonged (lasting longer than 20 min) cycling/running exercise increased plasma HSP70 concentrations. Individual aerobic capacity, exercise characteristics (intensity, duration, and modality), and environmental conditions (temperature, relative humidity, and the fraction of inspired oxygen) explained a considerable (~57%) degree of variation in exercise-induced plasma HSP70 expression. These exercise-associated factors can individually and collectively trigger the specific mechanisms for release of HSP70 into the circulation; however, this meta-regression found that the magnitude of exercise-induced plasma HSP70 responses is statistically influenced by the participant's peak (or maximal) oxygen uptake, alongside the exercise intensity and duration, as well as environmental temperature. Although our statistical analyses were not designed to examine how interactions between selected moderators, it is reasonable to infer that individuals with higher $\dot{V}O_{2max/peak}$ who exercise at higher intensities and/or for longer durations are likely to exhibit larger increases in circulating HSP70. We posit that other factors [e.g., age, sex, menstrual cycle phase, total work done, exercise protocols (continuous vs. programmed intermittent vs. self-paced), and durability] may play a role in plasma HSP70 responses to exercise, and recommend that future studies assess plasma HSP70 responses to exercise defined according to the exercise intensity domains. The findings from this systematic review, meta-analysis, and meta-regression contribute to our understanding of how acute prolonged cycling or running exercise affects plasma HSP70 concentrations and provide clearer insights into the influence of exercise-associated factors on the plasma HSP70 responses.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental Figs. S1 and S2 and Supplemental Tables S1–S4: https://doi.org/10.6084/m9.figshare.30792542.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.C., A.E.K., and E.M. conceived and designed research; T.C., A.M.S.B., and M.R.C. performed experiments; T.C., A.M.S.B., M.R.C., and T.S. analyzed data; T.C., A.E.K., M.R.C., T.S., and E.M. interpreted results of experiments; T.C. prepared figures; T.C. and E.M. drafted manuscript; T.C., A.E.K., A.M.S.B., M.R.C., T.S., and E.M. edited and revised manuscript; T.C., A.E.K., A.M.S.B., M.R.C., T.S., and E.M. approved final version of manuscript.

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