





# Exploration of possible sex bias in acute social stress research: a semi-systematic review

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## Review Article

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## Abstract

Stress can have a significant impact on the daily lives of individuals and can increase vulnerability to a number of medical conditions. This study aims to estimate the ratio of male to female participants in acute social stress research in healthy individuals. We examined original research articles published over the last 20 years. Each article was screened to determine the total number of female and male participants. We extracted data from 124 articles involving a total of 9539 participants. A total of 4221 (44.2%) participants were female, 5056 (53.0%) were male and 262 (2.7%) were unreported. Articles incorporating only females were significantly underrepresented compared to articles incorporating only males. Forty articles (63.5%) which presented data from both females and males, failed to analyse and interpret the results by sex, a significant methodological limitation. In conclusion, in the literature published over the last 20 years, female participants are significantly underrepresented. In the studies where females are represented, severe methodological limitations are apparent. Researchers should be conscious of sexual dimorphism, menstrual phase and use of hormonal contraception, which may impact the interpretation of their results.

## Summations

- The literature published within acute stress research over the last 20 years has an overall underrepresentation of women.
- Articles incorporating only females were significantly underrepresented compared to articles incorporating only males.
- More than half of the articles that included data from both females and males failed to analyse and interpret the results by sex, a significant methodological limitation.

## Considerations

- The current review included 9539 participants sourced from 124 original articles published over 20 years in recognised journals.
- We did not consider studies on pregnant women, patient populations, post-menopausal women or older adults.
- We did not provide additional demographic information. However, it is plausible that this information would demonstrate other biases in the field of acute social stress and warrants further investigation.

## Introduction

There are notable differences between the sexes in their susceptibility and response to stress (Kirschbaum *et al.*, 1999; Rohleder *et al.*, 2003; Uhart *et al.*, 2006; Heck and Handa, 2019). The coping mechanisms of men and women vary depending on the nature of the stressor (Kudielka and Kirschbaum, 2005). Men show a more substantial response to stressors involving competition, whereas women react to interpersonal stressors such as social rejection (Kudielka and Kirschbaum, 2005). In response to acute psychosocial stress, the differences between men and women are most pronounced when women are in the follicular phase of the menstrual cycle, characterised by low oestrogen levels (Heck and Handa, 2019; Kudielka and Kirschbaum, 2005). Women’s responses to stress are tightly connected with the gonadal hormones (Verma *et al.*, 2011) and thus vary considerably depending on menstrual phase and use of hormonal contraceptives (HCs) (Herman and Cullinan, 1997; Kirschbaum *et al.*, 1999). Furthermore, women are at a higher risk of developing stress-related diseases during adolescence and adulthood, supporting the idea that oestrogen and progesterone influence the manifestation of stress-related disorders in adult women (Heck and Handa, 2019).

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Original research should therefore be planned and powered to identify factors that may contribute to variations in psychological stress measures such as cortisol. Menstrual cycle phase and use of HCs are obvious candidates for scrutiny (Burrowes, 2021). Women are underrepresented in a number of fields, such as cardiology (Kentner and Grace, 2017), sports medicine (Hagstrom *et al.*, 2021), neuroscience (Beery and Zucker, 2011), autoimmune dysfunction (Lockshin, 2006; Fish, 2008), pharmacology (Beery and Zucker, 2011) and physiology (Beery and Zucker, 2011). The clinical studies focused only on males suggest the results to be applicable to both sexes. However, 80% of the drugs failing clinical trials from the US pharmaceutical market from 1997 to 2000 were due to side effects present solely in women (Burrowes, 2021). In 1997, the U.S. Food and Drug Administration (FDA), implemented a rule according to which pharmaceutical companies need to ensure the safety of the manufactured drug for both genders (Verma *et al.*, 2011); stating that it is unethical to prescribe a drug to women unless they are included in the studies that aim to understand the disease mechanism. The outcome of such a policy has not gone unnoticed. It has made a difference by increasing the overall number of female participants in the clinical research funded by the US National Institutes of Health (NIH). However, the lack of funding for the medical fields focused on women, such as stress-related disorders or reproductive systems, remains a significant challenge (Burrowes, 2021).

Researchers exploring the aspects of social stress commonly accept that there are physiological and psychological sex differences (Verma *et al.*, 2011). However, to the best of our knowledge, no authors have examined the number, ratio or percentage of male and female subjects participating in research in this field. Therefore, this study examines the sex of participants involved in social stress research published within the last 20 years.

## Materials and methods

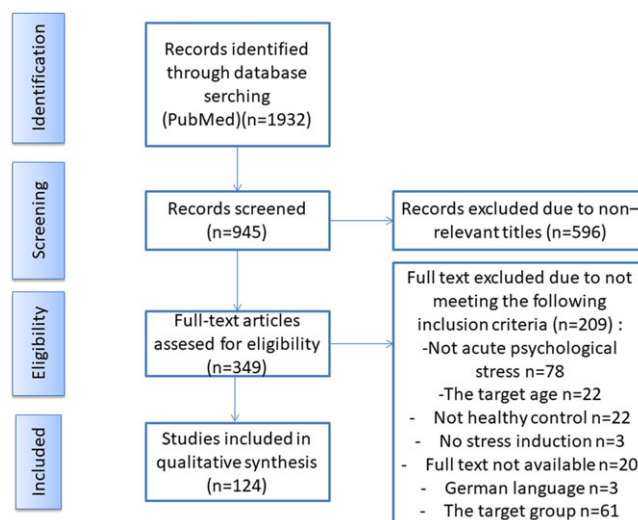
This is a semi-systematic review performed according to The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher *et al.*, 2009). In total, 124 publications were included in the quantitative synthesis.

### Database search

A systematic literature search was performed using the electronic database MEDLINE<sup>®</sup> via PubMed<sup>®</sup>. The search was conducted in December 2021, with the following combined set of keywords: [((cortisol) OR (HPA) OR (neuroendocrine) OR (hydrocortisone) OR (psychoneuroimmunology) OR (psychoimmunology) OR (psychoneuroendocrinology)) AND (“stress reactivity”) OR (“acute stress”) OR (“laboratory stress”) OR (“experimental stress”) OR (“psychological stress”) OR (“mood induction”) OR (“emotion”))] Filters: Abstract, Clinical Trial, Randomized Controlled Trial, from 2000 to 2021]. This search generated 1932 results.

### Study selection

One investigator selected the manuscripts based on the title and abstract, followed by a full-text assessment. See Fig. 1 for a graphical representation of the search and selection process. All selected papers met the following inclusion criteria: 1) healthy adult participants, 2) 18–50 years of age, 3) medication-free participants, 4) acute psychological stress, 5) salivary or blood cortisol and 6) at least two measurement points for cortisol. Papers were



**Figure 1.** The flow chart illustrates the search and selection process of studies for this semi-systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The process includes an initial database search, followed by the screening of titles and abstracts for relevance, and then full-text screening of potentially relevant studies. The excluded studies at each stage are documented along with the reasons for their exclusion. Finally, the included studies are assessed for quality, and the relevant data is extracted for the present semi-systematic review.

excluded if they contained at least one of the exclusion criteria such as: 1) pregnant women, 2) subjects under 18 or above 50 years of age, including menopausal women or post-menopausal women, 3) physical or psychological illnesses, 4) chronic stressor studies, 5) physical stressors, 6) physical–psychological combined stressor and 7) urinary or hair cortisol.

### Data extraction and analysis

The extracted data are number of women included, number of men included, year of publication and whether or not sex, menstrual cycle phase and HC were taken into account when analysing data. Data were analysed via Chi-square tests. *p*-Values < 0.05 were considered *a priori* as significant. Data are presented as percentages.

## Results

Data were extracted from 124 articles, listed in Table 1, involving 9539 participants. A total of 4221 (44.2%) participants were female, and 5056 were male (53.0%); the sex of 262 (2.7%) individuals was not reported (Table 2). Females were significantly underrepresented ( $\chi^2 = 75.283$ ,  $df = 1$ ,  $p < .001$ ). Figure 2 shows the sex of participants from 2000 to 2021.

Overall, only 15 (12.1%) of the articles incorporated only females, 41 (33.1%) included only males and 63 (50.8%) included both males and females; five articles (4.0%) did not report the sex of their participants (Table 3). Articles incorporating only females were significantly underrepresented compared to articles incorporating only males ( $\chi^2 = 29.109$ ,  $df = 1$ ,  $p < .001$ ). Figure 3 shows data of the use of male-only, female-only and both sexes as participants from 2000 to 2021.

Only 22 articles (36.5%), which presented data from both females and males, included sex as an experimental variable.

**Table 1.** Description of studies (N = 124)

Study	Females(n)	Males(n)	Year	Sex	Menstrual cycle	HC use
Skosnik <i>et al.</i> , 2000	10	10	2000	No	No	No
Honk <i>et al.</i> , 2000	0	40	2000	–	–	–
Young <i>et al.</i> , 2000	8	12	2000	No	No	No
Seeman <i>et al.</i> , 2001	17	9	2001	Yes	No	No
Wolf <i>et al.</i> , 2001	25	33	2001	No	Yes	No
Young and Nolen-Hoeksema, 2001	47	0	2001	–	No	No
Scarpa and Luscher, 2002	30	14	2002	No	No	No
Stroud <i>et al.</i> , 2002	26	24	2002	Yes	Yes	No
Gaab <i>et al.</i> , 2003	0	48	2003	–	–	–
Söderpalm <i>et al.</i> , 2003	0	28	2003	–	–	–
Gold <i>et al.</i> , 2003	83	0	2003	–	No	No
Rohleder <i>et al.</i> , 2003	25	0	2003	–	Yes	Yes
Kudielka, Schommer, <i>et al.</i> , 2004	81	99	2004	No	Yes	No
Kudielka, Buske-Kirschbaum, <i>et al.</i> , 2004	21	20	2004	Yes	Yes	No
Domes <i>et al.</i> , 2009	0	60	2004	–	–	–
Gruenewald <i>et al.</i> , 2004	47	34	2004	No	No	No
Roy, 2004	0	82	2004	–	–	–
Hellhammer <i>et al.</i> , 2004	40	40	2004	No	No	Yes
Young <i>et al.</i> , 2005	54	36	2005	No	No	No
Maheu <i>et al.</i> , 2005	0	19	2005	–	–	–
Jackson <i>et al.</i> , 2006	45	49	2005	Yes	No	No
Hammerfald <i>et al.</i> , 2006	–	–	2005	Yes	No	No
Känel <i>et al.</i> , 2006	0	30	2005	–	–	–
Hänsel and Känel, 2013	0	21	2005	–	–	–
Gaab <i>et al.</i> , 2005	0	81	2005	–	–	–
Wüst <i>et al.</i> , 2005	0	106	2005	–	–	–
Elzinga and Roelofs, 2005	44	0	2005	–	Yes	No
Hammerfald <i>et al.</i> , 2006	56	27	2006	Yes	No	Yes
Eller <i>et al.</i> , 2006	63	32	2006	Yes	No	No
Fries <i>et al.</i> , 2006	0	46	2006	–	–	–
Childs <i>et al.</i> , 2006	13	19	2006	No	No	No
Nater <i>et al.</i> , 2006	0	20	2006	–	–	–
Kumsta <i>et al.</i> , 2007	114	94	2007	Yes	No	Yes
Ditzen <i>et al.</i> , 2008	0	63	2007	–	–	–
Mikolajczak <i>et al.</i> , 2007	0	56	2007	–	–	–
van Dulmen <i>et al.</i> , 2007	48	9	2007	No	No	No
Nater <i>et al.</i> , 2007	0	20	2007	–	–	–
Bottaccioli <i>et al.</i> , 2020	0	54	2007	–	–	–
Blackhart <i>et al.</i> , 2007	122	137	2007	No	No	No
Tahara <i>et al.</i> , 2007	–	–	2007	No	No	No
Het and Wolf, 2007	44	0	2007	–	Yes	No
Bellingrath and Kudielka, 2008	33	20	2008	No	No	No
Scholz <i>et al.</i> , 2009	0	42	2008	–	–	–

(Continued)

Table 1. (Continued)

Study	Females(n)	Males(n)	Year	Sex	Menstrual cycle	HC use
Linnen et al., 2012	0	26	2008	–	–	–
Wirtz et al., 2008	0	42	2008	–	–	–
Zoccola et al., 2008	–	–	2008	No	No	No
Schoofs et al., 2008	0	36	2008	–	–	–
Dickerson et al., 2008	58	31	2008	No	No	No
Childs and de Wit, 2010	4	13	2009	No	No	No
Scholey et al., 2009	32	8	2009	No	No	No
Pierrehumbert et al., 2009	27	0	2009	–	Yes	No
Pace et al., 2009	32	29	2009	No	No	No
Wolf et al., 2002	18	22	2009	No	Yes	No
Weik et al., 2010	32	33	2010	Yes	No	No
Balodis et al., 2010	59	29	2010	No	No	No
Cornelisse et al., 2011	55	22	2010	Yes	Yes	Yes
von Dawans et al., 2011	0	25	2010	–	–	–
Starcke et al., 2011	18	22	2010	No	No	No
Raspopow et al., 2010	65	0	2010	–	No	No
Jönsson et al., 2010	0	10	2010	–	–	–
Zöller et al., 2010	0	89	2010	–	–	–
Lovallo et al., 2010	188	136	2010	Yes	No	No
Pilgrim et al., 2010	14	11	2010	No	No	No
Plessow et al., 2012	24	24	2011	No	No	No
Wolf et al., 2012	0	37	2011	–	–	–
Linnen et al., 2012	48	48	2011	Yes	No	No
Youssef et al., 2012	35	30	2011	Yes	No	No
Childs et al., 2011	0	25	2011	–	–	–
Sommer et al., 2011	0	24	2011	–	–	–
Almela et al., 2011	62	0	2011	Yes	No	No
Engert et al., 2011	0	50	2011	–	–	–
Mohan et al., 2011	0	32	2011	–	–	–
Bostock et al., 2011	40	0	2011	–	No	No
Hänsel and Känel, 2013	28	8	2012	No	No	No
Leininger and Skeel, 2012	0	58	2012	–	–	–
Nedeljkovic et al., 2012	32	17	2012	No	Yes	No
Het et al., 2012	84	148	2012	No	Yes	No
Kimura et al., 2013	9	30	2013	No	Yes	No
Walser et al., 2013	41	41	2013	Yes	No	No
Oei et al., 2014	0	37	2013	–	–	–
Polheber and Matchock, 2014	22	26	2013	No	No	No
Kimura et al., 2013	9	30	2013	Yes	Yes	No
Wiemers et al., 2013	31	32	2013	No	Yes	No
Pabst et al., 2013	0	41	2013	–	–	–
Nyklíček et al., 2013	62	26	2013	Yes	No	No
Pottier et al., 2013	–	–	2013	No	No	No

(Continued)

Table 1. (Continued)

Study	Females(n)	Males(n)	Year	Sex	Menstrual cycle	HC use
Smith, 2010	69	64	2013	No	No	No
Montero-López et al., 2018	42	0	2014	–	Yes	Yes
Mayer et al., 2017	–	–	2014	No	Yes	No
Bedgood et al., 2014	0	85	2014	–	–	–
Montoya et al., 2014	0	20	2014	–	–	–
Petrowski et al., 2014	14	17	2014	No	Yes	No
Burton et al., 2014	39	31	2014	Yes	No	No
Pilgrim et al., 2014	30	26	2014	No	No	No
Abelson et al., 2014	21	33	2014	No	Yes	No
Creswell et al., 2014	27	39	2014	No	No	No
Stephens et al., 2016	135	147	2015	Yes	Yes	No
Maki et al., 2015	40	0	2015	–	Yes	No
Spanakis et al., 2016	59	41	2015	Yes	Yes	No
Sollberger et al., 2016	0	80	2015	–	–	–
Herbison et al., 2016	360	438	2016	Yes	Yes	Yes
Busse et al., 2017	80	63	2017	Yes	No	No
Ali et al., 2017	23	23	2017	No	Yes	No
Lamarche et al., 2017	0	66	2017	–	–	–
Singer et al., 2017	0	50	2017	–	–	–
Gallego-Gómez et al., 2020	84	28	2019	No	No	No
Sep et al., 2019	0	100	2019	–	–	–
Ditzen et al., 2019	183	183	2019	Yes	Yes	No
Manigault et al., 2020	0	58	2020	–	–	–
Sandner et al., 2020	10	30	2020	No	No	Yes
Hermann et al., 2021	0	32	2020	–	–	–
Kothgassner et al., 2021	84	0	2020	–	No	No
Ali et al., 2020	59	58	2020	Yes	Yes	No
Meier et al., 2021	18	17	2020	No	No	No
Crane et al., 2020	82	39	2020	No	No	No
Bottaccioli et al., 2020	26	14	2020	No	No	No
Sladek et al., 2020	53	31	2020	No	No	No
Appiah-Kusi et al., 2020	29	29	2020	No	No	No
Scheuringer et al., 2020	69	0	2020	–	No	No
Gideon et al., 2020	0	58	2020	–	–	–
Sheppard et al., 2021	241	162	2021	Yes	No	No
Heimgartner et al., 2021	53	0	2021	–	No	No
Kuchenbecker et al., 2021	37	0	2021	–	Yes	No
Huebner et al., 2021	69	72	2021	No	No	No

The number of female and male participants is listed. Publication year indicates when the manuscript was first made available, for example, online, and may vary from reference. The extent that sex, menstrual cycle phase or hormonal contractions (HC) was included in the analysis is indicated with yes or no when applicable.

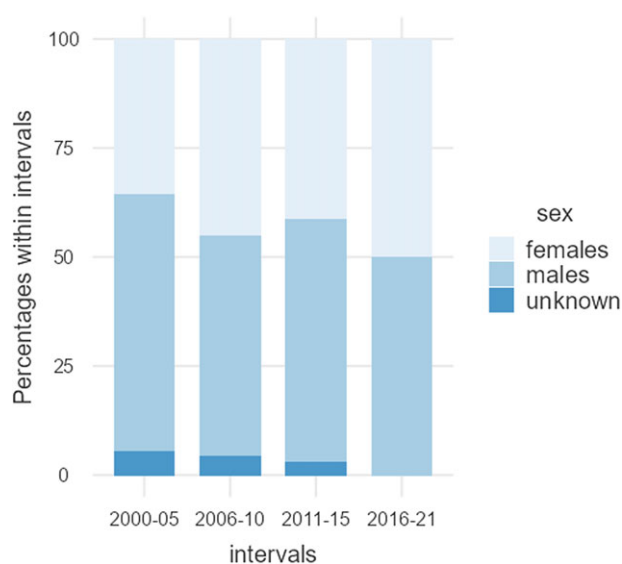
Twenty-six articles (33.3%) on females-only and both females and males included the stage of the participants' oestrous or menstrual cycle phase. Of the articles reporting womens' menstrual cycle phase, 13 articles (50.0%) only included women in the luteal phase, and 4 articles (15.4%) included women in both the follicular

and luteal phases. Fifty-two articles (66.7%) did not report menstrual cycle phase, nor was it an inclusion or exclusion criteria.

Forty-two articles, corresponding to 53.8% of all studies on females-only and both females and males, excluded women using HCs. Seven studies (9.0%) included women using HCs, of which

**Table 2.** The sex of participants from 2000 to 2021

		Intervals				
Sex		2000–05	2006–10	2011–15	2016–21	Total
Females	Observed	563	1095	1003	1560	4221
	% within column	35.6 %	45.2 %	41.4 %	50.1 %	44.2 %
Males	Observed	935	1223	1347	1551	5056
	% within column	59.1 %	50.5 %	55.6 %	49.9 %	53.0 %
Unknown	Observed	83	106	73	0	262
	% within column	5.2 %	4.4 %	3.0 %	0.0 %	2.7 %
Total	Observed	1581	2424	2423	3111	9539
	% within column	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

**Figure 2.** Male and female participants, as well as individuals where the sex was not reported. Between 2000 and 2021, the percentage of male participants remained relatively stable, while the percentage of individuals where the sex was not reported appeared to decrease slightly, in favour of female participants.

only two studies (2.6%) analysed the stress response of women using HCs separately from freely cycling (FC) women; 29 (37.2%) did not mention HCs as inclusion or exclusion criteria.

## Discussion

To our knowledge, this is the first study to examine the sex of participants in the acute social stress literature. Here, we present evidence that female participants are significantly underrepresented in the research from the last 20 years. In the studies where females are represented, severe methodological limitations are apparent, including lack of information of menstrual cycle phases or whether participants were HC users.

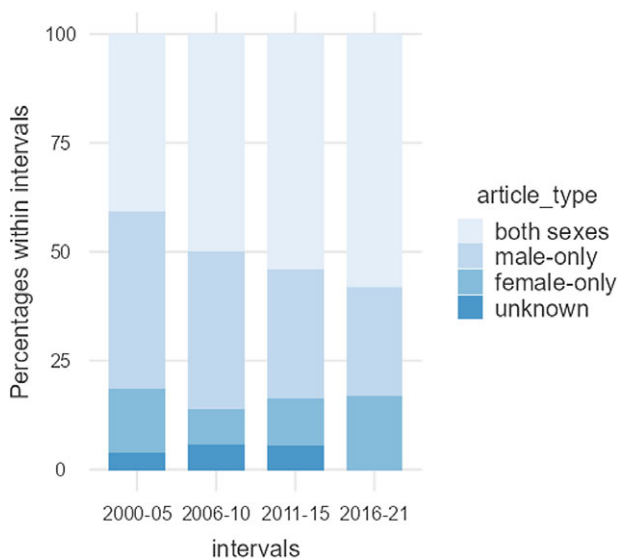
Research suggests that men and women may differ in their response to acute social stress, both in behaviour and physiology (Otte *et al.*, 2005). For example, men tend to exhibit “fight or flight” responses, which is characterised by increased arousal and readiness for physical action. This response may be due to the influence of higher levels of testosterone. On the other hand, women have a greater likelihood of exhibiting the “tend and befriend” response,

which is characterised by increased social behaviour and affiliation (Taylor *et al.*, 2000). This response is mediated by the release of oxytocin and is characterised by seeking support and protection of family and friends, nurturing and caring for others, and increased prosocial behaviour. This response may be due to the influence of higher levels of oestrogen and progesterone, which are associated with social behaviour and attachment to others (Lighthall *et al.*, 2012). Furthermore, research suggests that there may be differences in the hypothalamic–pituitary–adrenal (HPA) axis between men and women, which may contribute to behavioural differences triggered by acute social stress. For example, studies have shown that women tend to have higher baseline cortisol levels than men, which may make them more sensitive to stressors. Additionally, women may have a more sensitive HPA axis overall, with greater responsiveness to stressors and a more rapid return to baseline cortisol levels following a stressor (Kajantie and Phillips, 2006).

Females account for 44.2% of the total number of participants in the 124 articles reviewed. Interestingly, the average percentage of female participants was relatively consistent from 2000 to 2021, although both the number of articles and the total number of participants have consistently increased over time, see Table 2 and Fig. 1. Despite the fact that 50.8% of the articles published over this period incorporated both male and female participants, women are underrepresented. This is mainly due to the greater volume of articles consisting of male-only data (33.1%), compared to female-only data (12.1%), and secondly, articles reporting data from both sexes tend to overrepresent male participants and limit the number of female participants. The proportion of female-only articles was relatively consistent from 2000 to 2021, whereas the proportion of male-only articles seems to have experienced a slight decrease in favour of articles incorporating participants of both sexes, see Table 3 and Fig. 2. Furthermore, most of the studies conducted on males do not give a thorough justification of why females were excluded. Only two of the publications stated that females were excluded from the studies due to challenges in studying all women at the same phase of the menstrual cycle (Honk *et al.*, 2000; Jönsson *et al.*, 2010). Another publication mentions that females are excluded because the menstrual cycle and birth control pills alter the level of glucocorticoids corticosterone and cortisol (CRT) (Montoya *et al.*, 2014). Only 37.1% of the articles that incorporated male and female participants over the last 20 years included sex as an experimental variable. Thus, most articles failed to recognise potential sex-related differences in susceptibility and response to acute social stress, inadvertently adding variability to

**Table 3.** Use of male-only, female-only and both sexes as participants from 2000 to 2021

Article type		2000–05	2006–10	2011–15	2016–21	Total
Both sexes	Observed	11	18	20	14	63
	% within column	40.7 %	50.0 %	54.1 %	58.3 %	50.8 %
Male-only	Observed	11	13	11	6	41
	% within column	40.7 %	36.1 %	29.7 %	25.0 %	33.1 %
Female-only	Observed	4	3	4	4	15
	% within column	14.8 %	8.3 %	10.8 %	16.7 %	12.1 %
Unknown	Observed	1	2	2	0	5
	% within column	3.7 %	5.6 %	5.4 %	0.0 %	4.0 %
Total	Observed	27	36	37	24	124
	% within column	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

**Figure 3.** Articles using male-only, female-only, and both sexes as participants between 2000 and 2021. The percentage of female-only articles remained relatively stable, while those using only males appeared to decrease slightly, in favour of articles that included participants of both sexes.

their data in cases where sex-related differences exist. Although the increased inclusion of both sexes in the most recent years is encouraging, most of these studies still failed to investigate potential sex differences; they missed the opportunity to uncover how sex influences physiological parameters, for example, cortisol release.

According to Genazzini and co-workers (Genazzini *et al.*, 1975), adrenocorticotrophic hormone (ACTH) vary across the menstrual cycle phase. Women in the follicular and luteal phases do not have the same salivary-free cortisol response (Maki *et al.*, 2015). In order to compare the findings between men and women, it is first necessary to specify the phase of the menstrual cycle so the results can be interpreted. Progesterone has an inhibitory role in the HPA axis in women. In other words, the menstrual cycle phase impacts the response of the HPA axis to stress. However, it is still unclear how the sex hormones throughout the menstrual cycle affect the activity of the HPA axis. Consequently, there is a need

for further studies to investigate the interaction between sex hormones and the HPA axis. This will facilitate the design of future stress-related experiments. Twenty-six articles (33.3%) on females-only and both females and males included the stage of the participants' oestrous or menstrual cycle phase. Fifty-two articles (66.7%) did not report menstrual cycle phase, nor was it an inclusion or exclusion criteria. Seven studies (Gold *et al.*, 2003; Raspopow *et al.*, 2010; Zöller *et al.*, 2010; Almela *et al.*, 2011; Bostock *et al.*, 2011; Heimgartner *et al.*, 2021; Kothgassner *et al.*, 2021) conducted solely in female groups did not provide information about the phase of the menstrual cycle. On the other hand, 40 studies conducted in both sexes did not specify women's menstrual cycle phase. It is possible for researchers to include only women in a selected phase of the menstrual cycle to compare results between sexes while minimising cost and time, such as the study by Spanakis *et al.* (2016) that selected women during the follicular phase where sex hormone levels are thought to be comparable between men and women. In order to detect the differences between men and women, it has been recommended to compare the male group to two or more groups of women at different menstrual cycle phases (Rich-Edwards *et al.*, 2018). Of the articles reporting womens' menstrual cycle phase, 13 articles (50.0%) only included women in the luteal phase, 9 (34.6%) only included women in the follicular phase and 4 (15.4%) included women in both the follicular and luteal phases.

The women on HCs have a significantly different cortisol rhythm than FC women. In healthy adults, the cortisol level reaches a peak in the morning, approximately 30 minutes after waking up (Boisseau *et al.*, 2013; Roche *et al.*, 2013). On the other hand, the women on HCs have lower cortisol peaks; therefore, the cortisol level does not experience a significant drop (Boisseau *et al.*, 2013). In addition, the daily cortisol rhythms are blunted in pill-taking women; consequently, the daily cortisol curve seems to be flatter compared to naturally cycling women (Boisseau *et al.*, 2013). The high level of corticosteroid-binding globulins in HC users blunt the cortisol release in response to the stress (Wiegatz *et al.*, 2003; Kumsta *et al.*, 2007). It is suggested that birth control pills hyperactivate the HPA axis, and this causes the HPA axis to shut down. In reality, the pattern of HPA axis in pill-taking women is very similar to women with chronic stress (Miller *et al.*, 2007; Hertel *et al.*, 2017). However, this remains a hypothesis and requires research to identify the role that HCs may play and the

potential reversibility of effects upon cessation of HC use. Recent studies have found that hormonal contraception may impact the oxytocin system (Garforth *et al.*, 2020); however, more research is needed to understand the nature and magnitude of these effects on acute social stress, behaviour and cognition (Byg *et al.*, 2023). Forty-two articles, corresponding to 53.8% of all studies on females-only and both females and males, excluded women using HCs. Four studies (Walser *et al.*, 2013; Abelson *et al.*, 2014; Creswell *et al.*, 2014; Meier *et al.*, 2021) had HC use as an exclusion criterion for women but did not exclude men on exogenous androgens and anabolic steroids. Surprisingly, 29 studies (37.2%) did not mention HCs as inclusion or exclusion criteria. Only seven studies (9.0%) included women using HCs (Rohleder *et al.*, 2003; Hammerfald *et al.*, 2006; Kumsta *et al.*, 2007; Pilgrim *et al.*, 2014; Herbison *et al.*, 2016; Ditzen *et al.*, 2019; Sandner *et al.*, 2020) of which only two studies (2.6%) analysed the stress response of women using HCs (Boisseau *et al.*, 2013; Hertel *et al.*, 2017) separately from FC women and concluded that HC use impacts the HPA axis response to stress.

It is difficult to provide a specific rationale for the sex bias in the acute social stress literature, and a range of physiological and methodological issues likely contribute. Women have been excluded from biomedical research over the years under the assumption that the results from studies on men apply to females or that the variation of sex hormones throughout the menstrual cycle makes the interpretation challenging. However, other studies do not justify the exclusion of women (Beery and Zucker, 2011). Furthermore, there is still a misconception that women are more variable than men. Both males and females have variations in gonadal steroid hormones. Testosterone has a circadian rhythm, and its level is affected by factors such as physical exercise and age (Smith *et al.*, 2013). In comparison, reproductive females have a fluctuation of gonadal hormones throughout the phases of the menstrual cycle (Rich-Edwards *et al.*, 2018). Treating males as the norm comes with the consequence of placing women's health at risk. It is beneficial to science to include both sexes in order to get a full insight into the mechanisms of the HPA axis. It is not possible to elucidate stress response mechanisms by studying only males. The consideration of both sexes in stress-related research will advance our knowledge and lead to the development of safer products and therapies for stress-related disorders, minimising their side effects. The very least, the presentation of the data of both sexes can improve the design of future studies. The differences in methodological design are thought to be a main reason for inconsistent results across studies (Rich-Edwards *et al.*, 2018).

Original research should be planned and powered to identify factors that may contribute to variations in acute social stress measures such as cortisol, including sex, menstrual cycle and oral contraceptive use. By doing so, the findings will be more robust, generalisable and informative. Ensuring that a study has enough statistical power to distinguish different stress responses between, for example, men vs women, different phases of the menstrual cycle and hormonal contraception users vs natural cycling women, requires careful planning and consideration of several factors. Some key considerations include the following: using the correct statistical model, both analysis of variance (ANOVA) and a general linear model (GLM) can be used to model the relationship between a continuous dependent variable (such as cortisol levels or responses) and one or more independent variables (such as sex, menstrual cycle phase and hormonal contraception use). By including these variables in the model, the study would be able to test for main effects of sex, menstrual cycle phase and hormonal

contraception use on the measures of choice, as well as for any interactions between these variables. The main difference between ANOVA and GLM approach is that ANOVA is used to compare the means of two or more groups and assumes normality and homogeneity of variances, while GLM can be used to model a relationship between a continuous dependent variable and one or more independent variables and assumes linearity and normality of residuals. GLM can also be extended to include other explanatory variables such as age, BMI and lifestyle factors that may influence, for example, cortisol levels and can be used to model non-normal distributions of the response variable, and it allows to include categorical and continuous variables. Furthermore, the sample size of a study is one of the most critical factors in determining statistical power. Larger sample sizes increase the likelihood of detecting significant differences between groups, so it is important to ensure that the study has a large enough sample size to detect differences in stress hormones between men and women. It is important to control for any confounding variables that may affect stress hormones such as age, lifestyle and other hormones. Finally, as multiple comparisons are made with biological samples across different groups, it may be important to adjust the significance level to take into account the increased chance of finding false positives.

#### Limitations and future research

A strength of the current study is the number of participants (9539) sourced from original articles published over 20 years in recognised journals. However, the current review did not consider the following in the analysis: studies involving multiple publications based on data from the same population/sample, studies on pregnant women or patient populations or studies on postmenopausal women and older adults. Finally, due to the large number of participants, we did not provide additional demographic information. It is plausible that this information would demonstrate other biases in the field of acute social stress and warrants further investigation.

#### Conclusion

The current study demonstrated that female participants are still underrepresented in the acute social stress literature. Absolute numbers and percentage of female participants are significantly lower than males. More importantly, the ratio of articles that included only males to articles that only included females is two-fold greater across the literature. We stress the importance of reporting menstrual cycle phase information and HC use in studies presenting female data, and we encourage the comparison of male and female data in order to uncover potential important sex differences, which may be of utmost importance for drug development and providing appropriate medical care to both sexes.

**Data availability.** The data that support the findings of this study are available on request from the corresponding author.

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**Author contributions.** AR and MR conceptualised the study. AR extracted data, analysed and wrote the first draft under the supervision of MW and AL. All authors have edited and approved the submitted version and take responsibility for the integrity of the findings.



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