British Journal of Nutrition (2023), 130, 202–210 doi:10.1017/S0007114522003270

The effects of N-acetylcysteine on ovulation and sex hormones profile in women with polycystic ovary syndrome: a systematic review and meta-analysis

Zahra Shahveghar Asl[1,2], Karim Parastouei[3]* and Eslam Eskandari[3]

[1]*Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

(Submitted 27 February 2022 – Final revision received 14 September 2022 – Accepted 3 October 2022 – First published online 4 January 2023)

Abstract

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases characterised by unusual levels of sex hormones and dysfunction of the ovaries. The infertility rate is high among patients with PCOS. Unusual hormonal status can lead to the inability of ovaries to release functional and mature follicles. Clinical trials on the effects of N-acetylcysteine (NAC) supplementation on ovulation and sex hormones profile in women with PCOS have been controversial. We performed a systematic review and meta-analysis to evaluate the potential effects of NAC supplementation on ovulation and sex hormones profile. PubMed, Scopus, Embase, Web of Science and Cochrane Central library international databases were searched till September 2021. Meta-analysis was performed using a random-effects approach in case of significant between-study heterogeneity. Eighteen studies, including 2185 participants, were included in the present meta-analysis. NAC significantly reduced total testosterone (TT) levels (standardised mean difference (SMD): −0·25 ng/ml; 95 % CI (−0·39, −0·10); '$P < 0·001$', $I^2 = 53·9$ %, $P = 0·034$) and increased follicle-stimulating hormone (FSH) levels (SMD: 0·39 mg/ml; 95 % CI (0·07, 0·71); $P = 0·01$, $I^2 = 70·9$ %, $P = 0·002$). Oestrogen levels also increased after correcting publication bias. However, no significant effect was observed on the number of follicles, endometrial thickness, progesterone, serum luteinising hormone levels and sex hormone-binding globulin. The results indicated that NAC supplementation decreased TT levels and increased FSH levels. Overall, NAC supplementation might be effective in the improvement of reproductive system function in patients with PCOS.

Key words: **N-acetylcysteine: Polycystic ovary syndrome: Meta-analysis: Sex hormones**

Polycystic ovary syndrome (PCOS) is one of the most common hormonal, inflammatory and oxidant disorders among women of reproductive age worldwide[1]. PCOS has been known as the leading cause of infertility among women[2]. Approximately, 70 % of women with PCOS have infertility[3]. These patients may have missed, irregular or prolonged menstrual cycles or high levels of androgens. Follicles cannot grow and mature due to exceptional levels of sex hormone. Thus, ovaries may store numerous immature follicles and fail to release an egg regularly[4].

N-acetylcysteine (NAC) is a safe, economic and affordable drug which is used for the stimulation of ovulation[5]. This medication is an acetylated variant of L-cysteine, a great source of sulfhydryl groups. NAC is recognised as an antioxidant agent that can directly scavenge reactive oxygen species and is beneficial for PCOS patients. In a meta-analysis study, beneficial effects of NAC supplementation on IL-8, malondialdehyde and homocysteine levels have been shown. TNF-α and IL-6 levels could be positively impressed by NAC[6]. Also, NAC has several biologic impacts, such as reducing insulin resistance, regulating the insulin receptor in human erythrocytes and influencing the insulin secretion in pancreatic β-cells. Also, NAC has other anti-inflammatory characteristics by increasing the cellular levels of glutathione (GSH) and promoting apoptosis in PCOS patients[7].

Oligo ovulation or anovulation is the most common cause of infertility in PCOS women. Thus, induction of regular ovulation is vital for these patients. In subjects with PCOS, NAC can reduce insulin and testosterone levels and facilitate serum levels of homocysteine and lipid profile[2]. Teimouri *et al.* showed that adding NAC supplements to letrozole was efficient for fertility rate and the number of ovulatory follicles in women with PCOS[7].

Abbreviations: E2, oestradiol; FSH, follicle-stimulating hormone; GSH, glutathione; LH, luteinising hormone; NAC, N-acetylcysteine; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; TT, total testosterone; WMD, weighted mean difference.

* **Corresponding author:** Dr K. Parastouei, email parastouei@gmail.com

NAC supplementation could increase the average number of ovulatory follicles >18 mm and peak endometrial thickness in PCOS women[8]. Treatment with NAC induces ovulation, decreases miscarriage rates and increases live birth rates[9]. Also, among infertile males, NAC consumption improves the sperm parameters and oxidative stress as low levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH) and high levels of testosterone, increased sperm count and motility and decreased abnormal sperm morphology and DNA fragmentation[10]. Other potential effects of NAC for PCOS patients are lowering androgen levels, regulating menstrual cycles, increasing the follicle size, decreasing hirsutism, free testosterone and menstrual irregularity[11].

Indeed the role of NAC on sexual hormones is characterised as decreased serum levels of testosterone and androgen levels among PCOS women, reduced levels of FSH and LH and increased levels of testosterone in infertile men[5]. Also, serum levels of oestradiol (E2) and progesterone were improved in PCOS women after NAC consumption[9]. Results of a recent study showed no effect of NAC on levels of FSH, LH and FSH/LH ratio among infertile PCOS women[12]. In another study, NAC supplementation had no significant effect on serum levels of sex hormone-binding globulin (SHBG), LH, FSH, LH/FSH, E2 and testosterone in PCOS patients after 12 weeks[5,9,12,13].

In animal studies, E2 and progesterone levels were not changed after NAC supplementation in the serum of goats[14]. It has been also revealed that levels of hormones and spermatogenesis were not different among adult male Wistar rats following NAC consumption[15]. However, acrylamide and NAC supplementation enhanced FSH, LH and testosterone levels[15]. In another *in vivo* study, a reduction in levels of testosterone and an increase in levels of LH and FSH were seen after NAC supplementation among adult male Wistar rats[16].

Hyperandrogenism and anovulation are characterised by insulin resistance and hyperinsulinaemia. Thus, reducing insulin resistance is an efficient approach in these patients[17]. Numerous studies have indicated that NAC is effective for the induction of ovulation and pregnancy rates in PCOS patients. On the other hand, NAC might increase the chance of pregnancy[8,18].

Considering several benefits of NAC in patients with PCOS, there is still unresolved controversy among clinical trials investigating the efficiency of NAC supplementation in improving ovulation and sex hormones profile in PCOS patients. Therefore, the present meta-analysis aimed to evaluate the effects of NAC supplementation on infertility parameters such as ovulation biomarkers and serum levels of sex hormones in women with PCOS.

Materials and methods

A systematic search was conducted in the international scientific databases, including PubMed, Embase, Web of Science, Scopus and Cochrane Central Library, for relevant studies published up to September 2021. In the search strategy, only studies in English were included. MeSH terms and keywords were used. The search was conducted using the following search pattern: 'Acetylcysteine' [Mesh] OR 'acetylcysteine' [tiab] OR 'nacetylcysteine' [tiab] OR 'n-acetyl cysteine' [tiab]) OR 'NAC' [tiab] AND 'ovulation' [Mesh] OR 'total testosterone' [tiab] OR 'progesterone' [tiab] OR 'sexual function' [tiab] OR 'impotence/erectile/dysfunction' [tiab] OR 'sex hormone-binding globulin (SHBG) ' [tiab] AND 'Polycystic Ovary Syndrome' [tiab] OR 'Ovary Syndrome' [tiab] OR 'Polycystic Syndrome' [tiab] OR 'Polycystic Ovary' [tiab] OR 'Polycystic ovary disease' [tiab] OR 'Stein-Leventhal Syndrome' [tiab] OR 'Stein Leventhal Syndrome' [tiab] OR 'Syndrome, Stein-Leventhal' [tiab] OR 'Sclerocystic Ovarian Degeneration' [tiab] OR 'Ovarian Degeneration, Sclerocystic' [tiab]. The wild-card term'*' was used to increase the sensitivity of our search strategy. Only studies in English were included in the meta-analysis. In addition to searching in databases, we checked the references of related articles for any missing eligible articles (hand-search method). It should be noted that the protocol was not registered at the registry of systematic reviews/meta-analyses (PROSPERO).

Inclusion and exclusion criteria

We included potentially relevant studies if they met the following inclusion criteria: (a) original randomised clinical trials with either parallel or cross-over design; (b) supplementation with NAC in one group in comparison with a placebo group and (c) reporting means and SD at least for one of the following parameters: number of follicles, endometrial thickness, total testosterone (TT), progesterone, LH levels, FSH levels, E2 and SHBG.

In addition, the following studies were excluded: *in vitro*, *in vivo*, *ex vivo* studies, case reports, observational studies (cross-sectional, case–control, cohort) and quasi-experimental studies. Studies supplemented NAC along with other interventions. Besides, studies on pregnant and lactating women were excluded.

Data extraction

Two independent reviewers evaluated the articles following the inclusion criteria. As a first step, titles and abstracts were reviewed. Then, relevant studies were assessed to ensure the suitability of a study for full-text assessment. Any disagreement was resolved by discussion with the senior author.

From the selected studies, the following data were extracted: first author's name, publication year, sample size, study location, mean age of participants, intervention (type, dose, and duration of supplementation), study design and end-point values (as means and SD) for the number of follicles, endometrial thickness, TT, progesterone, serum LH levels, serum FSH levels, E2 and SHBG in both intervention and control groups.

Risk of bias assessment

The Cochrane Collaboration's risk of bias tool was employed to assess the risk of bias for each study[19]. The tool consists of seven domains, including random sequence generation, allocation concealment, performance bias, reporting bias, detection bias, attrition bias and other sources of bias. If the study contains a methodological defect that may affect its findings, the study

was given a 'high risk' score; if there was no defect for that domain, a 'low risk' score; if the information was insufficient to determine the effect, an 'uncertain risk' score. If the trial had 'low risk' for all domains, a high-quality study was considered a low risk of bias. Risk bias assessments were conducted independently by two reviewers.

Data synthesis and statistical analysis

To obtain the overall effect size, means and SD of after values in the NAC and control groups were analysed. We utilised a random-effects model to get the overall effect size if the amount of between-study heterogeneity was significant. Heterogeneity was determined using I^2 statistics and Cochrane's Q test. I^2 value >50 % or $P < 0{\cdot}1$ for the Q test was considered significant heterogeneity. If heterogeneity was not significant, the fixed-effects model was employed to estimate the overall effect size. To detect potential sources of heterogeneity, subgroup analyses were performed according to the predefined variables, including duration of the intervention, NAC dosage, study location and the type of control. The sensitivity analysis was used to identify the dependence of overall effect size on a single study. The small-study effect was evaluated by the formal tests of Egger's and Begg's. Publication bias was determined by visual inspection of the funnel plot. In the presence of publication bias, trim and fill analysis was used to simulate a model without publication bias presenting a new effect size by inserting new fictitious studies. All the statistical analyses were performed using Stata, version 16 (Stata Corp.). $P < 0{\cdot}05$ was considered as the significance level.

Results

Selected studies and systematic review

A total of 931 articles were obtained from a systematic search of electronic databases. After removing duplicate papers and screening the articles carefully by the title and abstract, thirty-five papers were included. Seventeen articles after the full-text review were excluded. Overall, eighteen articles met the inclusion criteria and enrolled in the meta-analysis. Two of them were from the hand-search of the related articles. The flow diagram of the literature search process is presented in online Supplementary Fig. 1. Overall, eighteen clinical trials published between 2002 and 2021 were included in the meta-analysis. Table 1 summarises the characteristics of the included studies. The mean age of participants was from 23 to 29 years. In addition, nine studies of these studies were performed in Egypt[13,18,20,21,22,9,23,8,24], four in Iran[12,5,7,25], two in Turkey[26,11], two in India[27,28] and one in Italy[29]. NAC was administered in various doses, from 1·2 to 1·8 g. The duration of the intervention varied from 5 d to 24 weeks among the studies.

Risk of bias assessment

The results of evaluating the quality of the included studies are presented in online Supplementary Fig. 2. Overall, five studies were defined as high quality[18,20,24,26,5]. In the study by Gayatri *et al.*[28], participants were not randomly assigned to the intervention and control groups, and seven studies described an allocation concealment method[18,20–22,23,5,8]. The blinding of subjects and researchers was reported in four studies[22,9,27,28]. In three studies, several patients did not complete the study and had a high risk of bias in the incomplete outcome data domain[11,26,29].

Effects of N-acetylcysteine on number of follicles

A pooled analysis of eight effect sizes with seven studies (subjects = 1888; intervention, 970; control, 918) on the number of follicles revealed no significant effect following the intervention (weighted mean difference (WMD): −0·10; 95 % CI (−1·02, 0·82), $P = 0{\cdot}829$) (online Supplementary Fig. 3). The level of heterogeneity was considerable ($I^2 = 99{\cdot}4$ %, '$P < 0{\cdot}001$'), which was reduced with subgrouping by country, treatment dosage, type of control and duration of studies. There were no significant changes in results following subgroup analysis (Table 2). Removing an individual research at a time by sensitivity analysis did not affect the results. Begg's test revealed no presence of small-study effects ($P = 0{\cdot}536$).

Effects of N-acetylcysteine on endometrial thickness

The effects of NAC on endometrial thickness were reported in eleven studies with thirteen effect sizes (subjects = 2663; intervention, 1356; control, 1307). The analysis indicated no significant change in endometrial thickness by NAC (WMD = 0·11 mm; 95 % CI (-0·95, 1·17), $P = 0{\cdot}839$) (online Supplementary Fig. 4(a)). A significant between-study heterogeneity was observed ($I^2 = 99{\cdot}3$ %, '$P < 0{\cdot}001$'). The type of control and study location were identified as sources of high heterogeneity following subgroup analysis. In addition, according to the subgroup analysis, there was a significant increase in endometrial thickness in studies conducted in Egypt ($P = 0{\cdot}025$) (Table 2). Sensitivity analysis determined that no particular study likely affected the pooled results. We found significant small-study effects using Egger's but not Begg's tests ('$P < 0{\cdot}001$' and $P = 0{\cdot}161$, respectively). Furthermore, visual inspection of the funnel plot revealed the asymmetric distribution (online Supplementary Fig. 4(b)). Thus, trim and fill analysis was conducted with five imputed studies. The corrected effect size for publication bias showed significant changes after trim and fill analysis (WMD = −1·385 mm; 95 % CI (−2·736, −0·034), $P < 0{\cdot}05$) (online Supplementary Fig. 4(c)).

Effects of N-acetylcysteine on luteinising hormone

Overall, ten effect sizes from eight studies with 990 participants (intervention, 509; control, 481) have examined the effects of NAC supplementation on LH, revealing no significant changes in NAC group compared with control group (standardised mean difference = 0·10 mg/ml; 95 % CI (−0·12, 0·33), $P = 0{\cdot}365$) (online Supplementary Fig. 5(a)). Significant heterogeneity was seen among the studies ($I^2 = 61$ %, $P = 0{\cdot}006$). Study location, treatment duration and type of control were detected as sources of heterogeneity following subgroup analysis (Table 2). Sensitivity analysis demonstrated that the overall effect size regarding the effects of NAC on LH did not depend on one

Table 1. Study characteristics of included studies

Citation (first author *et al.*, year)	Location	Study population	Sample size (control/ intervention)	Mean age (control/ intervention)	Intervention/ daily dose	Type of control	Duration (day or week)
Nasr *et al.*, 2010	Egypt	PCOS	30/30	28/29	NAC/1·2 g	Placebo	5 d
Hashim *et al.*, 2010	Egypt	PCOS	95/97	27/26·5	NAC/1·8 g	Metformin	5–6 weeks
Badawy *et al.*, 2006	Egypt	PCOS	404/400	28/28	NAC/1·2 g	Clomiphene citrate	5 d
Badawy *et al.*, 2007	Egypt	PCOS	260/210	27/27	NAC/1·2 g	Clomiphene citrate	5 d
Cheraghi *et al.*, 2014	Iran	PCOS	20/20	29/28	NAC/1·2 g	Placebo	6 weeks
			20/20	29·28	NAC/1·2 g	Metformin	6 weeks
Elgindy *et al.*, 2010	Egypt	Intracytoplasmic sperm injection	38/38	27/28	NAC/1·2 g	Standard long protocol	12 weeks
Ghomian *et al.*, 2018	Iran	PCOS	33/33	29/28	NAC/1·2 g	Clomiphene citrate	5 d
Hassan *et al.*, 2019	Egypt	PCOS	150/150	26/26	NAC/NR	Placebo	5 d
Maged *et al.*, 2015	Egypt	PCOS	40/40	26/25·8	NAC/1·2 g	Clomiphene citrate	5 d
Nemati *et al.*, 2017	Iran	PCOS	54/54	NR	NAC/1·8 g	Metformin	8 weeks
			54/54		NAC/1·8 g	Metformin	12 weeks
Teimouri *et al.*, 2021	Iran	PCOS	158/159	28/29	NAC/1·2 g	Letrozole	5 d
El Sharkwy *et al.*, 2018	Egypt	PCOS	82/80	26·5/26	NAC/1·8 g	L-carnitine	5 d
Fulghesu *et al.*, 2002	Italy	PCOS	17/6	25·5/25·5	NAC/1·8 g	Placebo	5–6 weeks
Köse *et al.*, 2015	Turkey	PCOS	17/17	25/28	NAC/1·8 g	Placebo	6 weeks
Oner *et al.*, 2011	Turkey	PCOS	45/30	24/23	NAC/1·8 g	Metformin	24 weeks
Elnashar *et al.*, 2019	Egypt	PCOS	31/31	28·5/28·5	NAC/1·8 g	Metformin	6 weeks
Gayatri *et al.*, 2010	India	PCOS	50/50	23/22·5	NAC/1·8 g	Metformin	12 weeks
Chandil *et al.*, 2019	India	PCOS	95/97	27/28	NAC/1·8 g	Metformin	24 weeks

NR, not reported; Con, control; PCOS, polycystic ovary syndrome; NAC, N-acetylcysteine.

individual study. No small-study effects were found using Egger's and Begg's tests ($P = 0{\cdot}927$ and 1·00, respectively). Moreover, funnel plot visual inspection revealed no sign of publication bias (online Supplementary Fig. 4(b)).

Effects of N-acetylcysteine on oestradiol

According to the eight studies with ten effect sizes on 2160 individuals (intervention, 1119; control, 1041), NAC supplementation did not significantly affect E2 levels (WMD = −15·96 pg/ml; 95 % CI (−35·54, 3·62), $P = 0{\cdot}110$) (online Supplementary Fig. 6(a)). There was a significant between-study heterogeneity ($I^2 = 94{\cdot}4$ %, '$P < 0{\cdot}001$'), which was decreased with subgrouping by type of control, study location, treatment dosage and duration of studies (Table 2). Subgroup analysis showed that NAC supplementation significantly decreased E2 levels when the supplementation was accompanied by metformin ($P = 0{\cdot}019$). Moreover, supplementation with a higher dosage of NAC (1·8 g/d) had a considerable impact on decreasing E2 compared with lower doses (1·2 g/d) ($P = 0{\cdot}049$). Sensitivity analysis demonstrated no evidence of the impact of a single study on the results. The Egger's and Begg's tests showed no significant small-study effect ($P = 0{\cdot}444$ and $P = 0{\cdot}721$, respectively). In addition, publication bias was revealed by visual inspection of the funnel plot (online Supplementary Fig. 6(b)). Thus, trim and fill analysis was done with four imputed studies resulting in a significant change in the results (WMD = −0·390 pg/ml; 95 % CI (−0·728, −0·052), $P < 0{\cdot}05$) (online Supplementary Fig. 6(c)).

Effects of N-acetylcysteine on follicle-stimulating hormone

A significant increase in FSH levels was observed by NAC supplementation in the combined analysis of six studies with seven effect sizes (subjects = 610; intervention, 319; control, 291) (standardised mean difference: 0·39; 95 % CI (0·07, 0·71); $P = 0{\cdot}01$) (online Supplementary Fig. 7(a)). Significant between-study heterogeneity ($I^2 = 70{\cdot}9$ %, $P = 0{\cdot}002$) was reduced by subgrouping by study location, treatment duration and type of control (Table 2). By removing El Sharkwy *et al.*[8] (effect size (ES): 0·37 mg/ml, 95 % CI (−0·04, 0·78)) and Nemati *et al.*[12] (ES: 0·34 mg/ml, 95 % CI (−0·41, 0·72)) studies, the significant effects of NAC on FSH levels became non-significant. The result of Begg's tests was not significant in identifying small-study effects ($P = 0{\cdot}764$).

Effects of N-acetylcysteine on total testosterone

Combining the data of six studies with eight effect sizes (subjects = 725; intervention, 369; control, 356) showed a significant effect of NAC on TT levels (standardised mean difference: −0·25; 95 % CI (−0·39, −0·10); '$P < 0{\cdot}001$') (online Supplementary Fig. 7(b)). There was a significant between-study heterogeneity ($I^2 = 53{\cdot}9$ %, $P = 0{\cdot}034$) in which the study location and duration of studies were revealed as the sources of heterogeneity following subgroup analysis. Performing subgroup analysis revealed that the effects of NAC on TT levels in studies with intervention duration ≥ 8 weeks were more robust than the entire sample (Table 2). Sensitivity analysis revealed that no single study likely

Table 2. Subgroup analyses for the effects of N-acetylcysteine on ovulation and sex hormones profile (95 % confidence intervals)

Variables	No. of study	WMD or SMD	95 % CI	*P*	I^2 (%)	*P* heterogeneity
Number of follicles						
Total dosage (g)	8	−0·10	−1·02, 0·82	0·829	99·4	0·000
1·2	5	0·32	−0·03, 0·67	0·075	91·0	0·000
1·8	3	−0·84	−2·81, 1·14	0·407	99·7	0·000
Intervention duration (week)						
≤1	4	0·12	−0·12, 0·38	0·333	79·9	0·002
≥6	4	−0·35	−2·13, 1·42	0·695	99·5	0·000
Country						
Iran	3	0·05	−0·10, 0·19	0·524	0·0	0·567
Egypt	5	−0·23	−1·55, 1·09	0·736	99·6	0·000
Type of control						
Placebo	1	1·10	0·72, 1·48	0·000	–	–
Metformin	3	−0·84	−2·81, 1·14	0·407	99·7	0·000
Clomiphene citrate	4	0·12	−0·12, 0·36	0·333	79·9	0·002
Endometrial thickness						
Total dosage (g)	13	0·11	−0·95, 1·17	0·839	99·3	0·000
1·2	7	0·61	−0·24, 1·47	0·158	95·0	0·000
1·8	5	−0·60	−2·06, 0·86	0·421	99·2	0·000
NR	1	0·10	−0·20, 0·40	0·507	–	–
Intervention duration (week)						
≤1	6	0·29	−0·46, 1·05	0·447	94·5	0·000
≥6	7	−0·04	−1·43, 1·35	0·954	99·2	0·000
Country						
Iran	6	−0·23	−0·43, −0·03	0·719	8·7	0·360
Egypt	7	0·31	−1·36, 1·97	0·025	0·0	0·747
Type of control						
Placebo	3	1·02	−0·47, 2·51	0·181	95·0	0·000
Metformin	4	−0·87	−2·46, 0·73	0·287	96·2	0·000
Clomiphene citrate	4	0·49	−0·71, 1·68	0·427	96·2	0·000
NR	2	−0·03	−0·53, 0·47	0·916	41·5	0·191
LH						
Total	10	0·10	−0·12, 0·33	0·365	61·0	0·006
Type of control						
Placebo	4	0·09	−0·64, 0·82	0·811	83·7	0·000
Metformin	5	0·15	−0·05, 0·34	0·135	2·3	0·394
L-carnitine	1	0·11	−0·20, 0·42	0·482	–	–
Intervention duration (week)						
≤ 1	2	0·07	−0·11, 0·25	0·463	0·0	0·738
1–8	4	0·10	−0·79, 1·00	0·818	83·7	0·000
≥8	4	0·15	−0·09, 0·38	0·215	25·9	0·256
Country						
Iran	4	0·03	−0·43, 0·49	0·897	72·5	0·012
Egypt	2	0·07	−0·11, 0·25	0·463	0·0	0·738
Other	2	−0·01	−0·37, 0·35	0·957	0·0	0·837
Turkey	2	0·54	−0·90, 1·98	0·462	90·8	0·001
FSH						
Total	7	0·39	0·07, 0·71	0·016	70·9	0·002
Type of control						
Placebo	2	0·08	−0·55, 0·70	0·811	19·5	0·265
Metformin	4	0·46	−0·04, 0·95	0·069	82·6	0·001
L-carnitine	1	0·45	0·14, 0·77	0·004	–	–
Intervention duration (week)						
< 8	3	0·31	−0·08, 0·69	0·118	28·1	0·249
≥8	4	0·46	−0·04, 0·95	0·069	82·6	0·001
Country						
Iran	2	0·84	0·47, 1·22	0·000	44·0	0·182
Turkey	2	−0·18	−0·57, 0·20	0·345	0·0	0·953
Other	3	0·40	0·16, 0·63	0·001	0·0	0·788
Total testosterone						
Total	8	−0·25	−0·39, −0·10	0·001	53·9	0·034
Country						
Iran	4	−0·02	−0·25, 0·20	0·834	0·0	0·789
Other	4	−0·40	−0·60, −0·21	0·000	62·1	0·048
Intervention duration (week)						
< 8	3	−0·10	−0·43, 0·2	0·544	0·0	0·552
≥8	4	−0·28	−0·45, −0·12	0·001	69·4	0·011
E2						
Total	10	−15·96	−35·54, 3·62	0·110	94·4	0·000

Table 2. (*Continued*)

Variables	No. of study	WMD or SMD	95 % CI	*P*	I^2 (%)	*P* heterogeneity
Country						
Iran	4	−2·72	−7·70, 2·25	0·284	0·0	0·000
Egypt	4	−33·38	−124·88, 58·12	0·475	97·0	0·830
Other	2	1·93	−2·69, 8·54	0·413	0·0	0·931
Dosage (g)						
1·2	2	−21·63	−90·14, 46·87	0·536	78·7	0·030
1·8	7	−22·86	−45·59, −0·12	0·049	95·8	0·000
NR		39·90	12·27, 67·53	0·005	–	–
Type of control						
Placebo	3	25·35	−0·16, 50·86	0·051	20·0	0·286
Metformin	5	−30·55	−56·11, −4·99	0·019	97·2	0·000
Clomiphene citrate	2	−21·63	−90·14, 46·87	0·536	78·7	0·030
Intervention duration (week)						
≤ 1	3	0·71	−61·36, 62·78	0·982	93·9	0·000
1–8	4	−35·10	−130·65, 60·45	0·472	87·9	0·000
≥8	3	−0·28	−3·68, 3·13	0·873	0·0	0·369

WMD, weighted mean difference; SMD, standardised mean difference; LH, luteinising hormone; FSH, follicle-stimulating hormone; E2, oestradiol; NR, not reported.

affected the pooled results. There were no significant small-study effects using Begg's test ($P = 0{\cdot}764$).

Effects of N-acetylcysteine on progesterone

The pooled results of five studies (subjects = 1842; intervention, 947; control, 895) indicated that there were no significant effects of NAC supplementation on progesterone (WMD = −0·29, 95 % CI (−2·47, 1·89); $P = 0{\cdot}794$) (online Supplementary Fig. 8(a)). There was considerable between-study heterogeneity ($I^2 = 99{\cdot}5$ %, '$P < 0{\cdot}001$'). No subgroup analysis was performed on studies. No significant difference in overall effect size was shown after removing each study using sensitivity analysis. The results of Begg's test revealed no presence of a small-study effect ($P = 0{\cdot}806$).

Effects of N-acetylcysteine on sex hormone-binding globulin

Pooling data from four studies with five effect sizes (subjects = 414; intervention, 220; control, 194) showed no significant effect of NAC supplementation on SHGB levels (WMD: 1·68 nmol/l, 95 % CI (−2·10, 5·45), $P = 0{\cdot}383$) (online Supplementary Fig. 8(b)). No heterogeneity was detected among studies ($I^2 = 0{\cdot}0$ %, $P = 0{\cdot}851$). Subgroup analysis was not performed due to the low number of studies. Sensitivity analysis revealed that the overall effect size did not depend on one individual study. No significant small-study effects were performing Begg's test ($P = 0{\cdot}806$).

Discussion

Pooled analysis of eighteen randomised clinical trials with a total population of 3161 in the current study revealed that NAC supplementation had significant increasing and decreasing effects on FSH and TT levels, respectively. However, the elimination of publication bias using trim and fill analysis revealed that NAC also could decrease E2 levels and endometrial thickness. Country, type of control and intervention duration were the possible sources of high heterogeneity in the pooled analyses.

Several studies have investigated the effectiveness of NAC in the reproductive performance of females. Mona *et al.* showed that NAC administration before the chemotherapy could improve reproductive functions and the gonadal hormone disturbance in female rats through its protective effects against oxidative stress[30]. Naimi *et al.* reported that NAC alone had no significant beneficial effect on the number of ovarian follicles and the levels of hormones in adult rats. However, in a dose-dependent manner, its combination with acrylamide led to the significant elevation of the number of ovarian follicles and the levels of FSH, E2 and progesterone[31]. This was in line with our finding on E2 levels. However, due to the limited range of administered dosages of NAC, subgroup analysis based on the dosage was not performed on LH, FSH and TT levels. Performed pooled analyses on the number of follicles and endometrial thickness revealed that the effects of NAC on these variables were not in a dose-dependent manner. Furthermore, it has been demonstrated that higher doses of NAC (1·8 g/d) have decreasing effects on E2 levels compared with lower doses. This result could be explained with respect to the fact that E2 might be dose-dependent in response to NAC. Previous studies showed that with different doses of NAC, different effects could be observed[32]. For example, NAC consumption at a dose of 1·8 g/d for 12 weeks showed improvement in ovulation and pregnancy rates among patients with PCOS compared with 1·2 g daily after 12 weeks[12,9]. Also, it has been shown that a longer duration of supplementation might be more effective. It is difficult to achieve desirable and continuous results in a short intervention time. For example, a study with supplementation of 1·8 g/d for 6 weeks compared with another NAC intervention with 1·8 g/d for 24 weeks; changes in fasting glucose or fasting insulin were not significant in the first study; however, these biochemical markers were improved significantly in the second study. A decline in TT was reported in both studies[21,27].

Subgroup analysis based on the country showed that the improving effects of NAC on the endometrial thickness are more significant in some countries. This could be due to the differences in ethnicity of participants. Epigenetic and environmental changes are various in different countries, and this issue

British Journal of Nutrition

may lead to different responses to NAC supplementation[33]. However, due to the limited number of countries in which studies have been performed, exact interpretation of this issue must be with precaution.

In addition, NAC increases the cellular levels of antioxidants and reduces GSH at higher doses. Therefore, NAC can potentially improve insulin receptor activity in human erythrocytes and improve insulin secretion in response to glucose[13].

It has been shown that BMI, alcohol use and smoking influence E2 serum levels[34]. Recent studies showed that BMI and E2 levels have positive association. Women with high BMI score had high E2 levels. Indeed, increased E2 levels among overweight and obese postmenopausal women were due to the increased fat mass and E2 production in adipose tissue[35]. Rinaldi *et al.* showed that intake of alcohol increased serum levels of dehydroepiandrosterone sulphate, testosterone, androstenedione and oestrone compared with non-consumers women[36,37]. Another study revealed that alcohol consumption is related to change in plasma sex hormone levels in premenopausal women[38]. In postmenopausal women, alcohol ingestion was related to increased plasma E2 and oestrone levels[39]. Cigarette smoking is related to increase in 2-hydroxylation pathway of E2 and decrease in bio-availability of oestrogen[40]. A review study proposed another mechanisms underlying the potential interaction of the smoking and oestrogen metabolites such as decrease in oestrogen activity, SHBG levels, aromatase enzyme activity, C-20, 22 desmolase enzyme levels and increase in free testosterone levels, hyperandrogenism and the hepatic metabolism of steroid[41].

NAC, compared with metformin, led to a decrease in E2 levels. However, the effects of NAC on the number of follicles, endometrial thickness, LH and FSH levels were not markedly different. Metformin, through the induction of 5' AMP-activated protein kinase and subsequently insulin sensitivity, has been widely used as a therapeutic agent in various conditions, including type 2 diabetes mellitus, PCOS and gestational diabetes[42]. These pathways could contribute to a synergistic effect of metformin and NAC on sex hormones profile. These factors have been ignored in the relationship between NAC and E2 levels. Therefore, a definite interpretation of more decreasing effects of NAC on E2 levels than metformin needs more studies. Nasr *et al.* reported that NAC supplementation increases the number of follicles in PCOS women[23]. According to the bias assessment using the Cochrane Collaboration's risk of bias tool, this study had a lower risk of bias; therefore, its results could be reliable. However, additional studies are needed to compare the effects of NAC with placebo on the number of follicles. El Sharkwy *et al.* demonstrated that NAC, in comparison with L-carnitine, led to a significant increase in FSH levels[24]. Due to the higher number of participants, this study was greatly weighted and influenced the overall results, so removing of mentioned study in sensitivity analysis changed the increasing effects of NAC on the FSH levels. Other mechanisms proposed for the effects of L-carnitine on FSH and LH levels could be attributed to the regulation of lipid peroxidation and activity of antioxidant enzymes[43]. A comparison of two meta-analyses with the subjects of NAC and L-carnitine supplementation on inflammatory and oxidative stress biomarkers revealed that NAC had better effects than L-carnitine[6,44]. Similarly, our results suggested that improving the effects of NAC on FSH levels was more significant than L-carnitine. However, due to the limited number of conducted studies, more conclusions must be declared with precaution. On the other hand, the hormone status and other metabolites levels, such as serum 25(OH)D in women younger than 30 years, are lower than that in women 30 years and older[45]. Thus, this situation also may affect the results[46].

NAC supplementation for ≥ 8 weeks led to a significant decrease in TT. In other investigated variables, extending supplementation duration was ineffective in the overall results. Regarding TT, studies with shorter duration had smaller sample sizes than studies with longer duration studies. Therefore, they might not have enough power to reach a significant result.

The risk of bias in one included study was low, in two was moderate and other included studies had a higher risk of bias. Also, publication bias could change the effects of NAC on E2 levels and endometrial thickness. As there was no unexplained heterogeneity, our results had no inconsistency. Participants of included studies in seventeen of eighteen studies had PCOS. Therefore, our results could be generalised to these patients.

Different underlying mechanisms have explained how NAC could affect reproductive biomarkers. NAC is the source of the sulfhydryl group that is essential for removing free radicals like H_2O_2, OH* and O_2^{-*}[47]. As well, NAC supplementation increased levels of GSH in individuals with low GSH levels and improved redox homoeostasis[48]. Moreover, reactive oxygen species-induced mitogen-activated protein kinase activation could be inhibited by NAC[49]. Therefore, NAC, through decreasing lipid peroxidation, could have beneficial effects on reproductive biomarkers. It must be noted that the GSH levels might be effective in the relationship between NAC and reproductive biomarkers[50]. Therefore, measuring GSH levels to determine the availability of cysteine as the limiting step must be considered in future studies. Badawy *et al.* reported that NAC induced ovulation in PCOS patients through increasing insulin sensitivity[13]. This was similar to the effects of metformin on PCOS.

Our systematic review and meta-analysis had some limitations that must be noted. First, only a limited number of the studies have assessed the effects of NAC on progesterone and SHBG levels. Consequently, subgroup analysis could not be performed on these biomarkers. Second, due to this cause, the comprehensive subgroup analysis based on dosage or type of control in some biomarkers was not performed. Therefore, a definite interpretation on the effects of different dosages of NAC should be considered cautiously. Third, in some cases, publication bias has changed the overall results. However, its impact was neutralised by performing trim and fill analysis. As well, the risk of bias was almost high in the included studies that might affect the quality of evidence. Therefore, future studies must focus on the proper design to diminish the possible sources of bias. As well, further studies are needed to compare the synergistic effects of NAC, metformin and L-carnitine on reproductive biomarkers in patients with PCOS. This study had some strengths too. First, to our knowledge, current study is the first comprehensive systematic review and meta-analysis addressing this issue, so far.

Second, all possible subgroup and bias-controlling analyses were performed to obtain reliable results on the effects of NAC on the reproductive biomarkers.

Conclusion

The results indicated that NAC supplementation decreased TT levels and increased FSH levels. Oestrogen levels also were increased after correcting publication bias. These might contribute to a better function of the reproductive system. However, this conclusion should be with precaution as the other sex hormones and ovulation parameters were not influenced by NAC supplementation, significantly. Overall, this study supports the efficacy and favourable effects of NAC supplementation on improvement of reproductive system function.

Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S0007114522003270

Acknowledgements

The authors would like to thank the Clinical Research Development Unit of Baqiyatallah Hospital, for all their support and guidance during carrying out this study.

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

K. P. was responsible for designing and coordinating the study. All authors were responsible for data collection, data analysis and data interpretation in the manuscript. E. E., Z. S. A., and K. P. were responsible for the statistical work and for writing the manuscript. K. P. was responsible for reviewing the manuscript. All authors read and approved the final manuscript.

The authors declare that they have no competing interests.

References

1. De Leo V, Musacchio M, Cappelli V, *et al.* (2016) Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol* **14**, 1–17.
2. Alchami A, O'Donovan O & Davies M (2015) PCOS: diagnosis and management of related infertility. *Obstet Gynaecol Reprod Med* **25**, 279–282.
3. Melo AS, Ferriani RA & Navarro PA (2015) Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. *Clinics* **70**, 765–769.
4. Jonard S & Dewailly D (2004) The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update* **10**, 107–117.
5. Ghomian N, Khadem N, Moeindarbari S, *et al.* (2019) Comparison of pregnancy rate in patients with polycystic ovary syndrome treated with clomiphene alone and in combination with N-acetyl cysteine: a randomized clinical trial. *Int J Women's Health Reprod Sci* **7**, 185–189.
6. Faghfouri AH, Zarezadeh M, Tavakoli-Rouzbehani OM, *et al.* (2020) The effects of N-acetylcysteine on inflammatory and oxidative stress biomarkers: a systematic review and meta-analysis of controlled clinical trials. *Eur J Pharmacol* **884**, 173368.
7. Teimouri B, Mollashahi S, Paracheh M, *et al.* (2021) Comparison of the effect of letrozole alone with letrozole plus n-acetylcysteine on pregnancy rate in patients with polycystic ovarian syndrome: a randomized clinical trial. *Int J Women's Health Reprod Sci* **9**, 75–79.
8. Maged AM, Elsawah H, Abdelhafez A, *et al.* (2015) The adjuvant effect of metformin and N-acetylcysteine to clomiphene citrate in induction of ovulation in patients with polycystic ovary syndrome. *Gynecol Endocrinol* **31**, 635–638.
9. Nasr A (2010) Effect of N-acetyl-cysteine after ovarian drilling in clomiphene citrate-resistant PCOS women: a pilot study. *Reprod Biomed Online* **20**, 403–409.
10. Jannatifar R, Parivar K, Roodbari NH, *et al.* (2019) Effects of N-acetyl-cysteine supplementation on sperm quality, chromatin integrity and level of oxidative stress in infertile men. *Reprod Biol Endocrinol* **17**, 1–9.
11. Oner G & Muderris II (2011) Clinical, endocrine and metabolic effects of metformin *v.* N-acetyl-cysteine in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* **159**, 127–131.
12. Nemati M, Nemati S, Taheri A-M, *et al.* (2017) Comparison of metformin and N-acetyl cysteine, as an adjuvant to clomiphene citrate, in clomiphene-resistant women with polycystic ovary syndrome. *J Gynecol Obstet Hum Reprod* **46**, 579–585.
13. Badawy A, State O & Abdelgawad S (2007) N-Acetyl cysteine and clomiphene citrate for induction of ovulation in polycystic ovary syndrome: a cross-over trial. *Acta Obstet Gynecol Scand* **86**, 218–222.
14. Luo J, Ao Z, Duan Z, *et al.* (2021) Effects of N-Acetylcysteine on the reproductive performance, oxidative stress and RNA sequencing of Nubian goats. *Vet Med Sci* **7**, 156–163.
15. Shahrzad E, Shariati M, Naimi S, *et al.* (2020) Protective effect of N-acetylcysteine on changes in serum levels of Pituitary–Gonadal axis hormones and testicular tissue in acrylamide-treated adult rats. *Adv Hum Biol* **10**, 16.
16. Moghadam MH, Shariati M, Naeimi S, *et al.* (2020) Influence of N-acetylcysteine on pituitary-gonadal axis hormones and protamine expression level in streptozotocin-induced diabetic male rats. *Asian Pac J Reprod* **9**, 89.
17. Carmina E & Azziz R (2006) Diagnosis, phenotype, and prevalence of polycystic ovary syndrome. *Fertil Steril* **86**, S7–S8.
18. Hassan M, Alalfy M, Hassan H, *et al.* (2019) Combined N-Acetylcysteine and clomiphene citrate for ovulation induction in polycystic ovary syndrome, a double blind randomized controlled trial. *Austin J Obstet Gynecol* **6**, 1134.
19. Higgins JP, Altman DG, Gøtzsche PC, *et al.* (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* **343**, d5928.
20. Badawy A, El Nashar A & El Totongy M (2006) Clomiphene citrate plus N-acetyl cysteine *v.* clomiphene citrate for augmenting ovulation in the management of unexplained infertility: a randomized double-blind controlled trial. *Fertil Steril* **86**, 647–650.
21. Elnashar A, Fahmy M, Mansour A, *et al.* (2007) N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study. *Fertil Steril* **88**, 406–409.
22. Hashim AH, Anwar K & El-Fatah RA (2010) N-acetyl cysteine plus clomiphene citrate *v.* metformin and clomiphene citrate in treatment of clomiphene-resistant polycystic ovary syndrome: a randomized controlled trial. *J Women's Health* **19**, 2043–2048.
23. Elgindy EA, El-Huseiny AM, Mostafa MI, *et al.* (2010) N-Acetyl cysteine: could it be an effective adjuvant therapy in ICSI cycles? A preliminary study. *Reprod Biomed Online* **20**, 789–796.
24. El Sharkwy IA & Abd El Aziz WM (2019) Randomized controlled trial of N-acetylcysteine *v.* l-carnitine among women with

clomiphene-citrate-resistant polycystic ovary syndrome. *Int J Gynecol Obstet* **147**, 59–64.
25. Cheraghi E, Mehranjani MS, Shariatzadeh MA, *et al.* (2016) N-Acetylcysteine improves oocyte and embryo quality in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection: an alternative to metformin. *Reprod Fertil Dev* **28**, 723–731.
26. Kose SA & Naziroglu M (2015) N-acetyl cysteine reduces oxidative toxicity, apoptosis, and calcium entry through TRPV1 channels in the neutrophils of patients with polycystic ovary syndrome. *Free Radic Res* **49**, 338–346.
27. Chandil N, Pande S, Sen SS, *et al.* (2019) Comparison of metformin and N acetylcysteine on clinical, metabolic parameter and hormonal profile in women with polycystic ovarian syndrome. *J Obstet Gynecol India* **69**, 77–81.
28. Gayatri K, Kumar JS & Kumar BB (2010) Metformin and N-acetyl cysteine in polycystic ovarian syndrome – a comparative study. *Indian J Clin Med* **1**, 7–13.
29. Fulghesu AM, Ciampelli M, Muzj G, *et al.* (2002) N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. *Fertil Steril* **77**, 1128–1135.
30. Helal MA (2016) The effects of N-acetyl-L-cysteine on the female reproductive performance and nephrotoxicity in rats. *Ren Fail* **38**, 311–320.
31. Naimi M, Shariati M, Naimi S, *et al.* (2019) N-Acetylcysteine improves acrylamide-induced changes in ovarian tissue and serum levels of pituitary-ovarian axis hormones in adult rats. *Eur J Biol* **78**, 75–81.
32. Cavallini G, Ferraretti AP, Gianaroli L, *et al.* (2004) Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. *J Androl* **25**, 761–770.
33. Diamanti-Kandarakis E, Kandarakis H & Legro RS (2006) The role of genes and environment in the etiology of PCOS. *Endocrine* **30**, 19–26.
34. Sriprasert I, Kono N, Karim R, *et al.* (2020) Factors associated with serum estradiol levels among postmenopausal women using hormone therapy. *Obstet Gynecol* **136**, 675–684.
35. Gruber CJ, Tschugguel W, Schneeberger C, *et al.* (2002) Production and actions of estrogens. *N Engl J Med* **346**, 340–352.
36. Rinaldi S, Peeters P, Bezemer I, *et al.* (2006) Relationship of alcohol intake and sex steroid concentrations in blood in pre-and post-menopausal women: the European prospective investigation into cancer and nutrition. *Cancer Causes Control* **17**, 1033–1043.
37. Shafrir AL, Zhang X, Poole EM, *et al.* (2014) The association of reproductive and lifestyle factors with a score of multiple endogenous hormones. *Horm Cancer* **5**, 324–335.
38. Sarkola T, Mäkisalo H, Fukunaga T, *et al.* (1999) Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol Clin Exp Res* **23**, 976–982.
39. Ginsburg ES, Mello NK, Mendelson JH, *et al.* (1996) Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA* **276**, 1747–1751.
40. Michnovicz JJ, Hershcopf RJ, Naganuma H, *et al.* (1986) Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. *N Engl J Med* **315**, 1305–1309.
41. Ruan X & Mueck A (2015) Impact of smoking on estrogenic efficacy. *Climacteric* **18**, 38–46.
42. Rice S, Elia A, Jawad Z, *et al.* (2013) Metformin inhibits follicle-stimulating hormone (FSH) action in human granulosa cells: relevance to polycystic ovary syndrome. *J Clin Endocrinol Metab* **98**, E1491–1500.
43. Rezaei N, Mardanshahi T, Shafaroudi MM, *et al.* (2018) Effects of l-Carnitine on the follicle-stimulating hormone, luteinizing hormone, testosterone, and testicular tissue oxidative stress levels in streptozotocin-induced diabetic rats. *J Evid Based Integr Med* **23**, 1–10.
44. Fathizadeh H, Milajerdi A, Reiner Ž, *et al.* (2020) The effects of L-carnitine supplementation on indicators of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. *J Diabetes Metab Disord* **19**, 1879–1894.
45. Nakamura K, Nashimoto M, Matsuyama S, *et al.* (2001) Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. *Nutrition* **17**, 921–925.
46. Steiner AZ, Herring AH, Kesner JS, *et al.* (2011) Antimüllerian hormone as a predictor of natural fecundability in women aged 30–42 years. *Obstet Gynecol* **117**, 798–804.
47. Sadowska AM, Manuel YKB & De Backer WA (2007) Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant *in vitro* and *in vivo* dose-effects: a review. *Pulm Pharmacol Ther* **20**, 9–22.
48. Paschalis V, Theodorou AA, Margaritelis NV, *et al.* (2018) N-acetylcysteine supplementation increases exercise performance and reduces oxidative stress only in individuals with low levels of glutathione. *Free Radic Biol Med* **115**, 288–297.
49. Xiong T, Zhang Z, Zheng R, *et al.* (2019) N-acetyl cysteine inhibits lipopolysaccharide-induced apoptosis of human umbilical vein endothelial cells via the p38MAPK signaling pathway. *Mol Med Rep* **20**, 2945–2953.
50. Atkuri KR, Mantovani JJ, Herzenberg LA, *et al.* (2007) N-Acetylcysteine – a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol* **7**, 355–359.