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Influence of *n*-3 fatty acid supplementation on inflammatory and oxidative stress markers in patients with polycystic ovary syndrome: a systematic review and meta-analysis

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Abstract

Polycystic ovary syndrome (PCOS) is defined as a reproductive endocrine disease that results in a low-grade inflammatory and pro-oxidant state. Dietary factors, including *n*-3 fatty acids, may have a key role in improving metabolic disorders in PCOS patients. The present study aimed to investigate the influence of *n*-3 fatty acid supplementation on inflammatory and oxidative stress (OS) markers in patients with PCOS. A systematic literature search of Medline/PubMed, Cochrane Central Register of Controlled Trials, Scopus and Lilacs, until November 2019, was conducted. Randomised clinical trials that reported inflammatory and OS markers as endpoints in women with PCOS receiving *n*-3 fatty acid supplementation were included. The pooled estimates of the weighted mean differences (WMD) and the standard mean differences (SMD) were calculated. Random effects models were adopted to measure the pooled outcomes. Among the 323 studies retrieved, ten fulfilled the inclusion criteria for a meta-analysis. We founded a significant decrease in high-sensitivity C-reactive protein (hs-CRP) (SMD –0·29 (95 % CI –0·56, –0·02) mg/l) and an increase in adiponectin (WMD 1·42 (95 % CI 1·09, 1·76) ng/ml) concentrations in the intervention group when compared with the placebo group. No statistically significant results were found in the meta-analysis for visfatin, nitric oxide, GSH or malondialdehyde levels or total antioxidant capacity. The data suggest that supplementation of *n*-3 fatty acids could reduce the inflammatory state in women with PCOS, through a decrease in hs-CRP and an increase in adiponectin levels.

Key words: **Polycystic ovary syndrome: *n*-3 Fatty acid supplementation: Inflammation: Oxidative stress markers: Meta-analyses**

Polycystic ovary syndrome (PCOS) is defined as an endocrine disease resulting from a hormonal imbalance that affects 5–18 % of women of reproductive age, making it an important public health problem in view of the co-morbidities and prevalence currently presented[1]. The syndrome is characterised by symptoms such as menstrual irregularity, anovulatory infertility, clinical and biochemical hyperandrogenism, as well as other metabolic manifestations, which affect from 30 to 70 % of women with PCOS[2–4].

The hormonal imbalance that occurs with PCOS can influence the reproductive, metabolic and psychological health of patients. Clinical manifestations may include precocious puberty, acne, alopecia, seborrhoea, irregular menstrual cycles, hirsutism, infertility and complications in pregnancy[5]. Anxiety, depression and non-acceptance of body image are frequent psychological co-morbidities[6]. Metabolic impairment includes the primary effect of insulin resistance (IR) on muscle and adipose tissue, with compensatory hyperinsulinaemia, associated with intrinsic *β*-cell dysfunction, type 2 diabetes mellitus and gestational diabetes, hyperlipidaemia, increased risk of CVD, obesity, sleep apnoea, non-alcoholic fatty liver disease and the metabolic syndrome (MetS)[7].

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance; MDA, malondialdehyde; MetS, metabolic syndrome; NO, nitric oxide; OS, oxidative stress; PCOS, polycystic ovary syndrome; RCT, randomised controlled trial; ROS, reactive oxygen species; SMD, standard mean difference; TAC, total antioxidant capacity.

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In 2018, a guideline for the assessment and management of PCOS was published by the American Society for Reproductive Medicine/European Society of Human Reproduction and Embryology, which endorsed the Rotterdam criteria for the diagnosis of PCOS in adult women who were not in menopause[8]. Among these criteria, PCOS can be diagnosed when at least two of the three proposed criteria are present, categorised as follows: (1) androgen status – clinical or biochemical hyperandrogenism; (2) menstrual history – oligo- or anovulation or (3) ovarian appearance – polycystic morphology on ultrasound[8].

The PCOS state of low-grade inflammation is similar to that of other non-communicable diseases, such as obesity, type 2 diabetes mellitus and CVD[9]. The role of inflammation in PCOS has been the subject of several studies, and associations have been found between increased levels of inflammation markers (high-sensitivity C-reactive protein (hs-CRP), ferritin, TNF and IL-6 and IL-18) and oxidative stress (OS) markers (malondialdehyde (MDA), total antioxidant capacity – TAC, nitric oxide (NO) and GSH) with PCOS[10–12].

In fact, a case–control study[12] that aimed to evaluate the relationship between polymorphisms in genes encoding inflammation-associated cytokines and the metabolic profiles of Brazilian women with PCOS (*n* 97) *v.* a control group (*n* 99) observed that fasting glucose levels varied according to IL-6 genotype, while the hirsutism score, 2-h glucose tolerance test, total cholesterol and TAG levels varied according to the IL-10 genotype. Serum lipid levels were also related to interferon-γ and transforming growth factor-*β* genotypes, suggesting that cytokine gene polymorphisms may promote abnormal metabolic features in PCOS[12].

Another case–control study[13] aimed to determine the relationship between OS markers and lipid profiles in patients with PCOS. This study included fifty PCOS patients and fifty healthy controls and revealed that serum MDA levels were significantly higher in PCOS patients than in controls and that TAC was significantly lower in the PCOS group[13]. It is known that TAC is also reduced in many diseases such as hypertension, type 2 diabetes mellitus, obesity and the MetS[14].

Among the environmental factors, numerous nutrients are known to modulate the inflammatory response and contribute to the protection and treatment of non-communicable diseases, such as MUFA and PUFA[14–16]. The long-chain *n*-3 fatty acids, namely *α*-linolenic acid (ALA), EPA and DHA, are commonly considered 'essential' fatty acids, since they are not synthesised in the human body and are mostly obtained from the diet[17]. EPA and DHA are found naturally in marine sources, including cold water fish, shellfish and seaweed. ALA can be converted to DHA or EPA after ingestion and is found in seeds such as chia, flaxseed and pumpkin seeds, as well as in vegetable and oilseeds like nut oils. In addition, it is also present in small amounts in other vegetable sources, such as spinach and kale[18].

Evidence suggests that *n*-3 fatty acid supplementation increases insulin sensitivity and plasma adiponectin levels, reduces hyperinsulinaemia, plasma TAG and liver fat and attenuates inflammation and the OS response in adults[19,20]. However, the role of *n*-3 fatty acid supplementation in controlling OS and chronic low-grade inflammation in women with PCOS is still uncertain. Therefore, the present study aimed to systematically review and meta-analyse randomised controlled trials (RCT) investigating the influence of *n*-3 fatty acid supplementation on inflammatory and OS markers in patients with PCOS.

Methods

The conduct and design of this systematic review and meta-analysis followed the predetermined protocol according to the Cochrane Handbook's recommendations[21]. Results were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[22]. The protocol of the current study was published on the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42019129199).

Search strategy

The PICO acronym search question was composed of: P (participants) = Women with Polycystic Ovary Syndrome; I (intervention) = *n*-3 fatty acid supplementation; C (control) = Placebo and O (outcomes) = Inflammatory and OS markers levels. A literature review was performed by searching the electronic databases Medline/PubMed (Medical Literature Analysis and Retrieve System Online), Cochrane Central Register of Controlled Trials (CENTRAL), Scopus and Lilacs (Latin American and Caribbean Health Sciences) until November 2019 to identify RCT, which reported the effect of *n*-3 fatty acid supplementation on inflammatory and OS markers in PCOS patients over 18 years of age. The initial search included the Medical Subject Headings terms 'Polycystic Ovary Syndrome' and 'Fatty Acids, Omega-3'. It also included the entry terms associated with a high-sensitivity strategy for the search of RCT developed by The Cochrane Collaboration[21]. Online Supplementary material 1 describes the search strategy used on the PubMed database.

The same terms were used to search for clinical studies in the National Institutes of Health (www.clinicaltrials.gov), the Brazilian Registry of Clinical Trials (www.ensaiosclinicos.gov.br) and the Turning Research into Practice (www.tripdatabase.com) databases. All potentially eligible studies were considered for review, regardless of the language and date of publication. A manual search was also implemented in the reference lists of relevant reviews[23–27].

Inclusion and exclusion criteria

We included only RCT that analysed the effect of *n*-3 fatty acid supplementation on inflammatory and OS markers in women with PCOS. The outcome was considered as changes in the concentration or activity of these markers from baseline until the end of the study. Studies that did not report the outcomes of interest, non-randomised studies and those that included children, adolescents (under 18 years of age) or pregnant women were excluded. Studies that did not present as endpoints inflammatory or OS markers were also excluded.

Study selection and data extraction

Initially, the studies retrieved from the databases were input into a single electronic library, and duplicates were excluded

using the EndNote® software. Two reviewers (J. A. G. T. and M. T. A.) independently analysed the titles and abstracts of the articles retrieved from the literature search, reviewed the full text of the published articles and extracted the data using a standardised data extraction tool. Any disagreements between the reviewers regarding the study data were resolved by a third investigator (K. B. G. or V. E. A.).

The extracted data included the number of participants, study design, trial duration and patients' demographic and anthropometric characteristics (age and BMI). *n*-3 Fatty acid supplementation data from the intervention and control groups were collected. Informative data about inflammatory and OS markers collected at baseline and the end of the study were extracted. Percentage changes in biomarker concentrations were calculated for the studies that presented baseline values.

Assessment of bias across studies and quality of evidence

The risk of bias of the studies and the quality of evidence were assessed independently by two reviewers (J. A. G. T. and M. T. A.) following the Cochrane guidelines[28], and a third reviewer (K. B. G. or V. E. A.) resolved any disparity. The Cochrane Risk of Bias Tool for Randomized Trials – Rob 2.0 was applied to assess the risk of bias in individual studies according to the recommendations of the Cochrane Collaboration[28,29]. The Rob 2.0 is structured into five domains: (1) bias arising from the randomisation process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in measurement of the outcome and (5) bias in selection of the reported result. The response options for the signalling questions are: (1) yes; (2) probably yes; (3) probably no; (4) no and (5) no information[29].

The Grading of Recommendations, Assessment, Development and Evaluation approach[30] was used to assess the quality of the evidence for each outcome: hs-CRP, adiponectin, visfatin, NO, GSH, MDA and TAC. This approach assesses the strength of the evidence quality by including factors that can decrease quality (e.g. methodological quality, directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias) or increase it (e.g. large magnitude of effect, reduction or spurious effect due to plausible confounding factors, dose–response gradient). Each evaluated factor was rated as high, moderate, low or very low[28,30].

Statistical analyses

Differences between the mean values and standard deviations at baseline and at the end of the study were used to report the changes in inflammatory and OS marker concentrations[31]. Heterogeneity between studies was assessed by Cochran's Q test, and $P \leq 0{\cdot}10$ was considered statistically significant. The I^2 test was also performed to evaluate the magnitude of heterogeneity, which was considered high if $I^2 \geq 50{\cdot}0\,\%$. The pooled estimates of the weighted mean differences (WMD) for NO, GSH, TAC and MDA and the estimates of the standard mean differences (SMD) for hs-CRP, adiponectin and visfatin between *n*-3 fatty acid supplementation and control groups were calculated using the random effects model[32], since significant heterogeneity among studies was identified in preliminary models. This approach also provided a more conservative assessment of the average effect size. Subsequently, sensitivity (subgroup) analyses were conducted by including variables to determine how much of the between-study difference could be explained by these variables. Publication bias was not accessed through funnel plot asymmetry, since according to the Cochrane Handbook[21], it should be used when there are at least ten studies included in the meta-analysis, and all outcomes evaluated in the present meta-analysis had, at maximum, seven studies included. The statistical analyses were performed using Review Manager 5® software. Significant values were considered as $P < 0{\cdot}05$ with the 95 % CI.

Results

Study characteristics

Fig. 1 shows the flow chart of the study selection process. The electronic database search identified 323 studies. After analysis of the titles, 210 abstracts were maintained and, subsequently, 178 were excluded after abstract reading. In this stage, the Kappa coefficient of agreement between the two investigators was 0·933. Eighteen RCT were selected for full-text reading after analysis of the abstracts; eight studies were excluded for not fulfilling the inclusion criteria, and thus, ten[33–42] randomised clinical trials were included in this systematic review with meta-analysis.

One study[42] was included as two independent reports because the findings were described by different interventions of interest (flaxseed and fish oils). The total sample size of all studies comprised 381 patients diagnosed with PCOS (nine studies[33–41] diagnosed by the Rotterdam criteria and one study[42] by the National Institutes of Health criteria) with a mean age of 27·05 years and 384 controls with a mean age of 27·10 years. All the included studies were parallel RCT that comprised 6–12 weeks of follow-up. Subgroup analyses based on sources of *n*-3 fatty acids (fish oil *v.* flaxseed and *n*-3 fatty acids *v.* *n*-3 fatty acids plus vitamins D and E) were performed to check the sources of heterogeneity between the studies. However, no significant differences were found between subgroups after the tests.

The included studies were grouped according to the assessed outcome: (1) inflammatory[33–39,41,42] and (2) OS[33,34,36,40,41] marker changes, when supplemented with just *n*-3 fatty acids[32,35–39,42] or co-supplemented with vitamin D[34] or E[40,41] in patients with PCOS. Table 1 presents the characteristics of the studies included. Online Supplementary material 2 shows the changes in biomarkers of the included studies. Online Supplementary material 3 presents the laboratory characteristics of the participants before and after *n*-3 fatty acid intervention.

Inflammatory markers – high-sensitivity C-reactive protein

Of the ten selected studies, six[33,34,36,37,41,42] investigated the effects of *n*-3 fatty acid supplementation on the circulating concentrations of hs-CRP compared with a placebo group. The mean follow-up time of the studies was 9 weeks (6–12 weeks), and they included thirty-four to sixty participants (mean age

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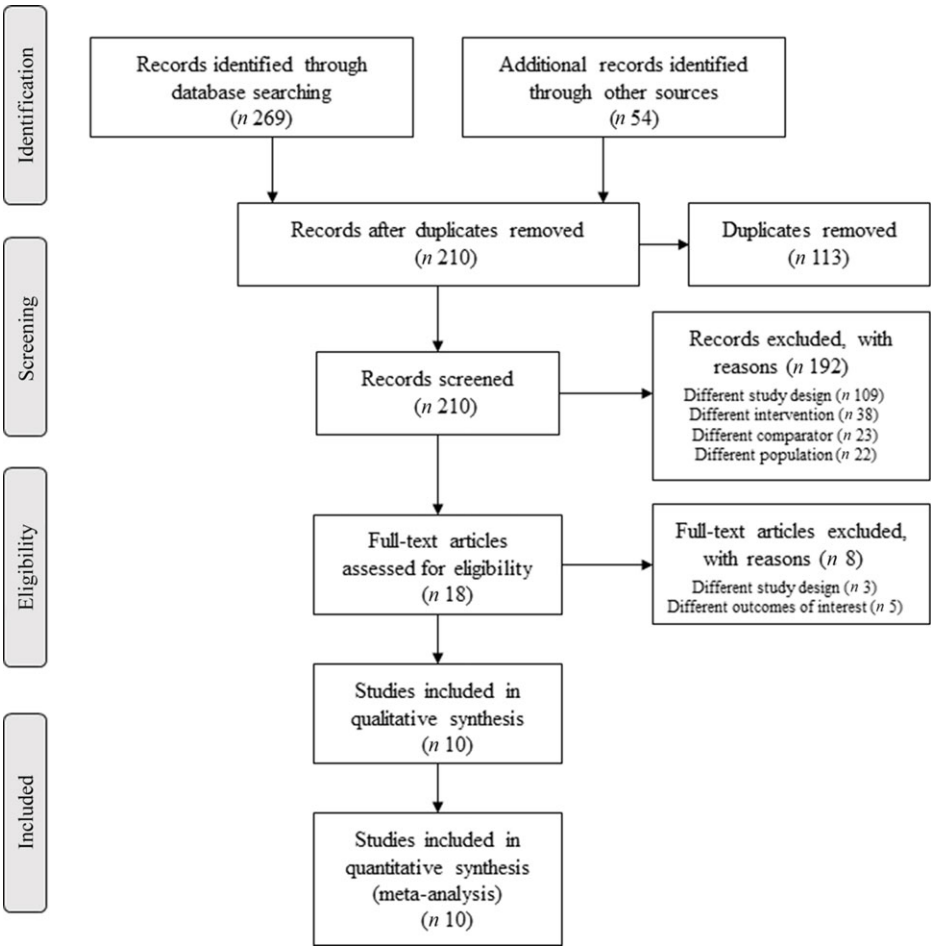

Fig. 1. Flow chart of the literature search and the study selection process.

28 years). The mean BMI was 29·57 kg/m^2, and one study[42] used National Institutes of Health criteria to diagnose PCOS. Two studies[33,42] had fish oil as the intervention, three[36,37,42] with flaxseed oil and two with *n*-3 fatty acid co-supplementation with D[34] and E[41] vitamins. One study[34] did not report the placebo used, four studies[33,36,37,41] used paraffin oil and one[42] soya oil as the placebo.

Overall, *n*-3 fatty acid intervention decreased significantly hs-CRP concentrations when compared with the control group (SMD –0·29 (95 % CI –0·56, –0·02) mg/l; $I^2 = 38$ %, $P_{\text{for heterogeneity}}$: 0·14; Fig. 2(a)).

Inflammatory markers – adiponectin

A total of four studies[35,37,38,42] were included in meta-analysis that evaluated the effects of *n*-3 fatty acid supplementation on the circulating concentrations of adiponectin compared with a placebo group. The studies included 34–195 participants (mean age 27 years), and one study[42] used National Institutes of Health criteria to diagnose PCOS. The mean follow-up time of the studies was 8 weeks (6–12 weeks), and the mean BMI was 27·76 kg/m^2. Four studies[35,37,38,42] had fish oil as the intervention and one[42] used flaxseed oil as *n*-3 fatty acid supplementation.

The pooled data from four studies showed a significant effect of *n*-3 fatty acid supplementation on increasing adiponectin concentrations (weighted mean difference 1·42 (95 % CI 1·09, 1·76) ng/ml; $I^2 = 5$ %, $P_{\text{for heterogeneity}}$: 0·38; Fig. 2(b)).

Inflammatory markers – visfatin

The meta-analysis that evaluated the concentrations of visfatin, under *n*-3 fatty acid supplementation when compared with a placebo group, included two studies[38,39]. Both studies used the Rotterdam criteria for PCOS diagnosis, and the intervention was composed of 180 mg of EPA and 120 mg of DHA. The mean follow-up time was 8 weeks. In addition, the mean age and BMI were 27·2 and 30·12 kg/m^2, respectively.

In the meta-analysis, we did not observe a decrease of visfatin concentrations in the *n*-3 fatty acid supplementation group when compared with the control group. (weighted mean difference –0·00 (95 % CI –0·05, 0·05) ng/ml; $I^2 = 0$ %, $P_{\text{for heterogeneity}}$: 0·86; Fig. 2(c)).

Oxidative stress markers – nitric oxide

Four studies[33,34,36,41] were included in the meta-analysis that evaluated serum levels of NO in the *n*-3 fatty acid group

Table 1. Characteristics of the studies investigating inflammation and oxidative stress markers concentrations from *n*-3 fatty acid supplementation (Mean values and standard deviations)

Author and country	Study duration (weeks)	Mean age (years)						Group characteristics		Baseline and at end-of-trial BMI (kg/m^2)				Diagnosis criteria of PCOS	Interest outcomes
		Intervention			Control					Intervention		Control			
		No. of patients	Mean	SD	No. of patients	Mean	SD	Intervention	Control	Mean	SD	Mean	SD		
Amini *et al.* (2018)[33]; Iran	12	27	27·2	6·2	27	28·9	4·2	2000 mg/d of fish oil per d	100 mg of paraffin oil per d	25·8 25·6	4·8 4·7	25·9 25·8	4·3 4·4	Rotterdam criteria	hs-CRP, NO, TAC, GSH and MDA
Jamilian *et al.* (2018)[34]; Iran	12	30	26·8	4·4	30	25·1	3·7	2000 mg/d of fish oil plus 50 000 IU of vitamin D every 2 weeks	Not reported	27·4 27·1	3·9 3·8	27·1 27·0	7·0 7·1	Rotterdam criteria	hs-CRP, NO, TAC, GSH and MDA
Mejia-Montilla *et al.* (2017)[35]; Venezuela	12	97	23·6	3·4	98	23·3	3·9	180 mg of EPA and 120 mg of DHA per d	1000 mg of paraffin oil per d	26·4 25·7	3·0 3·1	26·0 26·2	2·7 2·8	Rotterdam criteria	Adiponectin
Mirmasoumi *et al.* (2017)[36]; Iran	12	30	28·4	6·4	30	27·0	3·2	1000 mg/d of flaxseed oil	500 mg of paraffin oil per d	26·9 26·9	5·1 5·0	26·7 26·6	5·3 5·4	Rotterdam criteria	hs-CRP and NO
Mohammadi *et al.* (2012)[37]; Iran	8	30	27·3	4·27	31	27·7	4·53	720 mg EPA and 480 mg DHA per d	Four capsule contained 500 mg paraffin oil	28·7 28·6	3·21 3·30	28·8 28·8	2·90 2·94	Rotterdam criteria	hs-CRP and adiponectin
Nadjarzadeh *et al.* (2015)[38]; Iran	8	39	26·9	5·9	39	26·9	5·0	540 mg EPA and 360 mg DHA per d	1000 mg of paraffin oil per d	31·5 31·1	5·7 5·9	31·8 31·8	3·9 3·7	Rotterdam criteria	Visfatin and adiponectin
Rafraf *et al.* (2013)[39]; Iran	8	30	27·3	4·3	31	27·7	4·5	720 mg EPA and 480 mg DHA per d	Paraffin oil	28·7 28·6	3·2 3·3	28·7 28·8	2·9 2·9	Rotterdam criteria	Visfatin
Rahmani *et al.* (2016)[40]; Iran	12	34	24·9	5·5	34	26·6	5·6	400 mg of ALA plus 400 IU of vitamin E per d	Not reported	28·4 28·2	4·4 4·6	29·0 29·0	6·5 6·5	Rotterdam criteria	TAC, GSH and MDA
Talari *et al.* (2018)[41]; Iran	12	30	Not reported		30	Not reported		400 mg of ALA plus 400 IU of vitamin E per d	Paraffin oil	Not reported		Not reported		Rotterdam criteria	hs-CRP and NO
Vargas *et al.* (2011)[42]; USA	6	17	29·4	1·6	17	28·9	1·0	545 mg ALA per d	Soya oil	35·0 35·1	2·5 2·6	33·2 33·3	1·8 1·7	National Institutes of Health criteria	hs-CRP and adiponectin
Vargas *et al.* (2011)[42]; USA	6	17	31·7	1·9	17	28·9	1·0	358 mg EPA plus 242 mg DHA per d	Soya oil	36·3 36·6	1·9 1·8	33·2 33·3	1·8 1·7	National Institutes of Health criteria	hs-CRP and adiponectin

PCOS, polycystic ovary syndrome; hs-CRP, high-sensitivity C-reactive protein; NO, nitric oxide; TAC, total antioxidant capacity; MDA, malondialdehyde; IU, international units; ALA, α-linolenic acid.

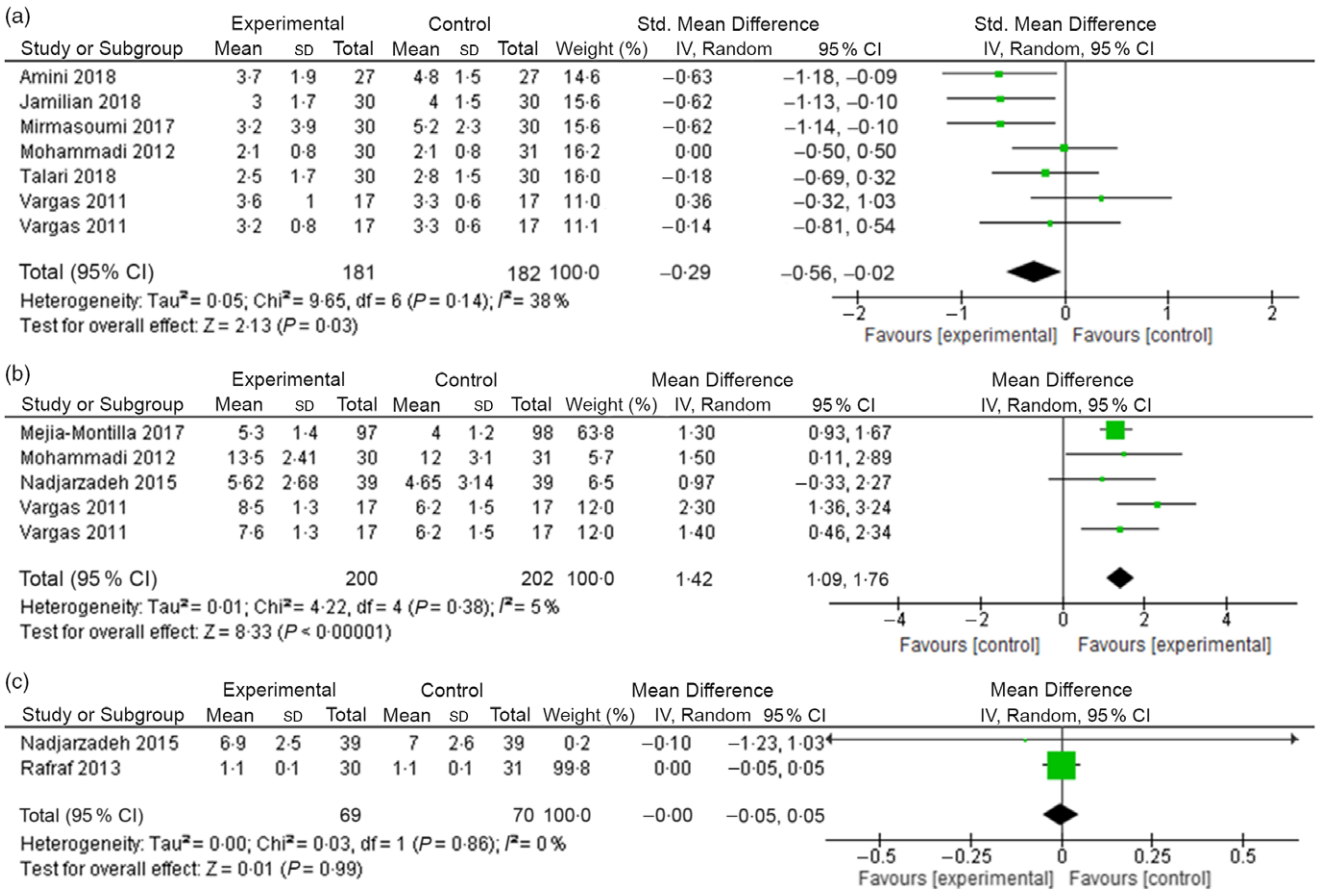

Fig. 2. (a) Change in high-sensitivity C-reactive protein (mg/l) concentrations due to *n*-3 fatty acid supplementation. (b) Change in adiponectin (ng/ml) concentrations due to *n*-3 fatty acid supplementation. (c) Change in visfatin (ng/ml) concentrations due to *n*-3 fatty acid supplementation.

compared with a placebo. The mean follow-up time of the studies was 12 weeks, and they included 54–68 participants (mean age 27 years). The mean BMI was 26·57 kg/m^2, and all studies used the Rotterdam criteria for PCOS diagnosis. Two studies[36,41] used flaxseed oil in the intervention group, and the Talari *et al.* study co-supplemented the group with vitamin E[41]. Another two studies used fish oil as *n*-3 fatty acid supplementation, and Jamilian *et al.*[34] co-supplemented with vitamin D. Three studies[33,36,41] used paraffin oil as the placebo.

n-3 fatty acid supplementation did not decrease serum levels of NO in the intervention group, when compared with the placebo group (SMD –0·01 (95 % CI –0·69, 0·67) (mmol/l); $I^2 = 85$ %, $P_{\text{for heterogeneity}}$: 0·0002; Fig. 3(a)).

Oxidative stress markers – GSH, malondialdehyde and total antioxidant capacity

For meta-analysis of these OS markers, three studies[33,34,40] were included that evaluated the influence of *n*-3 fatty acid supplementation on serum levels of GSH, MDA and TAC when compared with the placebo group. The mean follow-up of the studies was 12 weeks, and they included 54–68 participants (mean age 27 years). The mean BMI was equal to 27·19 kg/m^2, and all studies used Rotterdam criteria for PCOS diagnosis. Two studies used fish oil as *n*-3 fatty acid supplementation, and the study by Jamilian *et al.*[34] co-supplemented with vitamin D. Another study[40] included used flaxseed oil plus vitamin E as the intervention. One study[33] reported using paraffin oil as the placebo.

We did not observe a significant decrease in serum levels of GSH, MDA or TAC in the group *n*-3 fatty acids when compared with the placebo group (GSH – SMD 0·24 (95 % CI –0·42, 0·90) (mmol/l); $I^2 = 80$ %, $P_{\text{for heterogeneity}}$: 0·007; Fig. 3(b); MDA – SMD –0·17 (95 % CI –1·01, 0·66) (mmol/l); $I^2 = 87$ %, $P_{\text{for heterogeneity}}$: 0·0004; Fig. 3(c) and TAC – SMD 0·27 (95 % CI –0·45, 1·00) (mmol/l); $I^2 = 83$ %, $P_{\text{for heterogeneity}}$: 0·003; Fig. 3(d)).

Risk of bias and quality of the body of evidence

The risk of bias in the included studies is summarised in Fig. 4. In domain 1, regarding the process of randomisation, one study presented a high risk of bias because it did not report a randomisation process on methods. Domain 2, the risk of bias due to deviations from the intended interventions, was classified as low because no studies presented a possible negative effect of assignment to intervention. Domain 3, regarding the risk of bias due to missing outcome data, was classified as low risk related to the availability of data from 95 % of the participants in all included studies. In the risk of measuring the result – domain 4, we did not identify a likely directional bias, that is,

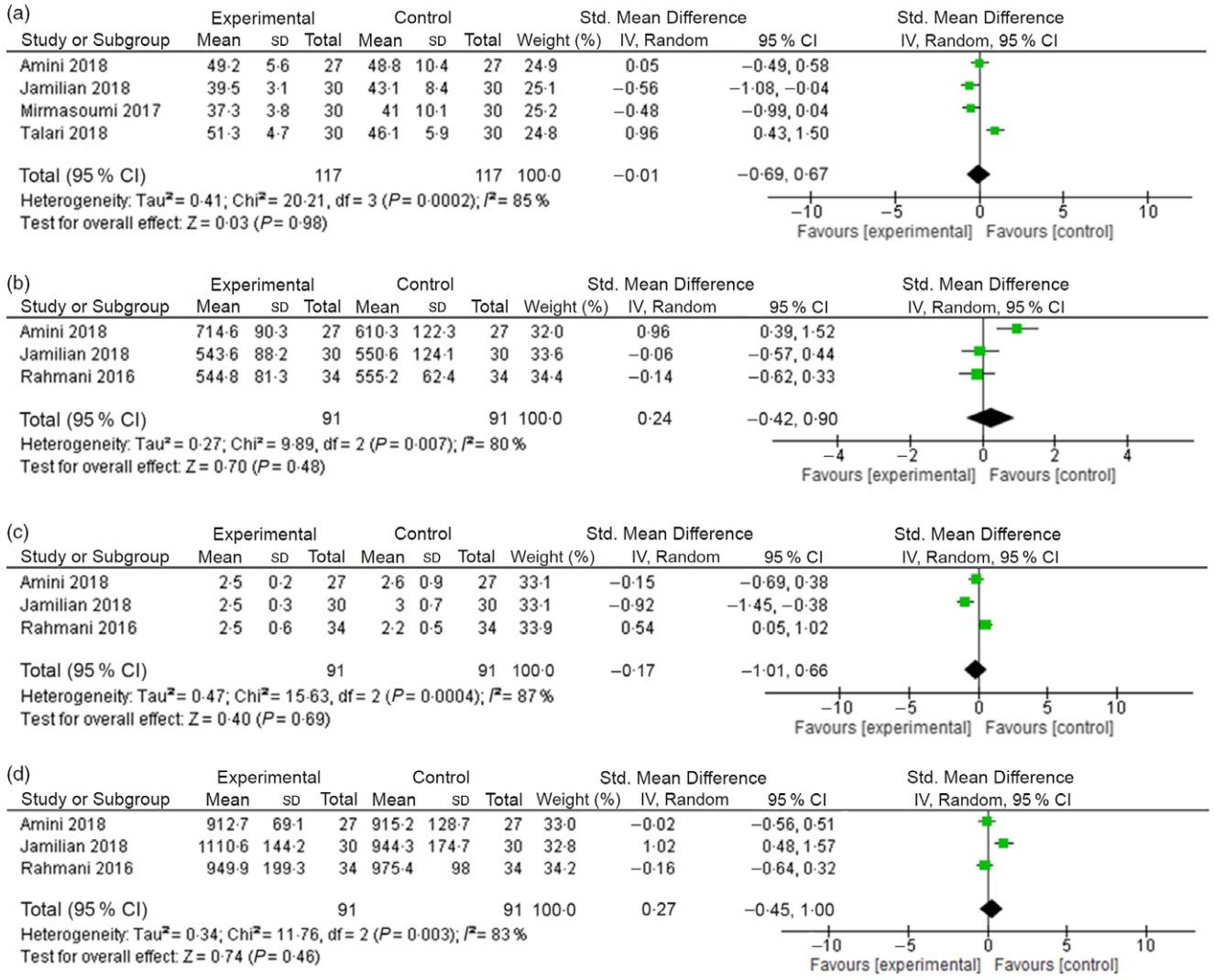

Fig. 3. (a) Change in nitric oxide (NO) (mmol/l) levels due to *n*-3 fatty acid supplementation. (b) Change in GSH (mmol/l) levels due to *n*-3 fatty acid supplementation. (c) Change in malondialdehyde (mmol/l) levels due to *n*-3 fatty acid supplementation. (d) Change in total antioxidant capacity (mmol/l) levels due to *n*-3 fatty acid supplementation.

measuring results that are not suitable for the outcomes that the authors planned to evaluate – directed to one of the interventions. For domain 5 – risk of selection of the reported result, we evaluated this domain as low risk because no individual study presented any evidence of selective reporting bias of results. Overall, eight[34–36,38–42] studies were evaluated as some concerns related to the intervention, one study[33] was evaluated as low risk and another study[37] as high risk, associated with evaluation of domains 1 and 2.

The quality of the body of evidence for each outcome of the current systematic review is described in online Supplementary material 4. The directness of evidence was classified as high considering the precision of the main effect estimates of interest. The results were highly heterogeneous, and no dose–response effect could be established. For one of the outcomes evaluated (inflammation biomarker changes), a clinically relevant effect of great magnitude was demonstrated. In summary, the quality of the body of evidence of this systematic review was classified as moderate.

Discussion

The present systematic review with meta-analysis of RCT analysed the role of *n*-3 fatty acid supplementation in PCOS patients considering plasma concentrations of inflammatory and OS markers. Intervention studies that compared *n*-3 fatty acid supplementation with a placebo were associated with no difference in visfatin levels nor in OS markers. However, it was observed that *n*-3 fatty acid supplementation significantly increased the circulating concentrations of adiponectin and decreased hs-CRP levels in PCOS patients when compared with placebo.

PCOS results in a pro-inflammatory state, and the development of metabolic dysfunction is supported by chronic low-grade inflammation. This state may be associated with the accumulation of visceral fat, glucose intolerance, IR and dyslipidaemia, all of which are presented in patients diagnosed with PCOS[43]. We observed in the present systematic review that *n*-3 fatty acid supplementation can reduce the levels of hs-CRP, an inflammatory maker known to be increased in

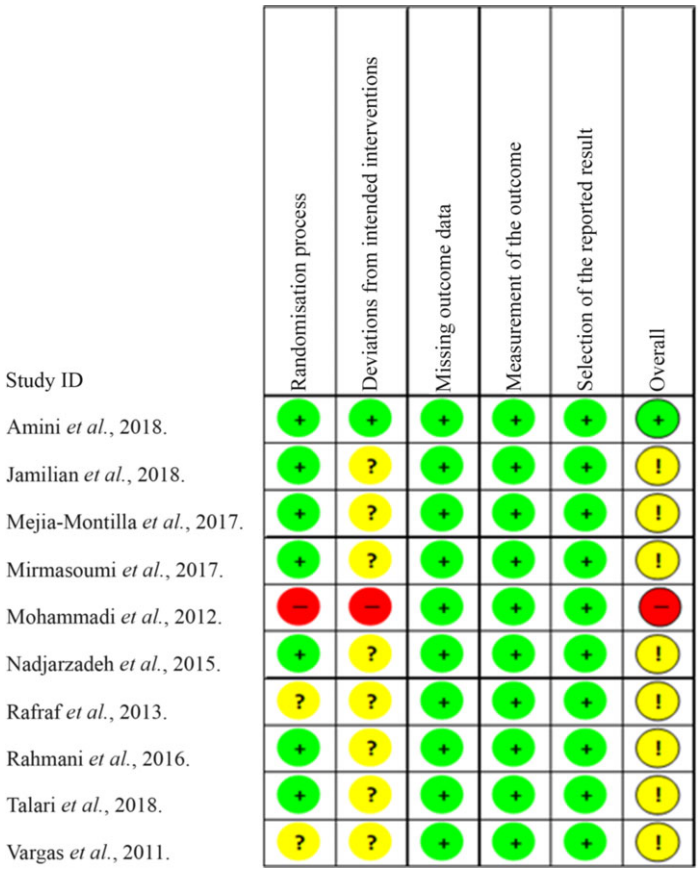

Fig. 4. Cochrane Collaboration bias risk graph. +, Low risk; ?, some concerns; −, high risk.

women with PCOS[43], which can predict a cardiovascular event[44]. hs-CRP is an acute-phase protein produced by the liver following stimulation by IL-6, the endocrine cytokine originating from adipocytes[45,46].

The mononuclear cells of women with PCOS are in an activated state, as evidenced by increased plasma hs-CRP, which may play a role in obesity[44]. A meta-analysis of thirty-one articles (*n* 3648 women) that aimed to evaluate the status of serum inflammatory markers in women with PCOS showed that circulating hs-CRP was 96 % higher in women with PCOS compared with controls (95 % CI 71 %, 122 %, $P < 0{\cdot}0001$) and it was associated with a high prevalence of obesity in the women with PCOS in the studies analysed[47]. *n*-3 Fatty-acid-derived mediators have been implicated in the resolution of inflammation, with a significant protective effect by inhibition of NF-κB activity, which rises in many inflammatory diseases[48]. In fact, our results corroborate the findings of a systematic review and meta-analysis that aimed to evaluate the effect of *n*-3 fatty acid supplementation on serum levels of inflammatory biomarkers in patients on haemodialysis. This study showed a significant decrease in serum levels of hs-CRP in the *n*-3 fatty acid supplementation group when compared with placebo (SMD −2·09; 95 % CI −3·62, −0·56, $P < 0{\cdot}05$)[49].

The state of low-grade inflammation is the result of the accumulation of visceral fat because this tissue is capable of producing cytokines, chemokines and other adipokines, such as adiponectin, which act, directly or indirectly, as mediators of systemic inflammation[50]. *n*-3 Fatty acid supplementation may be associated with a decrease in inflammation and in pro-inflammatory cytokine levels by stimulating the secretion of anti-inflammatory adipokines (such as adiponectin)[51]. Moreover, these effects of *n*-3 fatty acids could be related to their influence on signalling pathways that regulate the expression of genes encoding pro-inflammatory cytokines[52–54]. This regulation could be associated with NF-κB, one of the main transcription factors involved in up-regulation of the genes encoding pro-inflammatory cytokines, adhesion molecules and cyclo-oxygenase-2[53]. In fact, EPA decreases lipopolysaccharide-induced NF-κB activation in monocytes, and DHA reduces NF-κB activation in response to lipopolysaccharide in macrophages and dendritic cells[52–54]. Besides, the influence of EPA and DHA on NF-κB activation involves PPAR-γ, resulting in inhibition of NF-κB activation and reduced production of the pro-inflammatory cytokines TNF and IL-6 due to lipopolysaccharide stimulation[51].

In women, adiponectin controls steroidogenesis of ovarian granulosa and theca cells, oocyte maturation and embryo development beyond insulin sensitising and known anti-inflammatory effects, which also modulates folliculogenesis and androgen synthesis in ovaries[55]. Its receptor was also found in endometrial and placental cells, suggesting that this adipokine might play a crucial role in fetal growth, trophoblast invasion and

embryo implantation[55]. *n*-3 Fatty acids may improve insulin sensitivity by enhancing the production and secretion of anti-inflammatory adipokines, such as adiponectin, consequently reducing inflammation and proinflammatory cytokines[56]. A previous meta-analysis that assessed the effectiveness and safety of *n*-3 fatty acids for patients with PCOS included nine RCT (*n* 591 patients) and observed that, compared with the control group, *n*-3 fatty acids may improve adiponectin levels (weighted mean difference 1·34; 95 % CI 0·51, 2·17; $P = 0·002$)[25]. Plasma adiponectin concentrations correlate negatively with body weight and BMI in women with or without PCOS. Moreover, hypoadiponectinaemia is associated with higher degrees of hyperinsulinaemia and IR; hence, it could be related not only to obesity but also to the metabolic alterations that characterise PCOS[57].

It has been reported that circulating levels of visfatin and its gene expression were increased in women with PCOS, compared with controls matched by BMI and age[58]. Visfatin has been identified as a protein of 52 kDa produced by the bone marrow, liver and muscle and, during pregnancy, by the epithelium of the amniotic membrane, chorionic trophoblast and decidua[58]. Years later, it was proven that visfatin is produced by adipocytes and has insulin-mimetic action[59]. Visfatin has the ability to stimulate proinflammatory activity by enhancing TNF and IL-6 secretion, which further increases IR. On the other hand, the insulin-like effect of visfatin is not sufficient to counteract IR in conditions such as type 2 diabetes mellitus and PCOS, despite its high serum levels[59]. In fact, a meta-analysis with seventeen studies (1341 subjects – 695 cases and 646 controls) showed that visfatin levels are higher in women with PCOS compared with non-PCOS controls; furthermore, the study did not indicate a correlation between high visfatin levels and BMI, homoeostatic model assessment for IR or total testosterone levels in PCOS patients when compared with controls[59]. The changes in visfatin levels induced by *n*-3 fatty acids vary according to the type of dietary fat, supporting the hypothesis that visfatin up-regulation by EPA could be another mechanism by which *n*-3 fatty acids may improve insulin sensitivity[60].

OS is characterised by the imbalance between the capacity of the body to neutralise free radical molecules, using antioxidant enzymes, and their production. Excessive reactive oxygen species (ROS) generation promotes inflammation by activation of redox and inflammatory signalling pathways such the NF-κB pathway[57]. PCOS patients demonstrate OS due to hyperglycaemia, IR and chronic inflammation. Moreover, higher levels of NEFA lead to excess production of ROS[61]. A cross-sectional study suggested that excess androgen increases the generation of ROS from leucocytes, p47phox gene expression and the formation of MDA. OS increases chronic inflammation and vice versa[62]. Furthermore, OS and inflammation in the ovaries play an important role in the pathogenesis of PCOS and cause the development of atherosclerotic lesions in the ovary[63].

The mechanism by which OS could be reduced following *n*-3 fatty acid supplementation is still unclear, but it has been assumed that these effects may occur through immunomodulation and decreased leucocyte activation[64,65]. In this context, it is known that activated immune cells produce cytokines that consequently promote ROS generation. Moreover, EPA and DHA are effective as superoxide scavengers in an unsaturation-dependent manner, given the high unsaturation level of *n*-3 fatty acids[66].

PCOS patients have an increased risk of developing the MetS, which may be related to OS and cardiovascular events. While obesity can be a putative factor leading to the MetS, the relationship between the MetS and PCOS is attributed mainly to IR[67]. A prospective controlled study evaluated whether the presence of the MetS in PCOS patients could influence endoplasmic reticulum stress markers, OS and leucocyte endothelium interaction. The data highlight that ROS production, and therefore OS, is enhanced in PCOS, and it is associated with the presence of the MetS, which can increase CVD risk. Moreover, PCOS subjects with the MetS exhibited enhanced levels of proinflammatory cytokines, and these cytokines were correlated with homoeostatic model assessment for IR, reinforcing the importance of the MetS to IR and inflammation in PCOS[68].

The *n*-3 fatty acids are also known to improve the TAC and various associated signalling pathways, probably suppressing lipid peroxidation, which is represented by MDA[69]. MDA levels are significantly higher in PCOS patients and can be considered an important marker for OS[70]. In the present meta-analysis, no relationship was found between *n*-3 fatty acid supplementation and the decline of OS biomarker levels in PCOS. Another systematic review and meta-analysis that aimed to summarise the findings of RCT examining the effects of *n*-3 fatty acids on OS markers in healthy subjects, including thirty-nine trials (*n* 2875 participants), evidenced a significant increase of serum TAC and decrease of MDA in the intervention group when compared with the placebo group[71]. However, the study did not find significant results for NO and GSH, according to our results.

Although the literature search was conducted using multiple databases, without language restriction and following the protocol established in accordance with standardised recommendations, the present meta-analysis has some limitations. The lack of data on the actual consumption of *n*-3 fatty acids must be considered because it may influence inflammatory and OS markers. In addition, none of the studies included in the meta-analysis presented intention-to-treat analysis, a statistical approach that is usually associated with more conservative results. Moreover, only one study[33] showed a low risk of bias due to imprecision, suggesting that our results should have external validity.

In conclusion, the present systematic review with meta-analysis of RCT suggests that *n*-3 fatty acid supplementation in patients with PCOS was associated with a moderate increase in the concentrations of adiponectin and hs-CRP and no effect on the concentrations of visfatin or OS markers when compared with a placebo. Caution must be taken in interpreting these results because important sources of heterogeneity were found in the meta-analyses of *n*-3 fatty acid supplementation. Therefore, future RCT are necessary to confirm these findings.

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The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520003207

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