The Effect of n-3 Polyunsaturated Fatty Acid Supplementation on Androgen Status in Patients with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Clinical Trials

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Key words
- n-3 polyunsaturated fatty acids
- polycystic ovary syndrome
- systematic review
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Abstract

The anti-androgenic role of n-3 polyunsaturated fatty acids (PUFAs) among patients with polycystic ovary syndrome (PCOS) has recently been proposed. The present study aimed to systematically review clinical trials assessing the effects of n-3 PUFAs consumption on androgen status among adult females with PCOS. PubMed, ISI Web of Science, Google Scholar, and Scopus were searched up to December 2015. Clinical investigations assessing the effect of n-3 PUFAs on adult females with PCOS were included. Mean ± standard deviation of change in serum total testosterone, sex hormone binding globulin (SHBG), and dehydroepiandrostrone sulfate (DHEAS) were extracted. Eight clinical trials with 298 participants were eligible. Meta-analysis showed that n-3 PUFAs supplementation marginally reduces total testosterone (mean difference [MD]: −0.19 nmol/l; 95 % CI: −0.39 to 0.00; p = 0.054), but not SHBG (MD: 1.75 nmol/l; 95 % CI: −0.51 to 4.01; p = 0.129) or serum DHEAS levels (Hedes' g: −0.11 nmol/l; 95 % CI: −0.29 to 0.06; p = 0.19) among adult females with PCOS. Subgroup analyses showed that only before-after studies (Hedges' g: 0.15; 95 % CI: −0.27 to −0.04; p = 0.01) and long-term interventions (> 6 weeks) (Hedges' g: −0.17; 95 % CI, −0.29 to −0.05; p = 0.004) had reducing effects on serum DHEAS levels. The majority of long-term trials utilized a single group design (no control group). It does not appear that n-3 PUFAs supplementation significantly affects the androgenic profile of females with PCOS; however, some before-after and long-term intervention studies show reduced DHEAS levels. Future studies incorporating double blinded placebo controlled clinical trials with long follow-up periods are warranted.

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Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine metabolic disorder that occurs in females, in part due to genetic and environmental factors [1]. It is characterized by the presence of hyperandrogenism, menstrual cycle disturbances, infertility, and hirsutism [2], and is strongly associated with insulin resistance, metabolic syndrome (MetS), abdominal obesity, type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and dyslipidemia [3–7]. The prevalence of PCOS is highly variable from 2 to 28 % in different reports throughout the world [8–10].

It is believed that diet, exercise, and lifestyle modification can play an important role in PCOS control [11–14]. Recent studies have assessed the effect of calorie restriction, lower intake of trans and saturated fats, refined carbohydrates [15], and high dietary fiber on the management of PCOS [2, 14, 16]. Results from these studies contribute to a recommendation that weight loss, reduced caloric intake, adequate essential nutrient intake, and overall healthy food choices may help with PCOS management [17, 18].

Essential polyunsaturated fatty acids (PUFAs), particularly long-chain (LC) n-3 PUFAs, have been studied for their metabolic and health effects on females with PCOS [11, 19, 20]. Recent work indicates that n-3 PUFAs enhance insulin sensitivity and favorably affect hyperinsulinemia, plasma triglyceride, and liver fat content [21–23]. It has also been proposed that n-3 PUFAs might decrease inflammation and possibly obesity [24, 25]. Clinical trials reveal the possible anti-androgenic role of n-3 PUFAs along with their metabolic effects among females with PCOS [26, 27]. However, results from these studies are inconsistent [11, 19, 20, 24, 26, 28, 29], perhaps due to a decrease in the n-6:n-3 PUFAs ratio by competition or displacement [11, 30].

Although the effects of n-3 PUFAs on the metabolic profile have been extensively studied, we are not aware of any systematic review summarizing the published data regarding their effects on androgen levels among females with PCOS. Further, differences between the effects of essential n-3 PUFAs from plant origins [α-linolenic acid (ALA)] vs. marine-derived long chain n-3 PUFAs [eicosapentanoic acid (EPA) and docosahexanoicacid (DHA)] [26] have not been elucidated. The aim of the present study is 2-fold: 1) systematically review clinical trials assessing the effects of n-3 PUFA consumption on androgen levels [testosterone, sex hormone binding globulin (SHBG), and dehydroepiandrostrone sulfate (DHEAS)] among adult females with PCOS, and 2) conduct a meta-analysis to quantify the effect of n-3 PUFA consumption on androgen levels among females with PCOS, and find possible sources of heterogeneity between inconsistent results.

Materials and Methods

Search strategy

A systematic literature search was conducted in medical databases including PubMed, ISI Web of Science, Google Scholar, and Scopus up to December 2015 using the following medical subject headings (MeSH) and non-MeSH keywords: "Fatty Acids, Omega-3", "Eicosapentaenoic Acid", "Docosahexaenoic Acids", "n-3 PUFAs", "n-3 Polyunsaturated Fatty Acid", "alpha-Linolenic Acid", "fish oil", "Nuts", "nutrient", "Food" in combination with "Polycystic Ovary Syndrome", "Ovarian Hyperstimulation Syndrome", "PCOS". No restriction was set on time of publication, language, or study design. The reference list of related articles were hand-searched for additional relevant studies. Title and abstracts were screened for relevant studies by 2 authors (MH, ASA), and discrepancies were resolved through group discussion with 3 other authors (BI, RG, GA).

Inclusion criteria

Published studies were included if they met the following criteria: 1) original article, 2) clinical trial in design, 3) conducted among adult females, 4) use of any type of n-3 PUFA supplements [fish oil, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or α-linolenic acid (ALA)], or foods rich in n-3 PUFAs as the main independent variable, and 5) assessed serum total testosterone, SHBG, and/or DHEAS levels as outcome measures among adult females with PCOS.

Quality assessment

To determine the level of evidence, the studies were judged according to the quantitative 5-point Jadad scale. Clinical trials were evaluated on randomization (extra point for randomization generation and allocation concealment), double-blinding (extra point for describing the method of double-blinding) and reporting of withdrawals and dropouts with number and reasons. High-quality trials were defined as those that scored > 2, and low-quality trials were those scoring ≤ 2 out of maximum possible score of 5 [31].

Data extraction

First author's last name, publication year, country in which the study was conducted, study design, duration of follow-up, number of individuals in intervention and control groups, age of participants, and name and composition of the study diets were extracted from eligible studies. Mean ± standard deviation (SD) of change in serum total testosterone, SHBG, and DHEAS levels for intervention and control groups were presented by 3 studies [19, 26, 29]. One study conducted by Phelan et al. [11] did not report mean ± SD change of the above-mentioned androgen levels, therefore the values were extracted from the figures.

We calculated correlation coefficients (r) between, before and after the intervention period, using one of the articles that reported changes in mean and SD of hormone levels [19] and used it to calculate the SD mean changes and their corresponding SDs for other studies. Three other studies reported their results as mean difference in the change between intervention and control groups for serum total testosterone and SHBG values; these values were directly used for the meta-analysis [24, 28, 32]. A study by Vargas et al. [26] compared the effects of marine and plant originated n-3 PUFAs supplementation with placebo; we extracted 2 effect sizes from this study and included them as 2 separate studies in our meta-analysis.

Statistical analyses

The raw mean difference (MD) and SD of changes in serum total testosterone and SHBG levels between intervention and control groups were used as effect size for the meta-analysis. However, due to different measurement units of DHEAS levels in the studies, we calculated Hedges' g [33, 34] and its corresponding standard error (SE), and used it as effect size to conduct the meta-analysis. Summary mean estimates with their corresponding SDs were derived via a random effects model incorporating study variability [35]. Statistical heterogeneity between studies was evaluated using Cochran's Q-test and I^2 [36]. Subgroup analyses based on study design (controlled clinical trials vs. before-after studies), follow-up period (long-term vs. short-term), and sources of n-3 PUFA supplementation (plant-based vs. essential) were done to check the sources of between study heterogeneity. Source of n-3 PUFA supplementation was evaluated due to a study conducted by Vargas et al. suggesting that plant-based vs. essential n-3 PUFA supplementation may have different metabolic and endocrine effects on females with PCOS [26].

Sensitivity analysis was used to explore the extent to which inferences might depend on a particular study or group of studies. Publication bias was assessed by visual inspection of funnel plots [37]. Formal statistical assessment of funnel plot asymmetry was performed using Egger's regression asymmetry test and Begg's adjusted rank correlation test [38]. Statistical analyses were performed using STATA, version 11.2 software (StataCorp, College Station, TX, USA), with a level of significance of $p < 0.05$.

Results

Our search retrieved 283 articles, 15 of which were selected following screening the titles and abstracts. After reading full-texts, 7 articles were excluded because they did not meet the inclusion criteria: 5 articles did not measure androgen levels among adult females with PCOS [39–43], one article evaluated the effect of phytoestrogens of soybean on hormone levels among females with PCOS [44], and one article was not published as an original article [only its abstract was available in the American Society for Reproductive Medicine (ASRM) proceedings; we did not have luck when attempting to contact the authors] [45].

Eight clinical trials were eligible to be included in the systematic review and meta-analysis. One study was included in the meta-analysis as 2 trials because it assessed the effects of both plant- (flaxseed oil) and marine- (fish oil) originated n-3 PUFAs in comparison with soybean oil [26]. The study selection process is illustrated in **Fig. 1**.

Characteristics and main outcomes of the included studies are presented in **Table 1**. Two of the included studies examined the effect of n-3 PUFA supplementation on serum total testosterone, SHBG [11,24], and DHEAS values [11] by using a randomized placebo controlled crossover design. Three studies assessed the effect of n-3 PUFA supplementation [26,32] and replacement of dietary fat with walnut [19], by using a randomized placebo controlled parallel design. Three investigations [20,28,29] conducted a before-after study design. Among the 8 eligible studies, 4 were conducted in the United States [19,20,26,28], one in Australia [24], one in Ireland [11], one in Turkey [29], and one in Iran [32]. In total, the 8 studies included 298 adult females with PCOS. The age of participants ranged from 20 to 45 years. Duration of studies ranged from 6 to 24 weeks (wks). Dose of n-3 PUFA supplementation (EPA + DHA) ranged from 1.5 g/d [29] to 4 g/d [24]. The amount of daily nut consumption (ALA) ranged from 36 g/1800 kcal [19] to 48 g/800 kcal [20]. There were no reports of adverse effects of n-3 PUFA supplementation in the studies evaluated. All studies advised their participants to maintain their usual dietary and activity habits **Table 1**.

Assessment of PCOS

Four studies diagnosed PCOS according to the National Institute of Health (NIH) criterion that is defined as the combination of chronic anovulation and clinical hyperandrogenism and/or hyperandrogenemia [11,19,20,26]. The other 4 studies defined PCOS using Rotterdam criteria, which is characterized by the presence of 2 or more of the following signs: 1) oligo/anovulation, 2) hyperandrogenemia and/or hyperandrogenism, and 3) polycystic ovaries [24,28,29,32].

Assessment of androgen hormone levels

Four studies measured serum hormone levels (total testosterone, SHBG and DHEAS) via the RIA method [19,20,26,29]. The other 4 studies measured androgen values using solid phase RIA, chemiluminescense immunometric assay, electro-chemiluminescenese immunoassay, and ELISA method, respectively [11,24,28,32].

Effect of n-3 PUFAs on serum total testosterone levels

Eight clinical trials with a total of 298 females with PCOS evaluated the effect of n-3 PUFAs on serum total testosterone concentration [11,19,20,24,26,28,29,32]. Our meta-analysis showed that n-3 PUFA supplementation marginally reduces total testosterone values [mean difference (MD): −0.19 nmol/l; 95% confidence interval (CI): −0.39 to 0.00; p=0.054].

Significant heterogeneity was found between the effect sizes of included studies (Cochrane Q-test, p=0.062, I^2=46.2%) (**Fig. 2**). To find the source of heterogeneity, we categorized the studies based on their designs (cross-over, parallel, uncontrolled before-after studies), source of n-3 PUFAs (marine-derived, vegetable origins) and duration of intervention (≤6 weeks, >6 weeks). Subgroup analyses showed that the effect was not significant in all subgroups. Heterogeneity was detected in before-after studies (Q-test, p=0.004, I^2=82.3%) [20,28,29], marine-derived sources of n-3 PUFAs (Q-test, p=0.013, I^2=65.6%) [19,20,26], and >6 weeks interventions (Q-test, p=0.007, I^2=71.8%) [11,20,24,28,29] (**Table 2**). Heterogeneity disappeared after removing one study by Kuzmanov et al. [28] (Q-test, p=0.145, I^2=0.0% for before-after studies; Q-test, p=0.235, I^2=0.0% for source of n-3 PUFAs; Q-test, p=0.185, I^2=0.0% for studies >6 weeks of follow-up). **Table 2** summarizes the meta-analysis results regarding the effects of n-3 PUFA supplementation on serum total testosterone levels, as well as subgroup

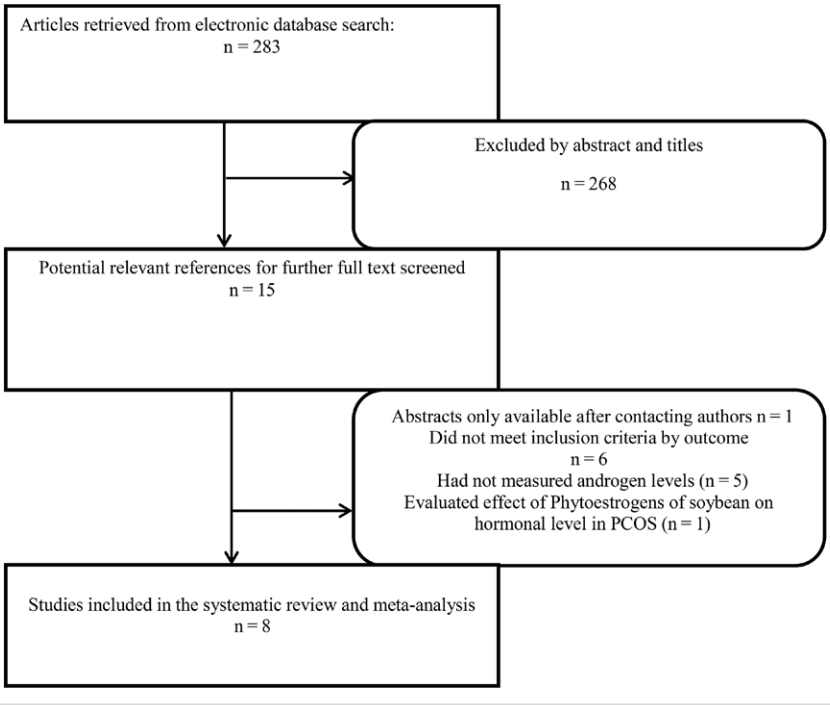

Fig. 1 Flow of study selection process.

Table 1 Randomized clinical trials eligible to be included in the systematic review and meta-analysis.

Author (year), Country	Participants (diagnoses criteria) Mean BMI category	Study design	Diet type		Duration (weeks)	Presented data	Result	JADAD score
			Intervention (number, name, and composition)	Control (number, name, and composition)				
Cussons AJ (2009) Australia (1)	F*: 25 (Rotterdam consensus) Obese	Randomized, crossover, double-blinded	n = 12 n-3 PUFA supplementation 4 g/d:56 %DHA, 27 % EPA	n = 13 Placebo (olive oil 4 g/d)	16 weeks, 8 week washout	Serum testosterone and SHBG levels No data for serum DHEAS level	No significant effect on total T and SHBG	3
Kalgaonkar S (2011) California,USA (2)	F: 31 (National institute of health) Obese	Randomized, double- blinded	n = 17 Replacement of 31 g/1800 kcal of dietary fat with 36 g walnut = 31 g oil: 2.9 g SFA, 4.5 g MUFA, 19.2 g LA, 4.3 g ALA	n = 14 Replacement of 31 g/1800 kcal of dietary fat with 46 g almond = 31 g oil	6 weeks	Serum testosterone, SHBG and DHEAS levels	Walnuts increased SHBG significantly but no effect on total T and DHEAS	3
Kasim-Karkas SE (2004) California, USA (3)	F: 17 (National institute of health) Obese	Uncontrolled before-after design	n = 17 Replacement of dietary fat with PUFA (walnut 48 g/800 kcal). 48 g of walnut: 311 kcal, 19 g LA, 3.3 g ALA.	–	12 weeks	Serum testosterone, SHBG and DHEAS levels	No significant effect on hormonal status	1
Kuzmanov A (2010) USA (4)	F: 12 (Rotterdam consensus) Not reported	Uncontrolled before-after design	n = 12 n-3 PUFA supplementation 2.3 g/d (2.1 g EPA + DHA)	–	12 weeks	Serum testosterone and SHBG levels	No significant effect on hormonal status	1
Nadjarzadeh A (2013) Iran (5)	F: 78 (Rotterdam consensus) Obese	Randomized, double- blinded	n = 39 n-3 PUFA supplementation 3 g/d	n = 39 Placebo (paraffin)	8 weeks	Serum testosterone and SHBG levels	Total testosterone decreased significantly and no effect on SHBG	4
Oner G (2013) Turkey (6)	F:45 (Rotterdam consensus) Normal weight	Uncontrolled before-after design	n = 45 n-3 PUFA supplementation 1 500 mg w-3/d		24 weeks	Serum testosterone, SHBG and DHEAS levels	Total testosterone decreased significantly and SHBG increased significantly	2
Phelan N (2011) Ireland (7)	F: 22 (National institute of health) Obese	Randomized cross-over double-blinded	n = 11 n-3 PUFA supplementation 4 g/d: 2.4 g n-3 PUFA, 1.9 g EPA & DHA EPA/DHA:1.49/1	n = 11 Placebo (olive oil 4 g/d)	12 weeks, 6 weeks washout	Serum testosterone, SHBG and DHEAS levels	No significant effect on hormonal status	4
Vargas (2011) California, USA (8)	F:34 (National institute of health) Obese	double-blinded, placebo- controlled study	n = 17 n-3 PUFA supplementation 3.5 g/d fish oil: 6 capsules/d (each capsule: 358 mg EPA, 242 mg DHA) flaxseed oil: 6 capsules/d (each capsule: 545 mg w-3)	n = 17 Placebo (soybean oil 6 capsules/d)	6 weeks	Serum testosterone, SHBG and DHEAS levels	No significant effect of both interventions on hormonal status compared to control group	3

[a] F: Female

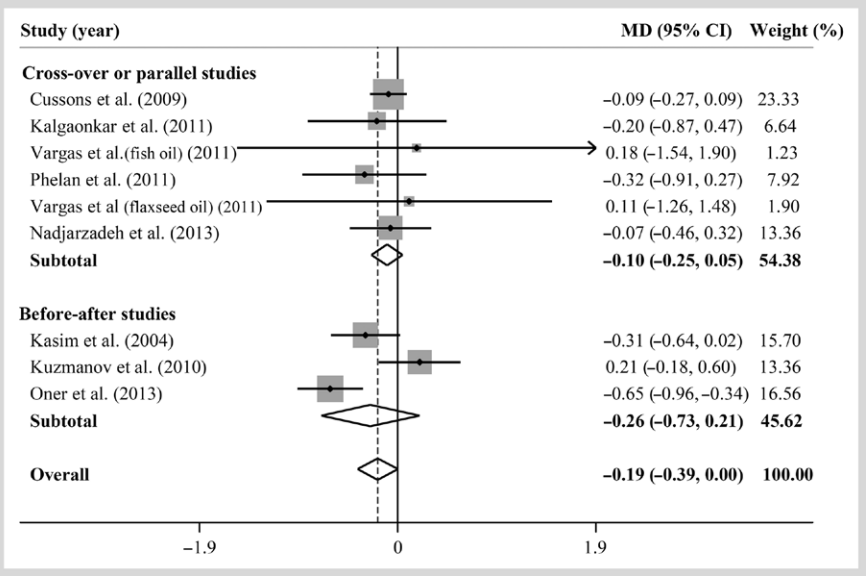

Fig. 2 Forest plot illustrating weighted mean difference in total testosterone concentrations change for all eligible studies as well as for subgroup analysis based on study design (controlled trials vs. before after studies). Analysis was conducted using random effects model.

Table 2 The effect of n-3 PUFAs on serum total testosterone levels stratified by study design, source of n-3 PUFA and duration of intervention in PCOS women.

Total testosterone	Number of effect sizes	Participants	Meta-analysis			Heterogeneity	
			Mean difference	Confidence interval	p-Value	I^2 %	Cochrane Q-test (p-Value)
Study design (scoring)							
Cross-over, parallel (>2)[a]	6	224	−0.10	(−0.25, 0.05)	0.178	0.0	0.974
Before-after (≤2)[b]	3	74	−0.26	(−0.73, 0.21)	0.277	82.3	0.004
Source of n-3 PUFA							
Marine derived	6	216	−0.17	(−0.44, 0.10)	0.208	65.6	0.013
Plant origins	3	82	−0.27	(−0.56, 0.02)	0.069	0.0	0.822
Duration							
Long term (>6 weeks)	6	199	−0.20	(−0.44, 0.03)	0.086	71.8	0.007
Short term (≤6 weeks)	3	99	−0.11	(−0.67, 0.46)	0.713	0.0	0.871

[a] High quality studies; [b] Low quality studies based on JADAD score

analyses based on study design, source of n-3 PUFA supplementation, and duration of intervention.

Effect of n-3 PUFAs on serum sex hormone binding globulin (SHBG) levels

The same 8 clinical trials (total n = 298 females with PCOS) assessed the effects of n-3 PUFAs on serum SHBG levels [11, 19, 20, 24, 26, 28, 29, 32]. n-3 PUFAs were associated with increased SHBG levels in 3 studies [19, 28, 29], however, our meta-analysis did not show a significant effect of n-3 PUFAs on SHBG levels (MD: 1.75 nmol/l; 95 % CI: −0.51 to 4.01; p = 0.129). Heterogeneity between studies was not significant (Q-test, p = 0.126, I^2 = 36.5 %). Subgroup analyses based on study design, source of n-3 PUFAs, and study duration revealed no statistically significant effects in any subgroups. Heterogeneity was observed in subgroup analyses in before-after studies [20, 28, 29] (Q-test, p = 0.006, I^2 = 80.3 %), studies using marine-derived sources of n-3 PUFAs [11, 24, 26, 28, 29] (Q-test, p = 0.041, I^2 = 56.5 %), and studies > 6 weeks intervention [11, 20, 24, 28, 29] (Q-test, p = 0.034, I^2 = 58.7 %). Heterogeneity disappeared after removing a study done by Oner et al. [29] (Q-test, p = 0.951, I^2 = 0.0 % for before-after studies; Q-test, p = 0.828, I^2 = 0.0 % for marine-derived source of n-3 PUFAs; Q-test, p = 0.743, I^2 = 0.0 % for studies > 6 weeks intervention). **Table 3** summarizes overall effect and subgroup analyses based on study design, source of n-3 PUFA supplementation, and duration of intervention regarding the effect of n-3 PUFAs on serum SHBG levels.

Effect of n-3 PUFAs on serum dehydroepiandrostrone sulfate (DHEAS) levels

Six of the evaluated clinical trials (total n = 183 females with PCOS) assessed the effects of n-3 PUFAs on serum DHEAS values [11, 19, 20, 26, 29]. None of the studies reported a significant effect of n-3 PUFAs on DHEAS levels compared to a control group. Further, our meta-analyses did not show a beneficial effect of n-3 PUFAs on DHEAS levels (Hedes' g: −0.11; 95 % CI, −0.29 to 0.06; p = 0.19). Heterogeneity between studies was not significant (Q-test, p = 0.22, I^2 = 28.5 %). Categorizing studies based on study design [20, 29] and duration [11, 20, 28, 29] showed a significant reduction of DHEAS levels (uncontrolled before-after studies: Hedes' g: −0.15; 95 % CI, −0.27 to −0.04; p = 0.01; > 6 weeks intervention: Hedes' g: −0.17; 95 % CI, −0.29 to −0.05; p = 0.004). There was no significant effect in subgroup analyses based on source of n-3 PUFAs however. **Table 4** shows the overall effect and subgroup analyses based on study design, source of n-3 PUFAs, and duration of intervention regarding the effects of n-3 PUFAs on serum DHEAS levels.

Table 3 The effect of n-3 PUFAs on serum SHBG value stratified by study design, source of n-3 PUFA and duration of intervention in PCOS women.

SHBG	Number of effect sizes	Participants	Meta-analysis			Heterogeneity	
			Mean difference	Confidence interval	p-Value	I^2 %	Cochrane Q-test (p-Value)
Study design (scoring)							
Cross-over, parallel (>2)[a]	6	224	1.21	(−0.33, 2.75)	0.125	0.0	0.972
Before-after (≤2) [b]	3	74	7.20	(−3.36, 17.76)	0.181	80.3	0.006
Overall							
Source of n-3 PUFA							
Marine derived	6	216	2.76	(−0.43, 5.95)	0.090	56	0.041
Plant origins	3	82	−0.12	(−3.95, 3.70)	0.949	0.0	0.925
Overall							
Duration							
Long term (>6 weeks)	6	199	2,39	(−0.92, 5.70)	0.157	58.7	0.034
Short term (≤6 weeks)	3	99	1.14	(−2.55, 4.82)	0.546	0.0	0.798

[a] High quality studies; [b] Low quality studies based on JADAD score

Table 4 The effect of n-3 PUFAs on serum DHEAS value stratified by study design, source of n-3 PUFA, and duration of intervention in PCOS women.

DHEAS	Number of effect sizes	Participants	Meta-analysis			Heterogeneity	
			Mean difference	Confidence interval	p-Value	I^2 %	Cochrane Q-test (p-Value)
Study design (scoring)							
Cross-over, parallel (>2)[a]	4	121	0.00	(−0.45, 0.46)	0.99	52.0	0.100
Before-after (≤2)[b]	2	62	−0.15	(−0.27, −0.04)	0.01	0.00	0.886
Source of n-3 PUFA							
Marine derived	3	101	−0.15	(−0.51, 0.21)	0.42	52.7	0.121
Plant origins	3	82	−0.05	(−0.32, 0.22)	0.70	15.7	0.305
Duration							
Long term (>6 weeks)	3	84	−0.17	(−0.29, −0.05)	0.004	0.0	0.392
Short term (≤6 weeks)	3	99	0.22	(−0.16, 0.59)	0.258	0.0	0.507
Overall	6	183	−0.11	(−0.29, 0.06)	0.195	28.5	0.221

[a] High quality studies; [b] Low quality studies based on JADAD score

Publication bias and sensitivity analyses

Although a slight asymmetry was seen in Begg's funnel plot for studies examining the effects of n-3 PUFAs on total testosterone, SHBG and DHEAS levels, there was no evidence of publication bias using Egger's test (p for bias: 0.97, 0.40, and 0.48, respectively) or Begg's test (p for bias: 0.75, 0.42, and 0.18, respectively).

Findings from sensitivity analyses revealed that none of the studies examining the effects of n-3 PUFAs on SHBG levels significantly affected the overall effect. Removing a study done by Kuzmanov et al. [28] altered the results to show significance regarding the effects of n-3 PUFAs on total testosterone levels (MD: −0.253; 95% CI, −0.441 to −0.066). Further, removing a study by Vargas et al. [26] subsequently showed a significant effect of n-3 PUFAs on DHEAS levels (Hedes' g: −0.15; 95% CI, −0.27 to −0.02).

Discussion

The present study provides the first systematic review and meta-analysis of clinical trials investigating the effects of n-3 PUFAs on androgen values in adult females with PCOS. The meta-analysis showed a slight reduction in serum total testosterone levels following n-3 PUFA supplementation among females with PCOS. However, the meta-analysis suggests SHBG and DHEAS levels were not affected by n-3 PUFA supplementation. Subgroup analyses based on study design and source of n-3 PUFAs (plant vs. marine) indicated no beneficial effect of n-3 PUFA supplementation on total testosterone and SHBG values within the subgroups. Stratified analyses based on study design and intervention duration showed that reductions in DHEAS levels were significant in trials with no control group and trials with long-term follow-up (most without control groups). In contrast, high quality randomized controlled clinical trials did not show significant effects of n-3 PUFA supplementation on serum testosterone, SHBG or DHEAS levels. It does not appear that n-3 PUFA supplementation significantly effects the androgenic profile among adult females with PCOS. Future studies using well-designed placebo controlled randomized trials with longer follow-up periods (>6 weeks) are necessary to confirm these findings.

In addition to the potential anti-androgenic benefits of n-3 PUFAs, there is evidence that n-3 PUFA supplementation provides beneficial effects on cardio-metabolic risk factors including insulin resistance, dyslipidemia, inflammation and obesity, the key pathogenic factors in PCOS [16,17,46–49]. Bioavailability of testosterone, a major ovarian androgen, depends on the

abundance of SHBG [50–52]. Studies show that insulin resistance decreases SHBG levels and testosterone binding that in turn increase free testosterone; unfavorable effects such as acne and excess hair growth may result [8,53,54]. There is some (inconsistent) evidence suggesting that n-3 PUFA supplementation may elevate fasting blood glucose concentration [27,55,56]. Epidemiological and clinical studies have reported a lower prevalence of T2DM in populations who consume considerable amounts of n-3 PUFA-rich seafood products [57–60]. However, there is conflicting evidence concerning the relationship between fish intake, dietary n-3 PUFAs, and risk of T2DM [61–64]. Overweight, obesity, and adipose tissue dysfunction are present in a large proportion of females suffering from PCOS [65]; there is potential for n-3 PUFAs playing an antiobesity role [66,67]. Botwood et al. suggested a negative correlation between SHBG concentrations and body mass index (BMI) as well as indices of central adiposity [68]. Further studies have suggested that insulin may be the hormonal mediator of the weight-related changes in SHBG. It should be noted that BMI, insulin and SHBG are interrelated in a different way in females with PCOS compared to normal subjects [68]. It has been proposed that there are increased levels of SHBG in women with normal WHR following the ingestion of n-3 PUFA [29] compared to obese or overweight women with PCOS [11,24,26]. These studies taken together suggest that n-3 PUFAs induce adipose tissue metabolism changes that may be a pre-factor for insulin sensitivity changes [30,41].

There is evidence showing that dietary n-3 PUFAs, including ALA, EPA, and/or DHA, may improve inflammation and MetS [49,69,70]. Recent studies suggest that females with PCOS experience an increase in lipogenesis and plasma n-6 PUFAs to n-3 PUFAs ratio that can lead to greater inflammatory biomarkers levels [30]. A potential mechanism is n-3 PUFAs suppress nuclear factor-κB activation and therefore the production of pro-inflammatory factors; subsequently, imposing their anti-inflammatory effects [71,72]. To support this theory, one study showed primary bovine theca cells treated with n-6 PUFAs and not n-3 PUFAs upregulated androstenedione secretion [11]. These results highlight that n-3 PUFAs not only have independent antiandrogenic effects due to insulin sensitizing, anti-inflammation and antiobesity properties, but can also reduce androgen levels by lowering the n-6:n-3 PUFAs ratio [2,11].

Due to the beneficial effects of n-3 PUFAs discussed above, and the fact that n-3 PUFAs are essential in the diet and unsynthesizable in the human body, dietary supplementation of n-3 PUFAs is believed to have favorable effects for the management of PCOS [26,29,32,73]. The limited number of studies with different study designs and potential confounding factors from different diagnosis criteria of PCOS, sources of n-3 PUFAs, and doses and intervention duration, limit the strength of the available data. Moreover, although we used a random effects model that takes between study variations into account, different methods of measuring androgen values might explain the between studies heterogeneity. Additionally, due to significant heterogeneity between interventions of control groups, we were unable to do subgroup analysis based on control groups.

In conclusion, the present analyses represent the first systematic review and meta-analysis suggesting that n-3 PUFAs might not significantly affect total testosterone, SHBG and DHEAS levels in females with PCOS. However, studies with no control group and longer follow-up periods did show significant reductions in DHEAS levels. Additional well-designed placebo controlled randomized controlled trials with longer follow-up periods (> 6 weeks) are required to establish optimal doses and quantify the effects of n-3 PUFA therapy on androgen levels among adult females with PCOS.

Author's Contribution

MH and ASA contributed in conception, design, data collection, statistical analysis, and drafting of the manuscript. BI, RG, GA, NB, and JT contributed to drafting of the manuscript. All authors approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

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