Effect of n-3 unsaturated fatty acid diet on C-reactive protein and erythrocyte sedimentation rate in the anti-inflammatory effect of rheumatoid arthritis: A meta-analysis

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ABSTRACT

The work was carried out to explore whether the anti-inflammatory effect of n-3 unsaturated fatty acids on patients with rheumatoid arthritis is related to the levels of inflammatory markers CRP and ESR. Studies on the treatment of rheumatoid arthritis with n-3 unsaturated fatty acid diet therapy and the outcome index containing CRP and/or ESR were included, and studies on the subjects suffering from other diseases affecting the outcome index were excluded. PubMed, Web of Science, EMBASE and Google Scholar were systematically searched, and all studies published from the establishment of the library to 2024 were collected. The Cochrane Bias Risk Assessment tool was used to evaluate the quality of the included studies. Data analysis was performed using Stata 16.0 software. Seven studies were included in this analysis. A total of 399 subjects were studied to explore the effect of an n-3 unsaturated fatty acid diet on rheumatoid arthritis. The results showed that there was no significant difference in CRP (Hedges’ $g = 0.06$, 95% CI: $-0.48$–$0.37$, $P = 0.79$) and ESR (Hedges’ $g = -0.14$, 95% CI: $-0.61$–$0.33$, $P = 0.55$) between the intervention and control groups. The results of this study showed that the anti-inflammatory effect of unsaturated fatty acids on rheumatoid arthritis was not correlated with CRP and ESR levels. Due to the small number of included studies, more high-quality studies are still needed to confirm this.

KEYWORDS

n-3 unsaturated fatty acids, diet therapy, rheumatoid arthritis, c-reactive protein, erythrocyte sedimentation rate

Highlighting: CRP and ESR are inflammatory markers of RA. Although the effect of dietary fatty acid control on ESR in patients with RA was noted as early as three decades ago, no study gave a summary answer to this question. Given that few studies to date have explored the effects of an unsaturated fatty acid diet on inflammatory response factors in RA, we summarised the effects of an unsaturated fatty acid ester diet on CRP and ESR in patients with RA, making a preliminary judgment and having a reference role in the clinical control of elevated CRP and ESR.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic inflammatory autoimmune disease characterised by joint pain and swelling, which can seriously damage physical function and quality of life (Sparks, 2019). Treatment approaches include the measurement of disease activity with a composite index, the application of treatment-target strategies, and the use of traditional, biological, and new abiotic disease-modifying anti-rheumatic drugs (Smolen et al., 2016). Diet therapy is a long-term chronic treatment with high safety.

The n-3 unsaturated fatty acids (n-3 PUFA) are potentially effective anti-inflammatory drugs (Calder, 2006). The CRP concentration is a very useful non-specific biochemical marker of inflammation (Pepys and Hirschfield, 2003), and this result obtained in the study is very valuable for the follow-up and treatment of the disease (Kaya et al., 2021). Elevated erythrocyte sedimentation rate (ESR) has traditionally been considered as a major indicator for the diagnosis and monitoring of inflammation in patients with RA (Abelson et al., 2009).

The results in research (Berbert et al., 2005) showed that fish oil as an n-3 PUFA had no effect on the CRP and ESR. The CRP and ESR in the indomethacin + n-3 PUFA combination treatment group significantly increased (Das Gupta et al., 2009). A study (Kremer et al., 1985)
conducted more than 30 years ago showed that dietary fatty acid manipulation had little effect on ESR in patients with RA, while another study (Sigaux et al., 2022) showed a significant decline in ESR, but the subjects had other diseases than RA. Studies lack analysis of the effect of n-3 PUFA on CRP and ESR. Our aim was to explore whether the anti-inflammatory effects of n-3 PUFA in RA were achieved by reducing the inflammatory marker CRP and ESR levels by summarising previous related studies.

2. METHODS

A meta-analysis of the published literature was performed according to the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) (Page et al., 2021).

2.1. Data collection and retrieval strategy

PubMed, Web of Science, EMBASE, and Google Scholar were systematically searched, and reports published up to 2024 were collected. All randomised, controlled, or cross-over trial reports exploring the effect of n-3 PUFAs (eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], docosapentaenoic acid [DPA], and α-linolenic acid [ALA]) on disease activity and systemic inflammation were included. We searched PubMed, Web of Science, EMBASE, and Google Scholar using the following search terms: (“n-3 PUFA” or “n-3 PUFAs” or “ω-3 fatty acids” or “eicosapentaenoic acid” or “docosahexaenoic acid” or “docosapentaenoic acid” or “linolenic acid” or “EPA” or “DHA” or “DPA” or “ALA”) and (“arthritis, rheumatoid” or “RA”). We have also searched the reference lists of the articles.

2.2. Inclusion and exclusion criteria

Inclusion criteria: 1. Study type: there were no strict restrictions on the study type; 2. Study subjects: RA patients; 3. Interventions: n-3 PUFA dietary therapy; 4. Outcome measures: CPR and (or) ESR.

Exclusion criteria: 1. Study subjects: patients had other diseases affecting outcome measures; 2. Other treatment measures were used in the control group; 3. Duplicate published data; 4. There were obvious errors in the data within the study.

2.3. Literature screening and data extraction

The literature search was performed according to the search strategy, and the literature screening was performed according to the PRISMA flow diagram. Literature was independently screened by 2 investigators before checking, and in case of disagreement a third investigator was arbitrated. Data extraction was performed independently by researchers, including the first author, region, characteristics of study subjects, intervention time, etc. After cross checking, controversial data were evaluated by a third researcher and unified by discussion.

2.4. Quality assessment

The Cochrane risk of bias assessment tool was applied for methodological quality assessment of the literature. These 6 entries were judged “low risk”, “high risk”, or “unclear” for the random
sequence generation, blinding of participants and assessors, allocation concealment, incomplete outcome data, selective outcome reporting, and other biases.

2.5. Statistical analysis

Meta analysis was performed using Stata 16.0. The analysis of heterogeneity among included studies was performed using the Q test. If $I^2 < 50\%$ and $P > 0.1$ indicated that there was no statistical heterogeneity among studies, then a fixed effects model was used for the analysis. If $I^2 \geq 50\%$ or $P \leq 0.1$ indicated large heterogeneity across studies, the random effects model was used for analysis. Sensitivity analysis was performed by examining the effect of individual studies on the total pooled effect size, and $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Results

After searching the database, a total of 1750 articles were obtained. The duplicate articles were eliminated by Endnote, and 1,372 articles remained. After reading the titles and abstracts, 1,171 articles did not meet the criteria, and 201 articles remained. After reading the full text, 192 articles were excluded according to the given criteria, and due to the incorrect result values and the lack of available data, 2 further articles were excluded, and the final number of articles included in the study was 7 (Sarzi-Puttini et al., 2000; Berbert et al., 2005; Galarraga et al., 2008; Das Gupta et al., 2009; Dawczynski et al., 2009; Veselinovic et al., 2017; Ghaseminasab-Parizi et al., 2022). A total of 399 cases were studied. The flowchart is shown in Fig. 1. The general characteristics of the included literature are detailed in Table 1.

3.1.1. Evaluation results. The quality evaluation of the 7 included studies showed that 4 studies did not clearly indicate the specific method of random allocation, so they were “uncertain”, and 1 study clearly stated that no blind method was used, so it was marked “high risk”. Three studies did not specify whether allocation concealment was used or not, so it was classified “uncertain”. The methodological quality evaluation results of the included studies are shown in Table 2.

3.1.2. Effect of dietary therapy on CRP in patients. The outcome indicators of the seven included studies all included CPR. There were 200 patients in the experimental group and 199 patients in the control group. The heterogeneity among the included studies was high ($I^2 = 77.49\%, P < 0.01$), so the random effect model was used for analysis. The results showed that (Hedges’ $g = 0.06$, 95% CI: $-0.48$–$0.37$, $P = 0.79$) there was no significant difference in CRP levels between the intervention group and the control group (see Fig. 2a for details).

3.1.3. Effect of dietary therapy on ESR of patients. The outcome indicators of 6 of the 7 included studies included ESR. There were 151 patients in the experimental group and 151 patients in the control group. The heterogeneity among the included studies was high ($I^2 = 75.65\%, P < 0.01$), so the random effect model was used for analysis. The results showed that the difference was not statistically significant (Hedges’ $g = -0.14$, 95% CI: $-0.61$–$0.33$, $P = 0.55$) (see Fig. 2b for details).
3.1.4. **Subgroup analysis.** According to the general characteristics, the studies were subgroup analysed by the sources of n-3 PUFA (plants, animals, or unknown) and therapy time (<24 weeks, ≥24 weeks). According to the results, n-3 PUFA from different sources or n-3 PUFA therapy time were not responsible for the heterogeneity. The analysis results of each subgroup are shown in Table 3.

3.1.5. **Sensitivity analysis.** Sensitivity analysis of the included studies showed no significant differences (see Fig. 3a and b for details).
<table>
<thead>
<tr>
<th>Study ID</th>
<th>Authors</th>
<th>Year</th>
<th>Area</th>
<th>Number of EG</th>
<th>Number of CG</th>
<th>Treatment (g/day)</th>
<th>Therapy time (week)</th>
<th>Inflammatory markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Berbert et al.</td>
<td>2005</td>
<td>Brazil</td>
<td>13</td>
<td>13</td>
<td>fish oil (3 g)</td>
<td>24</td>
<td>CRP, ESR</td>
</tr>
<tr>
<td>Study 2</td>
<td>Das Gupta et al.</td>
<td>2009</td>
<td>Bangladesh</td>
<td>40</td>
<td>41</td>
<td>omega-3 fatty acids (3 g)</td>
<td>12</td>
<td>CRP, ESR</td>
</tr>
<tr>
<td>Study 3</td>
<td>Dawczynski et al.</td>
<td>2009</td>
<td>Germany</td>
<td>21</td>
<td>21</td>
<td>n-3 fatty acids (2.4 g)</td>
<td>2 × 12</td>
<td>CRP, ESR</td>
</tr>
<tr>
<td>Study 4</td>
<td>Galarraga et al.</td>
<td>2008</td>
<td>Britain</td>
<td>49</td>
<td>48</td>
<td>cod liver oil (10 g)</td>
<td>36</td>
<td>CRP</td>
</tr>
<tr>
<td>Study 5</td>
<td>Veselinovic et al.</td>
<td>2017</td>
<td>Serbia</td>
<td>20</td>
<td>20</td>
<td>fish oil (5 g)</td>
<td>12</td>
<td>CRP, ESR</td>
</tr>
<tr>
<td>Study 6</td>
<td>Ghaseminasab-Parizi et al.</td>
<td>2021</td>
<td>Germany</td>
<td>35</td>
<td>35</td>
<td>flaxseed (30 g)</td>
<td>12</td>
<td>CRP, ESR</td>
</tr>
<tr>
<td>Study 7</td>
<td>Sarzi-Puttini et al.</td>
<td>2000</td>
<td>Italy</td>
<td>22</td>
<td>21</td>
<td>olive oil (unsaturated: saturated ≈2:1)</td>
<td>24</td>
<td>CRP, ESR</td>
</tr>
</tbody>
</table>

EG: experimental group; CG: control group; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate. * Cross experiment.
### Table 2. Risk of bias assessment of the included studies

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Random sequence generation</th>
<th>Blinding of participants and assessors</th>
<th>Allocation concealment</th>
<th>Incomplete outcome data</th>
<th>Selective outcome reporting</th>
<th>Other bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 2</td>
<td>Low</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 3</td>
<td>Unclear</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 4</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 5</td>
<td>Unclear</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 6</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Study 7</td>
<td>Unclear</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

### Fig. 2. Forest plot of CRP (a) and ESR (b)
3.2. Discussion

CRP and ESR are inflammatory markers of RA and often reflect disease activity, which are reduced to normal when the condition is in remission. Dietary components can affect disease activity in RA through several direct or indirect interactions with the immune system (Nelson et al., 2020). Many studies have focused on the classical proinflammatory cytokine tumour necrosis factor (TNF)-α, Interleukin (IL)-1β, and IL-6 effects (Calder, 2015). Few articles have focused on CRP and ESR as their primary concerns.

The animal experiment (Calder, 2017) and another study (Belch et al., 1988) have proved the positive role of unsaturated fatty acids in anti-inflammatory activity. In addition, studies (Edefonti et al., 2020; Vadell et al., 2020; Mazzucca et al., 2022) have proved that a diet containing unsaturated fatty acids has a positive effect on the disease of RA patients. The 3-series prostaglandins and 5-series leukotrienes produced by n-3-series fatty acids (mainly from EPA) have weak inflammation inducing properties (Wiktorowska-Owczarek et al., 2015). The anti-inflammatory effect of fish oil in patients with RA has been reported for a long time, such as the significantly reduced production of neutrophil LTB and macrophage IL-1 (Kremer et al., 1990), neutrophil LTB4 (Kremer et al., 1987; Cleland et al., 1988; van der Tempel et al., 1990), and monocytes (Cleland et al., 1988).

The result of this meta-analysis showed that the differences between the CRP (Hedges’s g = 0.06, 95% CI: −0.48–0.37, P = 0.79) and ESR (Hedges’s g = −0.14, 95% CI: −0.61–0.33, P = 0.55) values of the intervention group and the control group were not significant. The preliminary exploration of the anti-inflammatory mechanism of unsaturated fatty acid diet on

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Hedges’s g</th>
<th>[95% Conf. interval]</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP Sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plant</td>
<td>2</td>
<td>−0.123</td>
<td>−0.487</td>
<td>0.241</td>
</tr>
<tr>
<td>animal</td>
<td>3</td>
<td>0.195</td>
<td>−0.109</td>
<td>0.498</td>
</tr>
<tr>
<td>unclear</td>
<td>2</td>
<td>−0.267</td>
<td>−2.008</td>
<td>1.475</td>
</tr>
<tr>
<td>Overall</td>
<td>7</td>
<td>−0.059</td>
<td>−0.485</td>
<td>0.367</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24 weeks</td>
<td>3</td>
<td>−0.398</td>
<td>−1.179</td>
<td>0.384</td>
</tr>
<tr>
<td>≥24 weeks</td>
<td>4</td>
<td>0.228</td>
<td>−0.065</td>
<td>0.521</td>
</tr>
<tr>
<td>Overall</td>
<td>7</td>
<td>−0.058</td>
<td>−0.507</td>
<td>0.390</td>
</tr>
<tr>
<td>ESR Sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plant</td>
<td>2</td>
<td>0.25</td>
<td>−0.33</td>
<td>0.83</td>
</tr>
<tr>
<td>animal</td>
<td>2</td>
<td>−0.24</td>
<td>−0.71</td>
<td>0.24</td>
</tr>
<tr>
<td>unclear</td>
<td>2</td>
<td>−0.39</td>
<td>−1.63</td>
<td>0.86</td>
</tr>
<tr>
<td>Overall</td>
<td>6</td>
<td>−0.14</td>
<td>−0.61</td>
<td>0.33</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24 weeks</td>
<td>3</td>
<td>−0.187</td>
<td>−1.151</td>
<td>0.777</td>
</tr>
<tr>
<td>≥24 weeks</td>
<td>3</td>
<td>−0.060</td>
<td>−0.484</td>
<td>0.364</td>
</tr>
<tr>
<td>Overall</td>
<td>6</td>
<td>−0.144</td>
<td>−0.660</td>
<td>0.372</td>
</tr>
</tbody>
</table>
RA is helpful to understand the specific mechanism of anti-inflammatory effect of n-3 unsaturated fatty acid diet on patients with RA. To determine its effect on CRP and ESR in RA patients, has a relevance in the clinical control of CRP and ESR.
3.2.1. **Limitations.** 1. Many potential confounding factors such as gender, age, and disease severity are still present, which can affect our results. 2. Subgroup analysis was not conducted due to the small number of included studies and no strict classification of the type, dose, and duration of use of unsaturated fatty acids. 3. There is no strict control over the patient’s original basic treatment, and differences in the use of anti-inflammatory drugs may have an impact on the outcome. 4. Other outcome values in the included study (Das Gupta et al., 2009) appear to have obvious errors, but the seemingly correct CRP and ESR values were still included, and there was no standard deviation of the results in the literature, so it was not included in our study, which may affect the overall results.

3.2.2. **Innovative.** Although studies have pointed out the effect of dietary fatty acid consumption on ESR levels in patients with RA as early as thirty years ago (Kremer et al., 1985), no study has given a summative answer to this question. Given that there are currently few studies exploring the effects of unsaturated fatty acid diets on RA inflammatory response factors, we summarised the effects, made preliminary judgments, and provided a reference effect on the clinical control of C-reactive protein elevation and ESR. We hope that more studies will soon be available to compare with our results. At the same time, more researchers are called on to study this phenomenon, and the anti-inflammatory effect of polyunsaturated fatty acid diet cannot be denied just because the results of this paper showed that it had little effect on the level of inflammatory factors CRP and ESR.

4. **CONCLUSIONS**

Based on the results of the existing relevant studies in the literature, polyunsaturated fatty acid diet does not appear to have a significant effect on CRP and ESR levels in patients with RA. The findings provide evidence that the anti-inflammatory effects of unsaturated fatty acids are not associated with CRP and ESR levels. But it still needs to be confirmed in future high-quality studies.

**Declaration of interest:** The authors have no potential conflicts of interest to disclose.

**Author contributions:** Jimin Deng contributed to the discussion of content, Kexin Zhang contributed to the drawing, Chengbiao Ding and Yuanli Wang wrote the article, and Zhihua Zhang, Jiming Deng, and Ming Gao reviewed and/or edited the article before submission.

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