Exploring the link between dietary omega-3 and omega-6 fatty acid intake and rheumatoid arthritis risk: the NHANES 1999-2020 study

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Abstract

Objective

The association between the ingestion of n-3 and n-6 fatty acids and rheumatoid arthritis (RA) remains unclear. To address this, this study utilised data from the National Health and Nutrition Examination Survey (NHANES) spanning from 1999 to 2020.

Methods

Dietary intake information on n-3 and n-6 fatty acids was gathered through 24-hour interviews about dietary recall and adjusted based on weight. RA patient data was collected using questionnaires. Associations were evaluated using logistic regression and spline analyses. The study included a total of 50,352 participants in a cross-sectional manner.

Results

In the adjusted Model 2, higher odds ratios (ORs) of 0.72 (95% CI: 0.60–0.86) and 0.76 (95% CI: 0.62–0.92) were observed for n-3 and n-6 fatty acid intake, respectively, compared to the lowest category.

Conclusion

The results suggest a negative correlation between the ingestion of n-3 and n-6 fatty acids and the risk of rheumatoid arthritis in US adults.

Key words

rheumatoid arthritis, dietary n-3 fatty acid, dietary n-6 fatty acid, n-6:n-3 ratio, dose-response

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Introduction

RA is not confined to large joints but distinctly targets the smaller joints in the hands and feet, showcasing the unique nature of this immune-mediated disorder (1, 2). It is one of the most common autoimmune diseases globally, with a frequency spanning from 0.5% to 1.0%, and a comparatively greater occurrence among females (3). The development of RA is influenced by multiple factors, including genetics, environment, and stochastic elements (4). Research suggests that genetics contributes to a 50% susceptibility to RA (5). Additionally, environmental factors, like smoking and dietary choices, also play a crucial role in managing the disease. Smoking has been associated with the onset or progression of RA, whereas certain dietary modifications, for instance, refraining from the ingestion of red meat and incorporating more fruits and oily fish into the diet, may help reduce the risk of RA (6, 7). Interestingly, low to moderate alcohol ingestion has shown an inverse correlation with the development of RA, varying based on the dose, duration, and gender of the person in question (8).

While prior research has predominantly concentrated on factors that influence the adverse progression of RA, including prognosis and treatment effectiveness, it is crucial to investigate additional modifiable factors linked to the illness. (9). Research has shown that n-3 fatty acids, which are present in oily fish and fish oil supplements, may have potential benefits in treating RA symptoms due to their anti-inflammatory effects (10-12). Similarly, n-6 fatty acids have also been found to exhibit anti-inflammatory effects in certain studies (13-15). However, there is still contradictory evidence regarding the connection between n-3 and n-6 fatty acid ingestion and the risk of developing RA, with some studies reporting no significant reduction in risk with n-3 fatty acid intake (16, 17).

Until now, no previous studies have utilised the NHANES (National Health and Nutrition Examination Survey) database to investigate the correlation between the ingestion of n-3 and n-6 fatty acids and RA. As a result, our study aims to investigate the association between the intake of these fatty acids and RA in participants of the NHANES. Our hypothesis is that individuals with RA have a reduced ingestion of n-3 and n-6 fatty acids.

Materials and methods

Data source and study population

The National Health and Nutrition Examination Survey (NHANES) is conducted by the Centers for Disease Control and Prevention (CDC) and serves as a nationally representative survey in the United States. The main goal of this study is to assess the health and nutritional condition of the non-institutionalised civilian population. NHANES data provides valuable insights into various health conditions, risk factors, and their associations with socio-demographic factors. The survey employs a meticulous sampling methodology to ensure the findings can be extrapolated to the broader U.S. population. The Research Ethics Review Board of the National Center for Health Statistics approved this study, and all participants provided informed consent.

This study utilised data from eleven NHANES cycles conducted between 1999 and 2020. Initially, a total of 116,876 participants were surveyed. However, our analysis focused on 68,897 individuals aged 18 years and older. Exclusions were made for 1,758 pregnant and lactating women, 8,342 participants with incomplete arthritis data or arthritis types other than rheumatoid arthritis, 3,957 individuals without complete weight data, 3,890 participants did not have complete data for a 24-hour recall, while 598 participants had a significantly high or low total energy intake. Consequently, this cross-sectional study included a total of 50,352 participants in the final sample.

The characterisation of rheumatoid arthritis

The diagnosis of RA among participants was established by conducting personal interviews and utilising health condition questionnaires (MCQ160A) for self-reporting. Initially, the participants were asked, "Have you ever been informed by a healthcare profes-

sional that you have arthritis?" The participants were given response options of "Yes" or "No". The evaluation of rheumatoid arthritis involved further inquiry, "When you received your diagnosis, what type of arthritis did the doctors identify?". Response choices included "Psoriatic arthritis," "Rheumatoid arthritis", "Osteoarthritis", "Refused", "Other", and "Don't know".

Dietary n-3 and n-6 fatty acid intake

The dietary intake of n-3 and n-6 fatty acids was evaluated using 24-hour dietary recall interviews. The subtypes of n-3 fatty acids, including docosahexaenoic acid (22:6), clupanodonic acid (22:5), eicosatetraenoic acid (20:5), stearidonic acid (18:4), linolenic acid (18:3), were referenced from previous research (18). The different subtypes of n-6 fatty acids, such as arachidonic acid (20:4) and linoleic acid (18:2), were also taken into account.

To determine the average daily intake of n-3 and n-6 fatty acids in the diet, data from the USDA's Dietary Research Food and Nutrition Database (19) were utilised. The calculations were adjusted for body weight. Subsequently, the intake of n-3 and n-6 fatty acids in the diet, along with the ratio of n-6 to n-3, were divided into tertiles.

Covariates

Our study considered various characteristics as potential factors influencing RA. These covariates included gender, age (grouped into three categories: 18~44 years, 45~59 years, and 60 years and higher), ethnicity (including non-Hispanic Black, non-Hispanic White, other Hispanic, and Mexican American, and others), marital status (categorised as Widowed/Divorced/Separated/Never married, Married/Living with partner), annual household income (with subdivisions of (<$20,000 and ≥$20,000), educational level (classified as Above high school, High school, or Below high school, smoking status (divided into three categories based on their smoking habits, allowing for a comprehensive analysis: current smokers, who currently smoke on some days or every day after having smoked more than 100 cigarettes in their lifetime; former smokers, who have smoked more than 100 cigarettes in their lifetime but are currently nonsmokers; and never smokers, who have consumed less than 100 cigarettes in their lifetime), Alcohol use was classified as never (defined as having consumed fewer than 12 drinks in their lifetime), former (defined as having consumed 12 or more drinks in a year but not in the last year or having consumed 12 or more drinks in their lifetime), mild (defined as consuming 1 drink per day for women and up to 2 drinks per day for men), moderate (equivalent to consuming 2 drinks per day for women and up to 3 drinks per day for men; or intermittent binge drinking of 2 or more days per month but less than 5 days per month), and heavy (defined as consuming 3 drinks per day for women and up to 4 drinks per day for men; or frequent binge drinking of 5 or more days per month). Additionally, the initial interview involved gathering data on the complete amount of energy consumed in a day, collected via 24-hour dietary recall interviews.

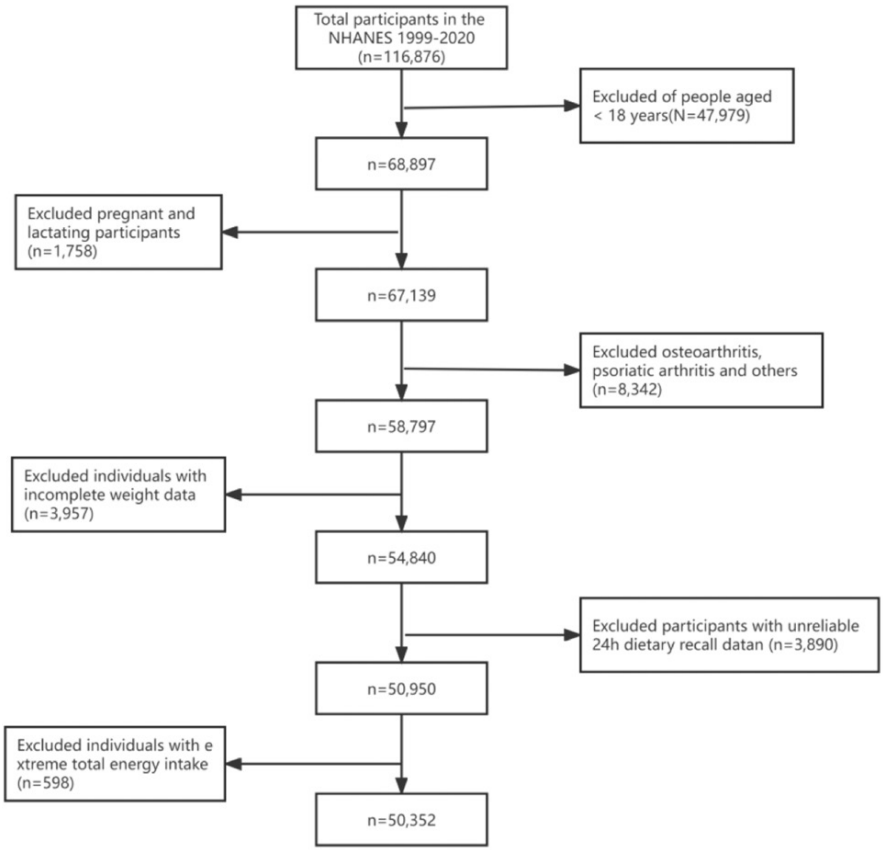

Fig. 1. Flowchart of the participant selection from NHANES 1999-2020.

Statistical methods

The statistical analyses in this study were conducted using R (v. 4.3.1). Given the complex and multi-stage sampling design of the NHANES database, we utilised dietary day one weights (WTDR4YR, WTDRD1) for analysis. Continuous variables were presented using weighted means and standard deviations, while categorical variables were expressed as weighted. To compare different groups, In the analysis of our data, we utilised the chi-square test to examine the relationships between categorical variables, whereas the t-test was applied to assess differences in continuous variables.

The dietary intakes of n-3 and n-6 fatty acids, as well as the n-6:n-3 ratio, were divided into tertiles: tertile 1 (representing the <33rd percentile), tertile 2 (ranging from ≥33rd to ≤67th percentile), and tertile 3 (representing the ≥67th percentile). Tertile 1 served as the reference category. To explore the association between the intake of n-3 and n-6 fatty acids in our research, we conducted binary logistic regression

analyses. Age and gender were included as adjustment variables in Model 1. In Model 2, we further adjusted for race, educational level, income, marital status, drinking status, smoking status, as well as total energy intake to account for potential confound, in addition to age and gender. The relationship between the ingestion of n-3 and n-6 fatty acids and the risk of RA was evaluated through a gender-stratified analysis. Furthermore, our team employed a constrained cubic spline to explore the dose-response relationship, while adjusting for the same confounding factors as in logistic regression Model 2.

Results

Participant characteristics and prevalence of rheumatoid arthritis

The fundamental attributes of the individuals included in the study, categorised by their RA status, are showed in Table I. In total, the study encompassed a sum of 50,352 participants, and the prevalence of RA was found to be 4.69%. It was observed that participants with RA tended to be older and predominantly female, with lower household income, educational level, and total energy intake when compared to those without RA. The prevalence of RA was higher among individuals with a history of smoking, whether former or current. Furthermore, it was noted that individuals with RA had lower intakes of both n-3 and n-6 fatty acids. As illustrated in Table II, the NHANES database only contains records for hsCRP/CRP during the periods 1999-2010 and 2015-2020. It is observed that the inflammatory markers, specifically hsCRP/CRP, are significantly higher in patients with RA compared to those without RA.

Table I. Characteristics of participants by rheumatoid arthritis, NHANES 1999-2020 (n=50,352).

	Non-rheumatoid arthritis	Rheumatoid arthritis	*p*-value
Number of participants (%)[1]	47345 (95.31)	3007 (4.69)	
Gender (%)[1]			<0.0001
Female	22657 (48.50)	1722 (56.99)	
Male	24688 (51.50)	1285 (43.01)	
Age group (%)[1]			<0.0001
18–44 years	23862 (53.13)	351 (17.46)	
45–59 years	11147 (27.89)	841 (35.81)	
≥60 years	12336 (18.98)	1815 (46.72)	
Race (%) [1]			<0.0001
Mexican American	8932 (9.28)	454 (6.79)	
Non-Hispanic Black	10508 (11.55)	922 (16.84)	
Non-Hispanic White	18961 (65.66)	1211 (65.11)	
Other Hispanic	4095 (6.02)	245 (5.48)	
Other race - including multi-racial	4849 (7.49)	175 (5.79)	
Marital status (%)[1]			0.6
Married/Living with partner	26122 (61.36)	1623 (60.60)	
Widowed/Divorced/Separated/Never married	18906 (38.64)	1355 (39.40)	
Household income (%)[1]			0.0001
<$20,000	3015 (5.40)	330 (11.75)	
≥$20,000	35504 (94.60)	2037 (88.25)	
Educational level (%) [1]			<0.0001
Above high school	23226 (58.60)	1212 (47.50)	
High school	11276 (24.79)	755 (27.32)	
Below high school	12427 (16.61)	1034(25.18)	
Smoke (%) [1]			<0.0001
former	9975 (22.65)	950 (32.19)	
now	9163 (21.23)	703 (26.79)	
never	25437 (56.12)	1353 (41.02)	
Alcohol use (%) [1]			<0.0001
former	5670 (11.67)	639 (22.11)	
heavy	9013 (23.81)	390 (16.98)	
mild	13741 (35.21)	871 (34.23)	
moderate	6512 (17.85)	349 (14.43)	
never	5887 (11.46)	425 (12.25)	
Total energy intake (kcal/day)[2]	2210.86 (6.62)	1986.52 (24.66)	<0.0001
Total adjusted n-3 fatty acid intake (mg/kg/day)[2]	23.50 (0.15)	20.93 (0.45)	<0.0001
Total adjusted n-6 fatty acid intake (mg/kg/day)[2]	213.43 (1.14)	187.73 (3.45)	<0.0001
n-6:n-3 ratio[2]	10.33 (0.09)	10.08 (0.13)	0.11

[1]Chi-square and [2]t-tests were applied to compare the differences between the rheumatoid arthritis group and the non-rheumatoid arthritis group.

Fatty acid intake and rheumatoid arthritis risk

In order to assess the disparities between the rheumatoid arthritis group and the non-rheumatoid arthritis group, chi-square tests were performed. Table III presents the odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) for rheumatoid arthritis concerning the ingestion of n-3 and n-6 fatty acids, along with the n-6:n-3 ratio. In the initial model of unadjusted binary logistic regression analysis, the highest intake group of n-3 fatty acids had an OR of 0.65 (95% CI 0.55, 0.75) compared to the reference group. Similarly, the highest intake group of n-6 fatty acids had an OR of 0.64 (95% CI 0.55, 0.75) compared to the reference group. After accounting for possible confounding factors in the Model 1, the association between the ingestion of both n-3 and n-6 fatty acids and the risk of rheumatoid arthritis remained significant.

In Model 2, in contrast to the reference group, individuals in the highest intake group of n-3 fatty acids exhibited an odds ratio (OR) of 0.72 (95% CI 0.60, 0.86), while those in the highest intake group of n-6 fatty acids showed an OR of 0.76 (95% CI 0.62, 0.92). Notably, the second intake group of n-3 fatty acids showed an OR of 0.82 (95% CI 0.69, 0.97). Regarding the n-6:n-3 ratio, in Model 1, the OR for the highest intake group in comparison to the reference group was 1.16 (95% CI 1.01, 1.34). However, this association was not observed in Model 2.

In Table IV, Model 3 adds an adjustment for hsCRP/CRP to the basis established by Model 2. The findings indicate that, during the 1999-2010 period, an increase in the intake of n-3 and n-6 fatty acids is associated with a decreased risk of developing RA, mirroring the results

Table II. CRP/hsCRP of participants by rheumatoid arthritis, NHANES 1999-2010/ 2015-2020.

	Non-rheumatoid arthritis	Rheumatoid arthritis	*p*-value
Number of participants (%)[1] IN 1999-2010	18593	1290	
CRP (mg/dL)	0.38 (0.01)	0.60 (0.03)	<0.0001
Number of participants (%)[1] IN 2015-2020	5696	388	
hsCRP (mg/L)	3.39 (0.11)	4.28 (0.34)	0.03

[1]t-tests were applied to compare the differences between the rheumatoid arthritis group and the non-rheumatoid arthritis group.

Table III. weighted odds ratios (95% confidence intervals) of rheumatoid arthritis across tertiles of adjusted dietary n-3, n-6 fatty acids intake and n-6:n-3 ratio, NHANES 1999–2020 (n=50,352).

	Crude	Model 1	Model 2
Adjusted n-3 (mg/kg/day)			
≤13.53	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.53 to<24.89	0.78 (0.67,0.90)**	0.81 (0.70,0.93)**	0.82 (0.69,0.97)*
≥24.89	0.65 (0.55,0.75)**	0.70 (0.60,0.82)**	0.72 (0.60,0.86)**
Adjusted n-6 (mg/kg/day)			
≤130.12	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
130.12 to<231.29	0.82 (0.71,0.95)**	0.85 (0.74,0.98)**	0.87 (0.74,1.03)
≥231.29	0.64 (0.55,0.75)**	0.75 (0.65,0.88)**	0.76 (0.62,0.92)**
n-6:n-3 ratio			
≤8.08	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
8.08 to<10.44	0.90 (0.79,1.02)	1.01 (0.88,1.16)	0.95 (0.82,1.10)
≥10.44	1.01 (0.88,1.16)	1.16 (1.01,1.34)*	1.11 (0.95,1.29)

Calculated using binary logistic regression. Model 1 adjusted for age and gender. Model 2 adjusted for age and gender, race, educational level, income, marital status, drinking status, smoking status,and total energy intake. *p<0.05; **p<0.01.

observed in Model 2. However, no such association was found during the 2015-2020 period.

Gender-specific analysis

A gender-stratified analysis was conducted using binary logistic regression to investigate the connection between the intake of n-3 and n-6 fatty acids, as well as the n-6:n-3 ratio, and the risk of rheumatoid arthritis. Two models were utilised in the analysis: Model 1, which accounting for age and gender, and Model 2, which also took into account race, income, educational level, marital status, drinking status, smoking status, and total energy intake in the adjustment. The levels of significance were determined at *p<0.05 and **p<0.01. The outcomes are presented in Table V. For females, the odds ratios (ORs) for rheumatoid arthritis were 0.76 (95% confidence interval [CI]: 0.61, 0.95) and 0.67 (95% CI: 0.53, 0.86) in Model 2 when considering n-3 and n-6 fatty acid intakes, respectively. Nevertheless, only a significant negative connection between n-3 fatty acid intake and the likelihood of developing rheumatoid arthritis in males was observed. The connection between the n-6:n-3 ratio and the risk of rheumatoid arthritis in females did not yield significant results across all three models. However, in males, the n-6:n-3 ratio was significantly linked with the risk of rheumatoid arthritis in both Model 1 and Model 2.

Dose-response relationship and recommended intake

The dose-response relationships can be observed in Figures 2 and 3. Applying the fully adjusted model, a non-linear connection between the ingestion of n-3 fatty acids and the prevalence of RA was observed (P for non-linearity <0.05). The reference value for n-3 fatty acid intake was set at log odds = 0, which corresponds to a daily intake of 38 mg/kg/day. This reference value could potentially be recommended as the daily intake of n-3 fatty acids for the prevention of RA. However, no similar changepoint in n-6 fatty acid intake was found. The confounding factors adjusted in the restricted cubic spline analysis remained consistent with those accounted for in logistic regression Model 2.

Table IV. weighted odds ratios (95% confidence intervals) of rheumatoid arthritis across tertiles of adjusted dietary n-3, n-6 fatty acids intake and n-6:n-3 ratio, NHANES 1999–2010/2015-2020.

NHANES 1999-2010	Model 3
Adjusted n-3 (mg/kg/day)	
≤13.53	1.00 (Ref.)
13.53 to<24.89	0.80 (0.65,1.00)*
≥24.89	0.75 (0.58,0.96)*
Adjusted n-6 (mg/kg/day)	
≤130.12	1.00 (Ref.)
130.12 to<231.29	0.82 (0.67,1.00)
≥231.29	0.75 (0.57,0.99)*
n-6:n-3 ratio	
≤8.08	1.00 (Ref.)
8.08 to<10.44	0.81 (0.64,1.02)
≥10.44	0.99 (0.78,1.26)
NHANES 2015-2020	
Adjusted n-3 (mg/kg/day)	
≤13.53	1.00 (Ref.)
13.53 to<24.89	0.90 (0.48,1.68)
≥24.89	0.91 (0.50,1.63)
Adjusted n-6 (mg/kg/day)	
≤130.12	1.00 (Ref.)
130.12 to<231.29	1.18 (0.69,2.03)
≥231.29	0.85 (0.53,1.37)
n-6:n-3 ratio	
≤8.08	1.00 (Ref.)
8.08 to<10.44	0.73 (0.51,1.06)
≥10.44	1.03 (0.72,1.47)

Calculated using binary logistic regression. Model 3 adjusted for age and gender, race, educational level, income, marital status, drinking status, smoking status, hsCRP/CRP, and total energy intake. *p<0.05; **p<0.01.

Discussion

This study is the initial examination that employs the NHANES database to explore the link between the ingestion of n-3 and n-6 fatty acids and the likelihood of developing RA in the general population of the United States. The findings suggest that higher ingestion of both n-3 and n-6 fatty acids are linked with a reduced risk of RA, with the associations being more prominent among females. By conducting a dose-response analysis, we identified a non-linear and U-shaped correlation between the intake of n-3 fatty acids and the risk of RA. On the other hand, a linear negative connection was observed between the intake of n-6 fatty acids and the risk

Table V. Weighted odds ratios (95% confidence intervals) of rheumatoid arthritis across tertiles of adjusted dietary n-3, n-6 fatty acid intake and n-6:n-3 ratio, stratified by gender, NHANES 1999-2020 (n=50,352).

	Female			Male		
	Crude	Model 1	Model 2	Crude	Model 1	Model 2
Adjusted n-3 (mg/kg/day)						
≤13.53	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.53 to<24.89	0.77 (0.64,0.93)**	0.77 (0.64,0.93)**	0.82 (0.67,1.02)	0.82 (0.66,1.02)	0.86 (0.69,1.07)	0.83 (0.65,1.06)
≥24.89	0.66 (0.55,0.79)**	0.69 (0.57,0.84)**	0.76 (0.61,0.95)*	0.66 (0.54,0.82)**	0.71 (0.57,0.89)**	0.70 (0.54,0.91)**
Adjusted n-6 (mg/kg/day)						
≤130.12	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
130.12 to<231.29	0.78 (0.65,0.93)**	0.77 (0.65,0.93)**	0.80 (0.65,0.98)*	0.94 (0.75,1.18)	0.98 (0.78,1.23)	1.01 (0.78,1.30)
≥231.29	0.59 (0.49,0.72)**	0.68 (0.55,0.82)**	0.67 (0.53,0.86)**	0.77 (0.61,0.97)*	0.87 (0.69,1.10)	0.91 (0.68,1.23)
n-6:n-3 ratio						
≤8.08	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
8.08 to<10.44	0.84 (0.71,0.99)*	0.93 (0.78,1.11)	0.88 (0.72,1.06)	1.01 (0.81,1.27)	1.14 (0.91, 1.43)	1.07 (0.85,1.36)
≥10.44	0.91 (0.77,1.08)	1.04 (0.87,1.25)	0.97 (0.80,1.18)	1.19 (0.95,1.49)	1.34 (1.06, 1.70)**	1.31 (1.02,1.67)*

Calculated using binary logistic regression. Model 1 adjusted for age. Model 2 adjusted for age, race, educational level, income, marital status, drinking status, smoking status, and total energy intake. *p<0.05; **p<0.01.

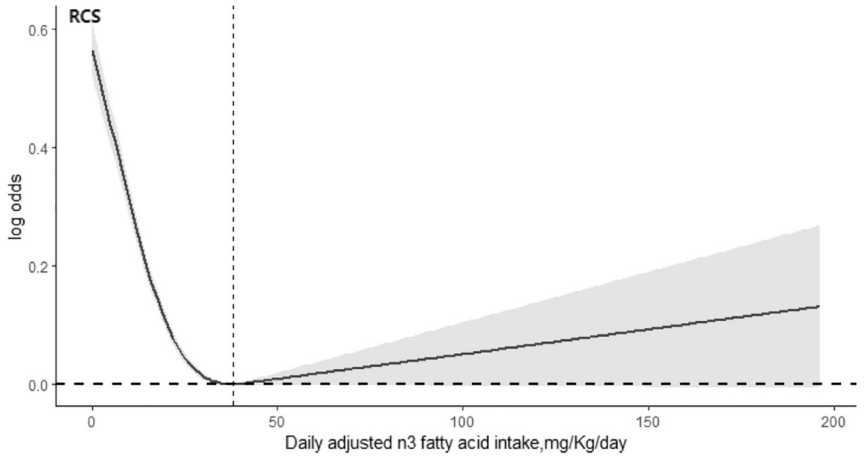

Fig. 2. Dose-response relationship between n-3 fatty acid intake and rheumatoid arthritis.

Fig. 3. Dose-response relationship between n-6 fatty acid intake and rheumatoid arthritis.

of RA. It is crucial to acknowledge that further research is needed to deepen our understanding of these relationships and to validate the findings. Prospective studies on a large scale and investigations utilising more precise dietary survey methodologies need to be employed in order to support and expand upon the current knowledge.

Increasing evidence suggests a potential link between polyunsaturated fatty acids and various human diseases (20). A prospective cohort study indicated that long-term dietary intake of n-3 fatty acids could reduce the risk of RA in women (21). Additionally, an observational study found that high consumption of fatty fish moderately decreases the risk of developing RA (odds ratio: 0.8, 95% confidence interval: 0.6–1.0) (22).

According to prior research, n-3 fatty acids offer a certain level of protection against the generation of autoantibodies related to RA (23); concurrently, the efficacy of n-3 fatty acids in reducing inflammation by lowering the levels of inflammatory biomarkers such as IL-6, TNF-α, and CRP has been supported by (24, 25). Additionally, dietary changes can have a direct beneficial effect on the function of the gut microbiome, improving the intestinal barrier and reducing the state of systemic inflammation. These studies also show that the intake of n-3 fatty acids is beneficial for the prevention and management of RA. For instance, Gan *et al*. (26) conducted a study on a population with a genetic risk to examine the relationship between the use of n-3 fatty acid supplements, the content of n-3 fatty acids in red blood cell membranes, and the presence of anti-CCP autoantibodies. The results indicated that the total content of n-3 fatty acids in red blood cells was inversely associated with the likelihood of producing anti-CCP antibodies (OR: 0.47, 95% CI: 0.24,0.92). This

suggests that n-3 fatty acids may have a protective role in the early stages of RA development.

Similarly, a large body of research indicates that n-3 fatty acids have a potential advantage in alleviating symptoms of RA (27-29). For instance, incorporating fish oil high in n-3 fatty acids into an anti-inflammatory diet is associated with a reduction in the number of joint pains and swellings as well as a decrease in the duration of morning stiffness among RA patients (30). Additionally, supplementing with n-3 fatty acids is related to a reduced dependency on analgesics and anti-rheumatic drugs (31).

In our study, we also investigated the impact of daily n-3 fatty acid ingestion on the risk of developing RA. We initially adjusted the intake based on participants' body weight and found results consistent with prior research. These findings further support the notion that higher ingestion of n-3 fatty acids may potentially lower the risk of RA development. Furthermore, our subsequent nonlinear analysis suggested that a daily intake of 38 mg/kg/day of n-3 fatty acids could be particularly beneficial for patients.

Our study also found a negative relationship between the ingestion of n-6 fatty acids and the risk of rheumatoid arthritis, which supports previous research. It is acknowledged that consuming more of the n-6 fatty acid arachidonic acid (ARA) or its precursor linoleic acid (LA) can increase inflammation. However, studies conducted on healthy adults have indicated that higher intake of ARA or LA does not always result in elevated levels of inflammatory markers (32). In fact, some epidemiological studies have suggested that ARA and LA may be associated with a reduction in inflammation (33). Additionally, a nested case-control study discovered a negative correlation between erythrocyte LA content and the stakes of RA (34).

Additionally, a cross-sectional study conducted on patients with RA found that a dietary pattern characterised by a high consumption of fatty acids derived from vegetables, such as alpha-linolenic acid (ALA) and linoleic acid (LA), was associated with a reduced likelihood of experiencing active disease (35). Similarly, in our own investigation, LA emerged as a significant contributor to the overall n-6 fatty acid pattern. The inverse correlation observed between n-6 fatty acids and the risk of rheumatoid arthritis could potentially be attributed to their anti-inflammatory properties. Notably, research conducted on non-RA populations has provided evidence suggesting that increased ingestion of n-6 polyunsaturated fatty acids is linked with decreased levels of C-reactive protein (CRP) and certain pro-inflammatory cytokines (36, 37). Furthermore, statistical analysis of hsCRP/CRP based on NHANES data from different periods indicates significant differences in inflammatory markers between RA patients and non-RA individuals. After adjusting for these markers, the intake of n-3 and n-6 was also found to reduce the risk of RA. However, this association was not significant during the 2015–2020 period, possibly due to the smaller sample size in this timeframe.

In addition, our analysis indicated that the beneficial effect of n-3 and n-6 fatty acids on the risk of RA may vary across genders, with a more noticeable effect observed in women compared to men. This gender discrepancy in the association can potentially be attributed to sex hormones. Sex hormones have been explained in a detailed review article as having an impact on the enzymes responsible for producing long-chain polyunsaturated fatty acids (LCPUFA) (38). Furthermore, research has shown that males have a notably lesser involvement of arachidonic acid (AA) and docosahexaenoic acid (DHA) in their overall plasma lipids and phospholipids when compared to females. The impact of sex hormones on the metabolism of essential fatty acids in humans has been extensively discussed in a review conducted by Childs *et al*. (39).

We have conducted a research study that possesses several noteworthy advantages. Firstly, our study stands out as the first investigation to examine the correlation between the ingestion of n-3 and n-6 fatty acids and RA by utilising a large sample size derived from the NHANES (National Health and Nutrition Examination Survey) database. By using a large sample size, the strength and applicability of our findings are greatly enhanced. Secondly, considering that the NHANES database incorporates multistage complex sampling data, we used a weighted logistic regression model to analyse the data. This approach enables us to provide an explanation for the intricacies of the intricate sampling framework, thereby ensuring the accuracy and reliability of our study's conclusions. Additionally, we made adjustments for other covariates in our analysis, further reinforcing the accuracy of our findings. Furthermore, in order to more precisely assess the impact of fatty acid ingestion, we initially made adjustments for body weight before conducting the analysis. This adjustment aids in isolating the specific effects of n-3 and n-6 fatty acid ingestion on RA. Finally, to explore the potential non-linear relationship between fatty acid ingestion and RA, we incorporated the use of restrictive cubic spline analysis. This technique allows us to capture any potential inflection points that may exist between fatty acid ingestion and RA.

Although our study has several strengths, it is important to acknowledge some limitations. Firstly, certain variables relied on questionnaires and self-reports, which may introduce bias and impact the accuracy of the results. For instance, similar to the outcomes from the study by Liu *et al*. (40), the prevalence among patients with RA was found to be unusually high, markedly surpassing the rate of around 0.5% as cited by others (41). Additionally, due to the cross-sectional nature of our study, we are unable to establish a direct cause-and-effect relationship between the intake of fatty acids and RA. Conducting longitudinal studies would provide a deeper understanding of this association. Our data gathering method, the 24-hour dietary recall interviews, possesses inherent limitations such as misrecordings and under-reporting due to conscious or unconscious biases, potentially leading to recall bias. Furthermore, the ingestion of n-3 and n-6 fatty acids from dietary supplements was not considered, despite its relevance.

In conclusion, while our study is advantageous due to its extensive sample

size, covariate adjustment, and the use of restrictive cubic spline analysis to explore non-linear relationships, it is crucial to recognise the limitations associated with self-reported data, cross-sectional design, and incomplete dietary information. Additional research is required to overcome these limitations and obtain a more comprehensive understanding of the correlation between the consumption of fatty acids and RA.

Conclusions

The study results indicate a negative association of the ingestion of n-3 and n-6 fatty acids and the prevalence of RA. However, it is crucial to acknowledge that additional large-scale studies conducted in a prospective manner and additional research employing more precise dietary survey methods is necessary to employed to verify and enhance the results.

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