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Are dietary vitamin D, omega-3 fatty acids and folate associated with treatment results in patients with early RA? Data from a population-based study.

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ABSTRACT

Background: Dietary intake of vitamin D and omega-3 fatty acids (FA) may associate with superior response to anti-rheumatic treatments. In addition, dietary folate intake may be associated with worse response to methotrexate (MTX). The aim of this study was to investigate the association between dietary vitamin D, omega-3 FA, folate and treatment results of disease modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis (RA) patients.

Methods: This prospective study was based on data from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, and included 727 early RA patients from 10 hospitals in Sweden. Data on dietary vitamin D, omega-3 FA and folate intake based on food frequency questionnaires were linked with data on European League Against Rheumatism (EULAR) response after three months of DMARD treatment. Associations between vitamin D, omega-3 FA, folate and EULAR response were analyzed with logistic regression adjusted for potential confounders.

Results: The majority of the patients (89.9%) were initially treated with MTX monotherapy and more than half of the patients (56.9%) with other DMARDs and glucocorticoids. Vitamin D and omega-3 FA were associated with good EULAR response (OR=1.80 [95% CI 1.14-2.83] and OR=1.60 [95% CI 1.02-2.53], respectively). Folate did not significantly associate with EULAR response (OR=1.20 [95% CI 0.75-1.91]). Similar results were seen in subgroup of patients who were initially treated with MTX monotherapy at baseline.

Conclusions: Higher intake of dietary vitamin D and omega-3 FA during the year preceding DMARD initiation may associate to better treatment results in early RA patients. Dietary folate intake was not associated with worse or better response to treatment, especially of MTX. Our results suggest that some nutrients may associate with enhanced treatment results of DMARDs.

ARTICLE SUMMARY

Strengths and limitations

- This is the first study to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA.

- This study may contribute to better understanding of dietary impact on anti-rheumatic treatment in order to achieve optimal treatment results.
- Reported dietary data is only an estimation of the actual dietary intake.

BACKGROUND

Treatment of rheumatoid arthritis (RA) has the aim of providing relief from the symptoms associated with inflammation, pain and stiffness, and of preventing long-term disability.[1] Disease modifying anti-rheumatic drug (DMARD) can be effective in treating inflammation, delaying joint damage and improving RA patient outcomes.[2 3] Methotrexate (MTX) is the most widely used DMARD for early RA. Low dose glucocorticoids (GC) can be added to the treatment with MTX in order to achieve better outcomes.[4]

The interest in dietary factors and RA has been increasing among researchers and patients with RA over the last decade. However, there is a lack of studies that have specifically investigated how certain nutrients may associate with treatment results in newly diagnosed patients with RA. Nutrients might influence the efficacy of DMARDs through influencing the gastrointestinal absorption of the drug and pharmacokinetics in other ways, or might influence the pharmacodynamic effects of the drugs.[5]

Vitamin D is involved in various aspects of inflammation and immunity. It is known that serum vitamin D levels are decreased in patients with RA, this has also been observed in RA patients in Sweden.[6-10] Some evidence suggests that vitamin D deficiency can trigger autoimmune response and therefore may provide an immunoregulatory effect.[11 12] Vitamin D deficiency has been associated with increased risk of RA.[10] The active metabolite of vitamin D ($1,25(OH)_2D$) inhibits the synthesis of pro-inflammatory interleukins (IL) such as IL-1, IL-6, IL-12 and tumor necrosis factor (TNF) by macrophages.[13] It also decreases major histocompatibility complex, class II (MHC-II) expression and cell surface molecules of MCH-II such as cluster of differentiation (CD)86, CD80 and CD40.[14] Vitamin D supplementation has been shown to decrease the disease activity in short-term in RA patients.[15]

Earlier data suggest that omega-3 FA is anti-inflammatory and can decrease disease activity in RA.[16-19] Omega-6 FA as well as omega-6:3 FA (ratio) have been found to be pro-inflammatory in RA.[20] Increased dietary intake of omega-3 FA has been associated with decreased serum levels of TNF- and C-reactive protein (CRP) in RA.[21] Resolvins are omega-3 FA derived eicosanoids with anti-inflammatory properties. They indirectly dampen the production of pro-inflammatory omega-6 FA derived eicosanoids, such as leukotrienes and prostaglandins, by inhibiting lysyl oxidase and

cyclooxygenase 1 and 2.[22] Therefore, it is of importance to have a good balance between omega-6 and omega-3.[23]

A well-known nutrient-drug interaction is between MTX and folate. MTX is a folate antagonist.[24] Folate stores are decreased in RA patients treated with MTX. Impaired folate status is also related to MTX toxicity. Gastrointestinal intolerance is one of the side effects of MTX, and folic acid and folinic acid supplementation have been shown to reduce the mucosal and gastrointestinal side effects during low dose of MTX treatment.[25] [26] Although MTX is believed to work through folate antagonism, it has been hypothesized that the anti-inflammatory effect of this drug might be due to its stimulation of adenosine release that prevents inflammatory cells from attaching to connective tissues.[27-29]

We hypothesized that higher dietary intake of vitamin D and omega-3 FA, may associate with superior response to anti-rheumatic treatments, whereas higher dietary folate intake might associate with a worse response to MTX. The aim of this study was to investigate the association between dietary intake of vitamin D, omega-3 FA, folate and treatment results of DMARDs in patients with early RA.

MATERIAL AND METHODS

Study participants

This study included initially 1,296 newly diagnosed RA patients (disease duration ≤12 months) from a population-based case-control study called Epidemiological Investigation of Rheumatoid Arthritis (EIRA). EIRA was initiated in May 1996 and the study design has previously been described.[30] Participants of EIRA were asked to complete food frequency questionnaires (FFQs) at inclusion/baseline. EIRA has been linked to the Swedish Rheumatology Quality register (SRQ) in order to provide clinical data. The study period for this report was from October 2005 to March 2012. Patients who did not fully complete the FFQ and/or had missing data on DMARD use at baseline as well as treatment results (EULAR response) after three months were excluded. After exclusions, 727 patients remained for analyses. (Figure 1.) This study was approved by the Regional Ethical Review Board at Karolinska institutet, Stockholm, Sweden.

Dietary assessment

EIRA participants were asked to complete a FFQ regarding their food intake at baseline. This self-administered, semi-quantitative FFQ included questions regarding frequency intake of 123 food items and beverages during the previous year before baseline. Pre-specified food frequency intake ranged over eight categories from *Never* to *≥3 times per day*. For frequently consumed foods, open questions were used and the participants could fill in number of slices, cups, glasses etc. The respondents could also specify their usual portion sizes (i.e. small, medium, large in relation to pre-specified medium size. Dietary nutrient intake of vitamin D (µg/day), omega-3 FA (g/day) and folate (µg/day) were calculated by multiplying the average frequency of consumption of each food item by the nutrient content of age and sex specific portion sizes.[31] Total omega-3 FA intake was calculated based on the most common FA of omega-3 ((alpha-linolenic acid, C18:3/10) + eicosapentaenoic acid, C20:5 + docosapentaenoic acid, C22:5 + docosahexaenoic acid, C22:6).[32 33] All dietary nutrients were energy-adjusted to the mean energy intake in the study population (1939 kcal) using the residual method.[34] This dietary assessment method and the validation of this FFQ have been described previously.[35 36] The FFQ included also questions regarding dietary supplement use of vitamin D, omega-3 FA/fish oil as well as folic acid (yes or no) during the previous year before baseline.

The estimated FFQ-based intakes of vitamin D-rich foods were validated in comparison to 4 x 1-week weighed dietary records among 129 persons. Pearson's correlations (*r*) between reported dietary vitamin D intake and vitamin D–fortified reduced-fat dairy products, vitamin D–fortified margarines and fatty fish were 0.60–0.70, 0.30-0.70 and 0.50, respectively (Wolk, A. et al., unpublished observations, 1992). Furthermore, serum concentrations of 25(OH)D among 116 persons have been significantly correlated with the FFQ-based vitamin D intake of fatty fish (r = 0.21, p = 0.02) and vitamin D-fortified reduced-fat dairy products (r = 0.20, p = 0.04).[37] Estimate of FFQ-based omega-3 FA intake has been validated in comparison to adipose tissue composition in 239 persons and in comparison to 4 x 1-week weighed dietary records in 184 persons. Pearson's correlation between the estimated intake of omega-3 FA and adipose tissue was 0.41[38] and 0.40 with dietary records.[39] Pearson's correlation was 0.50 between the estimated intake of folate and 4 x 1-week weighed dietary records of folate intake (n=129).[40]

Additional assessments

In addition to the FFQ, the EIRA participants were asked to report their age, weight, height, smoking habits, education and physical activity (PA) level. The EIRA questionnaire included questions regarding smoking status (never, former or current smoker) as well as smoking duration and intensity (pack-year). Pack-years were categorized into 0–9, 10–19 and 20+ pack-years; one pack-year was equal to 20 smoked cigarettes per day during one year.[41] Education level was categorized into high school and university degree. The participants reported the amount of PA performed during the previous year before inclusion in the EIRA study. PA was categorized into four groups: sedentary PA, moderate occasional PA, moderate regular PA and regular exercise.

Treatment results assessment

Treatment results were assessed using European League Against Rheumatism (EULAR) response criteria, which is validated tool for assessing treatment efficacy in RA.[42-44] It includes non-, moderate and good response to treatment. EULAR response was determined based on changes in 28-joint disease activity score (DAS28) from baseline/treatment initiation up to three months as well as the endpoint DAS28 at three months. EULAR response was studied primarily in all patients with any DMARD at baseline, and then specifically in all patients with MTX monotherapy at baseline. In this study, non- and moderate EULAR response were merged into one category to increase stratified power.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 23. Associations between dietary intake of vitamin D, omega-3 FA, folate and EULAR response, respectively, were analyzed with logistic regression. All multivariate analyses were adjusted for potential confounders such as age, sex, smoking, total energy intake, supplement use (vitamin D, omega-3 FA/fish oil and folic acid), BMI (weight (Kg)/[height (m)]^2), education, PA and anti-rheumatic treatment at baseline. Dietary intakes of vitamin D, omega-3 FA and folate were divided into quartiles based on intake of all the EIRA participants including the controls. The first quartile was the referent group. Age was categorized into eleven age groups of five-year range each, total energy intake into quartiles, supplement use into yes and no, BMI into below 25 and above 25 Kg/m², education into high school degree and university degree, and PA into sedentary to occasional moderate PA and moderate regular PA to regular exercise. Use of anti-rheumatic treatment was categorized into yes and no for each treatment. Smoking adjustment was based on pack-years.

RESULTS

Patient characteristics

This study included 727 RA patients. Baseline characteristics for the total study sample are presented in table 1. The proportion of current smokers was 31.9% and obtained university degree 25.2%. The proportions of patients who had performed moderate regular PA to regular exercise was 30.7%.

Table 1. Clinical baseline characteristics.

	N=727
Female, %	72.6
Age (years), mean ± SD	52.5 ± 13.1
BMI, (Kg/m²), mean ± SD	25.7 ± 4.6
Symptom duration (days), mean ± SD	302.3 ± 419.1
Rheumatoid factor, positive, %	51.4
ACPA positive, %	66.2
DAS28, mean ± SD	5.2 ± 1.3
HAQ, mean ± SD	1.0 ± 0.6
CRP (mg/L), mean ± SD	22.6 ± 29.8
Pain (VAS 0-100 mm), mean ± SD	53.5 ± 24.7
Patients' global assessment (VAS 0-100 mm), mean ± SD	51.0 ± 24.4
Physicians' global assessment (5-point scale), mean ± SD	2.2 ± 0.7
SJC, mean ± SD	9.2 ± 5.4
TJC, mean ± SD	8.2 ± 5.9

ACPA, anti–citrullinated protein antibody; BMI, body mass index; CRP, C-reactive protein; DAS28, 28-joint disease activity score; HAQ, health assessment questionnaire; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale

After three months, 399 patients (54.9%) had non- to moderate EULAR response and 328 patients (45.1%) had good EULAR response. Good responders, in comparison to non- to moderate responders, had significantly lower BMI (25.1 ± 4.4 versus 26.2 ± 4.8 Kg/m^2, p=0.005) and lower baseline TJC (TJC: 7.5 ± 6.3 versus 8.8 ± 6.3, p=0.019). The remaining baseline characteristics as well as smoking status did not differ significantly between non- to moderate and good responders.

Treatment use

The majority of the patients (89.9%) were initially treated with MTX monotherapy, 5.9% with sulfasalazine (SSZ) and 2.3% with triple therapy (MTX, SSZ, hydroxychloroquine (HCQ)). More than half of the patients (56.9%) combined their DMARD treatment with GC. Similar pattern of treatment use was seen at the three month follow-up. (Table 2) The comparison of treatment patterns between the different EULAR response groups after three months showed that triple therapy at baseline was more common in good responders than non- to moderate responders (3.7% versus 1.7%, p=0.046), and combined therapy of MTX and SSZ at three months was more common in non- to moderate responders (4.3% versus 1.0%, p=0.009).

Table 2. Treatment use at baseline and at three months follow-up.

	Baseline		3 months	
Treatment	**N (%)**	**GC use n (%)**	**N (%)**	**GC use n (%)**
MTX	653 (89.9)	373 (90.1)	579 (79.6)	351 (85.8)
SSZ	43 (5.9)	18 (4.3)	31 (4.3)	18 (4.4)
MTX + SSZ + HCQ	17 (2.3)	15 (3.6)	31 (4.3)	18 (4.4)
HCQ	8 (1.1)	3 (0.7)	9 (1.2)	5 (1.2)
LFM	2 (0.3))	1 (0.2)	0 (0)	0 (0)
AZA	1 (0.1)	1 (0.2)	0 (0)	0 (0)
MTX + SSZ	1 (0.1)	1 (0.2)	19 (2.6)	9 (2.2)
HCQ + SSZ	1 (0.1)	0 (0)	2 (0.3)	2 (0.5)
HCQ + AZA	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.2)
GC	414 (56.9)	-	432 (59.4)	-
Missing data	0 (0)	-	46 (6.3)	-

AZA, azathioprine; GC, glucocorticoids; HCQ, hydroxychloroquine; LFM, leuflunomide; MTX, methotrexate; SSZ, sulfasalazine.

Nutrient intake and treatment results

The mean intake of vitamin D, omega-3 FA and folate as well as supplement use in the whole study sample as well as in different EULAR response groups are presenteerd in table 3. Vitamin D intake between the different study centers were not significantly different due to their geographical locations (data not shown). Mean omega-3 FA intake at baseline was significantly higher in good responders. No difference between responders and non-responders was seen for vitamin D and folate

intake. Dietary supplement use did not differ significantly across EULAR response groups. The vitamin D intake was below the recommended daily intake (RDI) and the folate intake was borderline to the RDI according to Nordic Nutrition Recommendations 2012.[45]

Table 3. The mean intake of vitamin D, omega-3 FA, folate and dietary supplement use during the previous year from study inclusion in the whole study sample as well as in different EULAR response groups.

		EULAR response at three months		
Nutrient intake	**Total study sample (N=727)**	**Non-/Moderate (n=399)**	**Good (n=328)**	**p value**
Vitamin D				
Dietary intake, µg/day, mean ± SD	5.86 ± 2.30 [RDI: 10-20]	5.68 ± 2.15	6.07 ± 2.45	0.062
Supplementation, n (%)	57 (7.8)	29 (7.3)	28 (8.5)	0.580
Omega-3 FA				
Dietary intake, g/day, mean ± SD	0.68 ± 0.35 [RDI: ≥1 E%]	0.65 ± 0.30	0.71 ± 0.39	0.040
Supplementation, n (%)	142 (19.5)	85 (21.3)	57 (17.4)	0.222
Folate				
Dietary intake, µg/day, mean ± SD	308.39 ± 107.09 [RDI: 300-400]	308.28 ± 115.00	308.52 ± 101.57	0.417
Supplementation, n (%) *	113 (15.5)	67 (16.8)	46 (14.0)	0.355

RDI, Recommended daily intake accoring to Nordic Nutrition Recommendations 2012.[45] The recommendations are age and gender specific. Women and men ≥75 years are recommended a daily intake of 20 µg of vitamin D. Both women and men are recommended a daily intake of omega-3 fatty acids that equals 1 energy percent (E%) of the total daily fat intake. Women in fertile age are recommended a daily intake of 400 µg of folate, other women as well as men are recommended 300 µg.

* Folate supplementation use *before* treatment start. All patients who eventually started MTX treatment were also recieving folate supplements.

Dietary vitamin D and omega-3 FA intake were associated with good EULAR response, after adjustment for age, sex, smoking, total energy intake, supplementation, BMI, education and PA. However, dietary folate intake did not significantly associate with EULAR response. (Table 4) Additional analysis showed that omega-6:3 FA did not associate with EULAR response (OR=0.73 [95% CI 0.47-1.14]).

Table 4. Association between dietary intake of vitamin D, omega-3 FA, folate and EULAR response after three months.

Nutrient intake	**N**	**OR (95 % CI) Age and sex adj**	**OR (95 % CI) Multivariable adj**

Vitamin D	**727**		
1st quartile: ≤4.25 µg/day	182	1.00	1.00
2nd quartile: 4.26-5.42 µg/day	170	1.07 (0.70-1.64)	0.94 (0.60-1.49)
3rd quartile: 5.43-6.96 µg/day	184	1.15 (0.75-1.77)	1.13 (0.72-1.79)
4th quartile: >6.97 µg/day	191	1.75 (1.13-2.71)	1.60 (1.00-2.56)
p value, quartile 4 versus 1		0.012	0.048
Omega-3 FA	**727**		
1st quartile: ≤0.45 g/day	180	1.00	1.00
2nd quartile: 0.46-0.62 g/day	192	1.25 (0.82-1.89)	1.33 (0.85-2.06)
3rd quartile: 0.63-0.83 g/day	183	1.35 (0.89-2.07)	1.36 (0.87-2.15)
4th quartile: >0.84 g/day	172	1.64 (1.07-2.53)	1.71 (1.08-2.72)
p value, quartile 4 versus 1		0.024	0.023
Folate	**727**		
1st quartile: ≤244.88 µg/day	201	1.00	1.00
2nd quartile: 244.89-296.86 µg/day	182	1.32 (0.88-1.99)	1.35 (0.87-2.09)
3rd quartile: 296.87-365.70 µg/day	193	1.59 (1.07-2.38)	1.59 (1.03-2.45)
4th quartile: >365.71 µg/day	151	1.14 (0.74-1.75)	1.09 (0.68-1.75)
p value, quartile 4 versus 1		0.557	0.721

Multivariable adjustment for age (eleven 5-year age groups), sex, smoking pack-years, total energy intake (tertiles), supplementation (vitamin D, omega-3 FA/fish oil and folic acid), BMI (≤25 and >25), education level (high school and university) and PA (sedentary PA, moderate occasional PA, moderate regular PA and regular exercise), and use of DMARDs and GC baseline (yes or no).

p value: Comparison between 4th and 1st quartiles.

Similar results were seen in subgroup of the 653 patients who were initially treated with MTX monotherapy at baseline (OR=1.63 [95% CI 1.03-2.57] for vitamin D, OR=1.65 [95% CI 1.05-2.60] for omega-3 FA and OR=1.20 [95% CI 0.76-1.89] for folate, after adjustment for age and gender).

Dietary supplementation alone did not associate with EULAR response (OR=1.27 [95% CI 0.73-2.20] for vitamin D, OR=81 [95% CI 0.56-1.87] for omega-3 FA and OR=0.82 [95% CI 0.54-1.23] for folate, after adjustment for age and gender). In addition, patients who took supplements did not have significantly higher dietary intake of the three nutrients, compared to the patients without supplementation.

DISCUSSION

This study investigated the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. Omega-3 FA intake was significantly higher in patients with good response compared to patients with non- to moderate response. Higher vitamin D and omega-3 FA intakes were associated with good EULAR response. However, no association was found between folate intake and EULAR response. Simular results were observed in subgroup of patients who were initially treated with MTX monotherapy at baseline. Dietary supplementation of vitamin D, omega-3 FA and folate alone did not associate with EULAR response.

Several nutrients and drugs interact, and these nutrient-drug interactions can lead to nutrient imbalances or interfere with drug effectiveness.[46] Nutrients obtained from food intake may act differently during a long-term treatment. Drugs may modify the absorption, metabolism, and excretion of nutrients and vice versa; nutrients may interact with the absorption, metabolism, and excretion of drugs. Patients with chronic diseases, such as RA, might be experiencing important adverse nutrient-drug interactions since drugs are taken over long periods.

Vitamin D, omega-3 FA, folate and EULAR response

We found that higher intake of dietary vitamin D was associated with good EULAR response. A study, performed in Philadelphia, USA, showed no association between vitamin D concentration levels and clinical response to therapy using American College of Rheumatology (ACR) response in treatment naïve RA patients,[47] however, in contrast to our study the vitamin D intake in particular was not in focus. Only 15-20% of the vitamin D in blood originate directly from diet, the rest is produced during sunlight exposure.[48] Vitamin D deficiency measured in blood is common in patients with RA and has been associated with disease activity and inflammatory markers.[12 49] Vitamin D deficiency in RA patients in Sweden might be an issue due to less sun exposure, there is enough UV radiation from the sun to produce vitamin D during only 6 months per year. Therefore, increased vitamin D intake through either diet or supplementation might be of importance. Our results suggest that increased dietary intake of vitamin D before and/or during DMARD start may be associated with improved treatment outcome in RA patients. This finding requires confirmation.

Dietary omega-3 FA intake was associated with good EULAR response. Evidence suggests that long-chain omega-3 FA have anti-inflammatory properties and are beneficial in the treatment of autoimmune and inflammatory conditions.[50 51] Combination of MTX and omega-3 has shown a

significant reduction in liver enzyme activities.[52] In line with our results, a recent study has suggested that biomarkers of omega-3 FA may predict clinical outcomes relevant to standard drug treatment of RA patients.[53] In addition to the anti-inflammatory effect of the DMARDs, omega-3 FA may have a supplementary role in reducing inflammation and/or achieving better treatment outcome in early RA.

MTX was used by the majority of the patients. We did not find that higher dietary folate intake before starting with MTX and/or other DMARDs was associated with worse treatment response. Folate fortification in food items such as flour, rice, pasta, and other grain products has been common in the last two decades in order to primarily prevent neural tube defect in unborn children,[54 55] but not in Sweden. Folic supplementation in RA patients using MTX has shown to prevent side effects of the drug.[25] Dietary folate intake during MTX treatment might have adverse effects on MTX efficacy. Folic acid fortification in foods has been associated with requirement for higher MTX dose in a small study of RA patients.[56] However, in Sweden there is no obligatory fortification of food. Results from our study, performed in a country where there is no obligatory fortification, suggest that dietary folate intake before starting with MTX and/or other DMARDs did not associate with inferior EULAR response.

This study did not show any association between dietary supplementation and EULAR response, although, several studies have reported beneficial effect of supplementation of vitamin D and omega-3 FA/fish oil) in particular.[50 51 57 58] This could partly be explained due higher bioavailability of the nutrients in foods rather than supplements.

Strengths and limitations

This study is to our knowledge the first to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. The study included a large number of participants that were representative of RA patients in Sweden. The FFQ used in this study was highly validated.

Serum levels of vitamin D, omega-3 FA and folate were not taken into account in this study. Dietary data from FFQ were based on estimated dietary consumption as assessed close to the start of treatment. Under and over reporting may have occurred when completing the FFQ. This may have introduced non-differential misclassification of exposure, which would result that the odds ratios for

the comparisons between the extreme groups (4th versus 1st quartile) were biased towards the null value. Dietary patterns were assumed to be unchanged during the first three months from baseline. Clinical RA manifestations, treatment history, doses, adherence and side effects of DMARDs from treatment initiation were not considered in this study due to lack of data. The majority of the patients were treated with MTX, the number of patient with other DMARDs were limited in order to perform sensitivity analysis in subgroups of other DMARDs.

Conclusions

Higher intake of dietary vitamin D and omega-3 FA during the previous year from DMARD initiation may associate to better treatment results in early RA patients. Higher dietary folate intake was not associated with worse response to MTX. These results, if confirmed, suggest that dietary interventions may be of interest in the management of RA, not only for reasons of optimizing general health but also for achieving optimal results with anti-rheumatic medications.

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Competing interests

We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Authors' contribution

LA was instrumental in establishing the EIRA study. LN provided data from the EIRA master file. RVV played a major role in the study design and drafting the manuscript together with CL. CL performed all the statistical analyses. RVV, AW and LA contributed to the interpretation of the results from a clinical, nutritional and epidemiological aspect, respectively. All authors approved the final manuscript.

Data sharing

No additional data available.

REFERENCES

1. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24.
2. Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73(3):492-509. doi: 10.1136/annrheumdis-2013-204573
3. Cohen S, Cannon GW, Schiff M, et al. Two-year, blinded, randomized, controlled trial of treatment of active rheumatoid arthritis with leflunomide compared with methotrexate. Utilization of Leflunomide in the Treatment of Rheumatoid Arthritis Trial Investigator Group. *Arthritis Rheum* 2001;44(9):1984-92. doi: 10.1002/1529-0131(200109)44:9<1984::AID-ART346>3.0.CO;2-B
4. Svensson B, Hafstrom I. [Evidence for low-dosage prednisolone in early rheumatoid arthritis. Prevents destruction of the joints and relieves symptoms]. *Lakartidningen* 2006;103(47):3704-9. [published Online First: 2007/01/11]
5. Zhang L, Brett CM, Giacomini KM. Role of organic cation transporters in drug absorption and elimination. *Annu Rev Pharmacol Toxicol* 1998;38:431-60. doi: 10.1146/annurev.pharmtox.38.1.431
6. Costenbader KH, Feskanich D, Holmes M, et al. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008;67(4):530-5. doi: 10.1136/ard.2007.072736
7. Merlino LA, Curtis J, Mikuls TR, et al. Vitamin D intake is inversely associated with rheumatoid arthritis - Results from the Iowa Women's Health Study. *Arthritis and rheumatism* 2004;50(1):72-77. doi: Doi 10.1002/Art.11434
8. Sabbagh Z, Markland J, Vatanparast H. Vitamin D Status Is Associated with Disease Activity among Rheumatology Outpatients. *Nutrients* 2013;5(7):2268-75. doi: 10.3390/nu5072268 [published Online First: 2013/06/28]
9. Kerr GS, Sabahi I, Richards JS, et al. Prevalence of Vitamin D Insufficiency/Deficiency in Rheumatoid Arthritis and Associations with Disease Severity and Activity. *Journal of Rheumatology* 2011;38(1):53-59. doi: 10.3899/jrheum.100516
10. Song GG, Bae SC, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. *Clinical Rheumatology* 2012;31(12):1733-39. doi: 10.1007/s10067-012-2080-7
11. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun Rev* 2011;11(2):84-7. doi: 10.1016/j.autrev.2011.08.003
12. Cutolo M, Straub RH. Insights into endocrine-immunological disturbances in autoimmunity and their impact on treatment. *Arthritis Res Ther* 2009;11(2) doi: ARTN 21810.1186/ar2630
13. Luo J, Wen H, Guo H, et al. 1,25-dihydroxyvitamin D3 inhibits the RANKL pathway and impacts on the production of pathway-associated cytokines in early rheumatoid arthritis. *Biomed Res Int* 2013;2013:101805. doi: 10.1155/2013/101805
14. Almerighi C, Sinistro A, Cavazza A, et al. 1 alpha,25-Dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in Human Monocytes. *Cytokine* 2009;45(3):190-97. doi: 10.1016/j.cyto.2008.12.009
15. Chandrashekara S, Patted A. Role of vitamin D supplementation in improving disease activity in rheumatoid arthritis: An exploratory study. *Int J Rheum Dis* 2015 doi: 10.1111/1756-185X.12770
16. Wall R, Ross RP, Fitzgerald GF, et al. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010;68(5):280-9. doi: 10.1111/j.1753-4887.2010.00287.x
17. Ariza-Ariza R, Mestanza-Peralta M, Cardiel MH. Omega-3 fatty acids in rheumatoid arthritis: an overview. *Semin Arthritis Rheum* 1998;27(6):366-70.

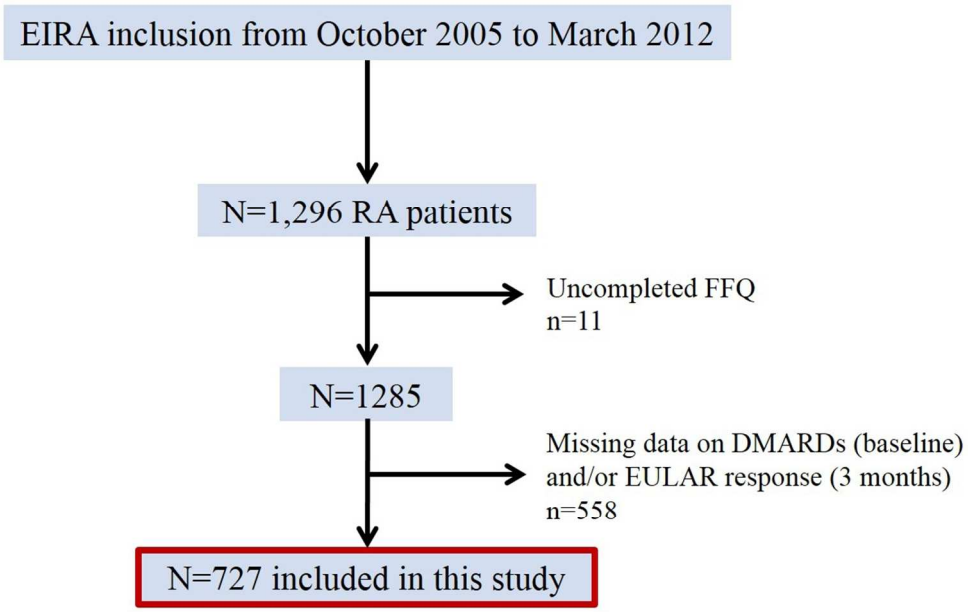
18. Adam O, Beringer C, Kless T, et al. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol Int* 2003;23(1):27-36. doi: 10.1007/s00296-002-0234-7
19. Sperling RI. The effects of dietary n-3 polyunsaturated fatty acids on neutrophils. *Proc Nutr Soc* 1998;57(4):527-34.
20. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Semin Arthritis Rheum* 1997;27(2):85-97.
21. Sundrarjun T, Komindr S, Archararit N, et al. Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble tumour necrosis factor receptor p55 in active rheumatoid arthritis. *J Int Med Res* 2004;32(5):443-54.
22. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie* 2009;91(6):791-5. doi: 10.1016/j.biochi.2009.01.008
23. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002;56(8):365-79.
24. Dijkmans BA. Folate supplementation and methotrexate. *Br J Rheumatol* 1995;34(12):1172-4.
25. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121(11):833-41.
26. Ortiz Z, Shea B, Suarez Almazor M, et al. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *Cochrane Database Syst Rev* 2000(2):CD000951. doi: 10.1002/14651858.CD000951
27. Cronstein BN, Eberle MA, Gruber HE, et al. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci U S A* 1991;88(6):2441-5.
28. Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest* 1993;92(6):2675-82. doi: 10.1172/JCI116884
29. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacol Rev* 2005;57(2):163-72. doi: 10.1124/pr.57.2.3
30. Stolt P, Bengtsson C, Nordmark B, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Annals of the Rheumatic Diseases* 2003;62(9):835-41. doi: DOI 10.1136/ard.62.9.835
31. Bergström L KE, Hagman U, Eriksson HB, Bruce Å. Livsmedelsdatasystemet KOST - livsmedelsverkets system för information om näringsvärden i livsmedel. *Vår Föda* 1991;43(8):439-47.
32. Gerster H. Can adults adequately convert alpha-linolenic acid (18 : 3n-3) to eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic acid (22 : 6n-3)? *International Journal for Vitamin and Nutrition Research* 1998;68(3):159-73.
33. Davis BC, Kris-Etherton PM. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. *American Journal of Clinical Nutrition* 2003;78(3):640s-46s.
34. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65(4 Suppl):1220S-28S; discussion 29S-31S.
35. Messerer M, Johansson SE, Wolk A. The validity of questionnaire-based micronutrient intake estimates is increased by including dietary supplement use in Swedish men. *Journal of Nutrition* 2004;134(7):1800-05.
36. Larsson SC, Hakansson N, Giovannucci E, et al. Folate intake and pancreatic cancer incidence: A prospective study of Swedish women and men. *Journal of the National Cancer Institute* 2006;98(6):407-13. doi: 10.1093/jnci/djj094
37. Burgaz A, Akesson A, Oster A, et al. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. *American Journal of Clinical Nutrition* 2007;86(5):1399-404.

38. Wallin A, Di Giuseppe D, Burgaz A, et al. Validity of food frequency questionnaire-based estimates of long-term long-chain n-3 polyunsaturated fatty acid intake. *Eur J Nutr* 2014;53(2):549-55. doi: 10.1007/s00394-013-0563-1 [published Online First: 2013/07/28]
39. Wolk A, Ljung H, Vessby B, et al. Effect of additional questions about fat on the validity of fat estimates from a food frequency questionnaire. *European Journal of Clinical Nutrition* 1998;52(3):186-92. doi: DOI 10.1038/sj.ejcn.1600538
40. Larsson SC, Giovannucci E, Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. *J Natl Cancer Inst* 2004;96(5):396-402.
41. Kallberg H, Ding B, Padyukov L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Annals of the Rheumatic Diseases* 2011;70(3):508-11. doi: 10.1136/ard.2009.120899
42. van Gestel AM, Prevoo ML, van 't Hof MA, et al. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis and rheumatism* 1996;39(1):34-40.
43. van Gestel AM, Anderson JJ, van Riel PL, et al. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. *Journal of Rheumatology* 1999;26(3):705-11.
44. Verhoeven AC, Boers M, van Der Linden S. Responsiveness of the core set, response criteria, and utilities in early rheumatoid arthritis. *Annals of the rheumatic diseases* 2000;59(12):966-74.
45. Tetens I, Pedersen, A.G., Schwab, U., Fogelholm, M., Thorsdottir, I., Gunnarsdottir, I., Anderssen, S.A., Möltzer, H.M., Becker, W., Koivisto Hursti, U.K., Wirfält, E., editor. *Nordic Nutrition Recommendations 2012*. 5th edition ed: Nord 2014:002, 2014.
46. Sorensen JM. Herb-drug, food-drug, nutrient-drug, and drug-drug interactions: mechanisms involved and their medical implications. *Journal of alternative and complementary medicine* 2002;8(3):293-308. doi: 10.1089/10755530260127989 [published Online First: 2002/08/08]
47. Baker JF, Baker DG, Toedter G, et al. Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. *Clinical and Experimental Rheumatology* 2012;30(5):658-64.
48. Reboul E. Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food Funct* 2015;6(2):356-62. doi: 10.1039/c4fo00579a
49. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmunity reviews* 2011;11(2):84-7. doi: 10.1016/j.autrev.2011.08.003 [published Online First: 2011/08/26]
50. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;83(6 Suppl):1505S-19S.
51. Stamp LK, James MJ, Cleland LG. Diet and rheumatoid arthritis: a review of the literature. *Semin Arthritis Rheum* 2005;35(2):77-94. doi: 10.1016/j.semarthrit.2005.05.001
52. Mohammad BI, Hadi NR, Thwainee AS. The effect of Omega-3 and Selenium on Methotrexate-Induced Hepatotoxicity. *Drug Metabolism Reviews* 2010;42:251-51.
53. Proudman SM, Cleland LG, Metcalf RG, et al. Plasma n-3 fatty acids and clinical outcomes in recent-onset rheumatoid arthritis. *British Journal of Nutrition* 2015;114(6):885-90. doi: 10.1017/S0007114515002718
54. Jacques PF, Selhub J, Bostom AG, et al. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *The New England journal of medicine* 1999;340(19):1449-54. doi: 10.1056/NEJM199905133401901
55. Kim YI. Folic acid supplementation and cancer risk: point. *Cancer Epidemiol Biomarkers Prev* 2008;17(9):2220-5. doi: 10.1158/1055-9965.EPI-07-2557
56. Arabelovic S, Sam G, Dallal GE, et al. Preliminary evidence shows that folic acid fortification of the food supply is associated with higher methotrexate dosing in patients with rheumatoid arthritis. *Journal of the American College of Nutrition* 2007;26(5):453-55.

57. Kostoglou-Athanassiou I, Athanassiou P, Lyraki A, et al. Vitamin D and rheumatoid arthritis. *Ther Adv Endocrinol Metab* 2012;3(6):181-7. doi: 10.1177/2042018812471070

58. Racovan M, Walitt B, Collins CE, et al. Calcium and vitamin D supplementation and incident rheumatoid arthritis: the Women's Health Initiative Calcium plus Vitamin D trial. *Rheumatol Int* 2012;32(12):3823-30. doi: 10.1007/s00296-011-2268-1

Figure 1. Overview of patient exclusions.



Overview of patient exclusions.

208x128mm (150 x 150 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓
Objectives	3	State specific objectives, including any prespecified hypotheses ✓
Methods		
Study design	4	Present key elements of study design early in the paper ✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ✓ *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes ✓, exposures ✓, predictors ✓, potential confounders ✓, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓
		(*b*) Describe any methods used to examine subgroups and interactions ✓
		(*c*) Explain how missing data were addressed ✓
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓
		(b) Give reasons for non-participation at each stage ✓
		(c) Consider use of a flow diagram ✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓
		(b) Indicate number of participants with missing data for each variable of interest ✓
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure ✓
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). ✓ Make clear which confounders were adjusted for and why they were included ✓
		(*b*) Report category boundaries when continuous variables were categorized ✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ✓
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ✓
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. ✓ Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Are dietary vitamin D, omega-3 fatty acids and folate associated with treatment results in patients with early RA? Data from a Swedish population-based prospective study.

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Are dietary vitamin D, omega-3 fatty acids and folate associated with treatment results in patients with early RA? Data from a Swedish population-based prospective study.

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ABSTRACT

Background: Dietary intake of vitamin D and omega-3 fatty acids (FA) may be associated with superior response to anti-rheumatic treatments. In addition, dietary folate intake may be associated with worse response to methotrexate (MTX). The aim of this study was to investigate the association between dietary vitamin D, omega-3 FA, folate and treatment results of disease modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis (RA) patients.

Methods: This prospective study was based on data from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, and included 727 early RA patients from 10 hospitals in Sweden. Data on dietary vitamin D, omega-3 FA and folate intake based on food frequency questionnaires were linked with data on European League Against Rheumatism (EULAR) response after three months of DMARD treatment. Associations between vitamin D, omega-3 FA, folate and EULAR response were analyzed with logistic regression adjusted for potential confounders.

Results: The majority of patients (89.9%) were initially treated with MTX monotherapy and more than half (56.9%) with other DMARDs and glucocorticoids. Vitamin D and omega-3 FA were associated with good EULAR response (OR=1.80 [95% CI 1.14-2.83] and OR=1.60 [95% CI 1.02-2.53], respectively). Folate was not significantly associated with EULAR response (OR=1.20 [95% CI 0.75-1.91]). Similar results were seen in a subgroup of patients who were initially treated with MTX monotherapy at baseline.

Conclusions: Higher intake of dietary vitamin D and omega-3 FA during the year preceding DMARD initiation may be associated with better treatment results in early RA patients. Dietary folate intake was not associated with worse or better response to treatment, especially to MTX. Our results suggest that some nutrients may be associated with enhanced treatment results of DMARDs.

ARTICLE SUMMARY

Strengths and limitations

- This is the first study to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA.

- This study may contribute to better understanding of dietary impact on anti-rheumatic treatment in order to achieve optimal treatment results.
- Reported dietary data are only an estimation of the actual dietary intake.

BACKGROUND

Treatment of rheumatoid arthritis (RA) aims at providing relief from the symptoms associated with inflammation, pain and stiffness and preventing long-term disability.[1] Disease modifying anti-rheumatic drug (DMARD) can be effective in treating inflammation, delaying joint damage and improving RA patient outcomes.[2 3] Methotrexate (MTX) is the most widely used DMARD for early RA. Low dose glucocorticoids (GC) can be added to the treatment with MTX in order to achieve better outcomes.[4]

The interest in dietary factors and RA has been increasing among researchers and patients with RA over the last decade. However, there is a lack of studies that have specifically investigated how certain nutrients may associate with treatment results in newly diagnosed patients with RA. Nutrients might influence the efficacy of DMARDs through influencing the gastrointestinal absorption of the drug and pharmacokinetics in other ways, or might influence the pharmacodynamic effects of the drugs.[5]

Earlier studies in RA focused on vitamin D, omega-3 fatty acids (FA) and folate . Vitamin D deficiency has been associated with an increased risk of RA.[6] Serum vitamin D levels are decreased in patients with RA, and this was also observed in RA patients in Sweden.[6-10] Some evidence suggests that vitamin D deficiency can trigger autoimmune responses and therefore may provide an immunoregulatory effect.[11 12] The active metabolite of vitamin D ($1,25(OH)_2D$) inhibits the synthesis of several interleukins and tumor necrosis factor (TNF).[13] It also decreases the expression of pro-inflammatory surface molecules.[14] Vitamin D supplementation has been shown to decrease disease activity short-term in RA patients.[15]

Earlier data suggest that omega-3 FA is anti-inflammatory and can decrease disease activity in RA.[16-19] Omega-6 FA as well as omega-6:3 FA (ratio) have been found to be pro-inflammatory in RA.[20] Therefore, it is of importance to have a good balance between omega-6 and omega-3.[21] Increased dietary intake of omega-3 FA has been associated with decreased serum levels of TNF- and C-reactive protein (CRP) in RA.[22]

MTX is a folate antagonist.[23] Folate stores are decreased in RA patients treated with MTX.[24] Impaired folate status is also related to MTX toxicity. Gastrointestinal intolerance is one of the side effects of MTX, and folic acid and folinic acid supplementation have been shown to reduce the

mucosal and gastrointestinal side effects during low dose MTX treatment.[25 26] Of note, excessive doses of folinic acid, but not folic acid during MTX therapy for RA can impair efficacy. Although MTX is believed to work through folate antagonism, it has been hypothesized that the anti-inflammatory effect of this drug might be due to its stimulation of adenosine release that prevents inflammatory cells from attaching to connective tissues.[27-29] Folic acid fortification in foods is mandatory in some countries (i.e. US, Canada), but not in Sweden.

We hypothesized that higher dietary intake of vitamin D and omega-3 FA may be associated with superior responses to anti-rheumatic treatments, whereas higher dietary folate intake might be associated with worse response to MTX. The aim of this study was to investigate the association between dietary intake of vitamin D, omega-3 FA, folate and treatment results of DMARDs in patients with early RA.

MATERIAL AND METHODS

Study participants

This study included initially 1,296 newly diagnosed RA patients (disease duration ≤12 months) from a population-based prospective case-control study called Epidemiological Investigation of Rheumatoid Arthritis (EIRA). EIRA was initiated in May 1996 and the study design has previously been described.[30] Participants of EIRA were asked to complete a food frequency questionnaire (FFQ) at inclusion/baseline.[31 32] EIRA has been linked to the Swedish Rheumatology Quality register (SRQ) in order to provide clinical data. The study period for this report was from October 2005 to March 2012. Patients who did not fully complete the FFQ and/or had missing data on DMARD use at baseline as well as treatment results (European League Against Rheumatism (EULAR) response) after three months were excluded. After exclusions, 727 patients remained for analyses. (Figure 1.) This study was approved by the Regional Ethical Review Board at Karolinska institutet, Stockholm, Sweden.

Dietary assessment

EIRA participants were asked to complete a FFQ at baseline. This self-administered, semi-quantitative FFQ included questions regarding frequency intake of 123 food items and beverages during the previous year before baseline. Pre-specified food frequency intake ranged over eight

categories from *Never* to *≥3 times per day*. For frequently consumed foods, open questions were used and the participants could fill in number of slices, cups, glasses etc. Dietary nutrient intake of vitamin D (µg/day), omega-3 FA (g/day) and folate (µg/day) were calculated by multiplying the average frequency of consumption of each food item by the nutrient content, the nutrient content values were obtained from the Swedish Food Administration Database.[33] Total omega-3 FA intake was calculated based on the most common FA of omega-3 ((alpha-linolenic acid, C18:3/10) + eicosapentaenoic acid, C20:5 + docosapentaenoic acid, C22:5 + docosahexaenoic acid, C22:6).[34 35] All dietary nutrients were energy-adjusted to the mean energy intake during the precious year from baseline in the study population using the residual method.[36] This dietary assessment method and the validation of this FFQ have been described previously.[31 32] The FFQ included also questions regarding dietary supplement use of vitamin D, omega-3 FA/fish oil as well as folic acid (yes or no) during the previous year before baseline.

The estimated FFQ-based intakes of vitamin D-rich foods were validated in comparison to 4 x 1-week weighed dietary records among 129 persons. Pearson's correlations (*r*) between reported dietary vitamin D intake and vitamin D–fortified reduced-fat dairy products, vitamin D–fortified margarines and fatty fish were 0.60–0.70, 0.30-0.70 and 0.50, respectively (Wolk, A. et al., unpublished observations, 1992). Furthermore, serum concentrations of 25(OH)D among 116 persons have been significantly correlated with the FFQ-based vitamin D intake of fatty fish (r = 0.21, p = 0.02) and vitamin D-fortified reduced-fat dairy products (r = 0.20, p = 0.04).[37] Estimate of FFQ-based omega-3 FA intake has been validated in comparison to adipose tissue composition in 239 persons and in comparison to 4 x 1-week weighed dietary records in 184 persons. Pearson's correlation between the estimated intake of omega-3 FA and adipose tissue was 0.41[38] and 0.40 with dietary records.[39] Pearson's correlation was 0.50 between the estimated intake of folate and 4 x 1-week weighed dietary records of folate intake (n=129).[40] The validity of a FFQ can differ between different populations.

Additional assessments

In addition to the FFQ, the EIRA participants were asked to report their age, weight, height, smoking habits, education and physical activity (PA) level. The EIRA questionnaire included questions regarding smoking status (never, former or current smoker) as well as smoking duration and intensity (pack-year). Pack-years were categorized into 0–9, 10–19 and 20+ pack-years; one pack-year was equal to 20 smoked cigarettes per day during one year.[41] Education level was categorized into high

school and university degree. The participants reported the amount of PA performed during the previous year before inclusion in the EIRA study. PA was categorized into four groups: sedentary PA, moderate occasional PA, moderate regular PA and regular exercise.

Treatment result assessment

Treatment results were assessed using EULAR response criteria, which is validated tool for assessing treatment efficacy in RA.[42-44] It includes non-, moderate and good response to treatment. EULAR response was determined based on changes in 28-joint disease activity score (DAS28) from baseline/treatment initiation up to three months as well as the endpoint DAS28 at three months. EULAR response was studied primarily in all patients with any DMARD at baseline, and then specifically in all patients with MTX monotherapy at baseline. In this study, non- and moderate EULAR response were merged into one category to increase stratified power.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 23. Baseline characteristics were compared between non-/moderate responders and good responders after three months with Mann-Whitney U test or Kruskal-Wallis test for continuous variables (mean and standard deviation (SD)) as well as Pearson's chi-square test for proportions (%). Further analyses included comparisons of dietary intake of vitamin D, omega-3 FA and folate between different subgroups of patients based on age, sex, smoking, supplement use, BMI, education, PA, geographical locations and season (spring/summer versus fall/winter) at EIRA inclusion, with Mann-Whitney U test and Kruskal-Wallis test.

Associations between dietary intake of vitamin D, omega-3 FA, folate and EULAR response, respectively, were analyzed with logistic regression. All multivariate analyses were adjusted for potential confounders such as age, sex, smoking, total energy intake, supplement use (vitamin D, omega-3 FA/fish oil and folic acid), BMI (weight (Kg)/[height (m)]^2), education, PA and anti-rheumatic treatment at baseline. Dietary intakes of vitamin D, omega-3 FA and folate were divided into quartiles based on intake of all the EIRA participants including the controls. The first quartile was the referent group. Age was categorized into eleven age groups of five-year range each, total energy intake into quartiles, supplement use into yes and no, BMI into ≤25 and >25 Kg/m², education into high school degree and university degree, and PA into sedentary to occasional

moderate PA and moderate regular PA to regular exercise. Use of anti-rheumatic treatment was categorized into yes and no for each treatment. Smoking adjustment was based on pack-years. The analyses were primarily including the total study sample regardless of treatment (n=727). The same analyses were also performed separately on patients with only MTX monotherapy (n=653). In addition, association between omega-3/fish oil supplementation and EULAR response were analyzed.

RESULTS

Patient characteristics

This study included 727 RA patients. Baseline characteristics for the total study sample are presented in table 1. The proportion of current smokers was 31.9% and obtained university degree 25.2% (no one had an education level below high school degree). The proportions of patients who had performed moderate regular PA to regular exercise was 30.7%.

Table 1. Clinical baseline characteristics.

	N=727
Female, %	72.6
Age (years), mean ± SD	52.5 ± 13.1
BMI, (Kg/m²), mean ± SD	25.7 ± 4.6
Symptom duration (days), mean ± SD	302.3 ± 419.1
Rheumatoid factor, positive, %	51.4
ACPA positive, %	66.2
DAS28, mean ± SD	5.2 ± 1.3
HAQ, mean ± SD	1.0 ± 0.6
CRP (mg/L), mean ± SD	22.6 ± 29.8
Pain (VAS 0-100 mm), mean ± SD	53.5 ± 24.7
Patients' global assessment (VAS 0-100 mm), mean ± SD	51.0 ± 24.4
Physicians' global assessment (5-point scale), mean ± SD	2.2 ± 0.7
SJC, mean ± SD	9.2 ± 5.4
TJC, mean ± SD	8.2 ± 5.9

ACPA, anti–citrullinated protein antibody; BMI, body mass index; CRP, C-reactive protein; DAS28, 28-joint disease activity score; HAQ, health assessment questionnaire; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale

After three months, 399 patients (54.9%) had non- to moderate EULAR response and 328 patients (45.1%) had good EULAR response. Good responders, in comparison to non- to moderate responders, had significantly lower BMI (25.1 ± 4.4 versus 26.2 ± 4.8 Kg/m^2, p=0.005) and lower baseline TJC (TJC: 7.5 ± 6.3 versus 8.8 ± 6.3, p=0.019). The remaining baseline characteristics as well as smoking status did not differ significantly between non- to moderate and good responders.

Treatment use

The majority of the patients (89.9%) were initially treated with MTX monotherapy, 5.9% with sulfasalazine (SSZ) and 2.3% with triple therapy (MTX, SSZ, hydroxychloroquine (HCQ)). More than half of the patients (56.9%) combined their DMARD treatment with GC. Similar pattern of treatment use was seen at the three month follow-up. (Table 2) The comparison of treatment patterns between the different EULAR response groups after three months showed that triple therapy at baseline was more common in good responders than non- to moderate responders (3.7% versus 1.7%, p=0.046), and combined therapy of MTX and SSZ at three months was more common in non- to moderate responders (4.3% versus 1.0%, p=0.009).

Table 2. Treatment use at baseline and at three months follow-up.

	Baseline		3 months	
Treatment	**N (%)**	**GC use n (%)**	**N (%)**	**GC use n (%)**
MTX *	653 (89.9)	373 (90.1)	579 (79.6)	351 (85.8)
SSZ	43 (5.9)	18 (4.3)	31 (4.3)	18 (4.4)
MTX + SSZ + HCQ	17 (2.3)	15 (3.6)	31 (4.3)	18 (4.4)
HCQ	8 (1.1)	3 (0.7)	9 (1.2)	5 (1.2)
LFM	2 (0.3))	1 (0.2)	0 (0)	0 (0)
AZA	1 (0.1)	1 (0.2)	0 (0)	0 (0)
MTX + SSZ	1 (0.1)	1 (0.2)	19 (2.6)	9 (2.2)
HCQ + SSZ	1 (0.1)	0 (0)	2 (0.3)	2 (0.5)
HCQ + AZA	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.2)
GC	414 (56.9)	-	432 (59.4)	-
Missing data	0 (0)	-	46 (6.3)	-

AZA, azathioprine; GC, glucocorticoids; HCQ, hydroxychloroquine; LFM, leuflunomide; MTX, methotrexate; SSZ, sulfasalazine.

* Oral treatment

Nutrient intake and treatment results

The mean (standard deviation) of the energy intake was 1939 (693) kcal, the median (interquartile range) was 1838 (1480-2266). The mean intake of vitamin D, omega-3 FA and folate as well as supplement use in the whole study sample as well as in different EULAR response groups are presenteerd in table 3. Mean omega-3 FA intake at baseline was significantly higher in good responders. No difference between responders and non-responders was seen for vitamin D and folate intake. Dietary supplement use did not differ significantly across EULAR response groups. The intakes of both vitamin D and omega-3 were below the recommended daily intake (RDI) and the intake of folate was borderline to the RDI according to Nordic Nutrition Recommendations 2012.[45]

Table 3. The mean intake of vitamin D, omega-3 FA, folate and dietary supplement use during the previous year from study inclusion in the whole study sample as well as in different EULAR response groups.

		EULAR response at three months		
Nutrient intake	**Total study sample (N=727)**	**Non-/Moderate (n=399)**	**Good (n=328)**	**p value ***
Vitamin D				
Dietary intake, µg/day, mean ± SD	5.86 ± 2.30 [RDI: 10-20]	5.68 ± 2.15	6.07 ± 2.45	0.062
Supplementation, n (%)	57 (7.8)	29 (7.3)	28 (8.5)	0.580
Omega-3 FA				
Dietary intake, g/day, mean ± SD	0.68 ± 0.35 [RDI: ≥2.15 **]	0.65 ± 0.30	0.71 ± 0.39	0.040
Supplementation, n (%)	142 (19.5)	85 (21.3)	57 (17.4)	0.222
Folate				
Dietary intake, µg/day, mean ± SD	308.39 ± 107.09 [RDI: 300-400]	308.28 ± 115.00	308.52 ± 101.57	0.417
Supplementation, n (%) ***	113 (15.5)	67 (16.8)	46 (14.0)	0.355

RDI, Recommended daily intake accoring to Nordic Nutrition Recommendations 2012.[45] The recommendations are age and gender specific. Women and men ≥75 years are recommended a daily intake of 20 µg of vitamin D. Both women and men are recommended a daily intake of omega-3 fatty acids that equals 1 energy percent (E%) or more of the total daily energy intake. Women in fertile age are recommended a daily intake of 400 µg of folate, other women as well as men are recommended 300 µg.

* Mean intakes and proportions of supplementation between non-/moderate and good EULAR response were compared with Mann-Whitney U test and Pearson's chi-square test, respectively.

** RDI for this particular study sample equals ≥1 E% of the total energy intake of the study sample (1939 kcal).

*** Folate supplementation use *before* treatment start. All patients who eventually started MTX treatment were also recieving folate supplements.

Further, vitamin D intake was found to be significantly higher in males and patients with higher BMI (>25). Omega-3 and folate intakes were higher in patients with a university degree and higher PA, but lower in smokers. In addition, nutrient intakes between the different study centers did not significantly differ due to their geographical locations or the season at EIRA inclusion. (Data not shown.)

Dietary vitamin D and omega-3 FA intake were associated with good EULAR response, after adjustment for age, sex, smoking, total energy intake, supplementation, BMI, education and PA. However, dietary folate intake did not significantly associate with EULAR response. (Table 4) Additional analysis showed that omega-6:3 FA did not associate with EULAR response (OR=0.73 [95% CI 0.47-1.14]). Further adjustments for season of EIRA inclusion did not change the OR's markedly (results not shown).

Table 4. Association between dietary intake of vitamin D, omega-3 FA, folate and EULAR response after three months.

Nutrient intake	N	OR (95 % CI) Age and sex adj	OR (95 % CI) Multivariable adj
Vitamin D	**727**		
1st quartile: ≤4.25 µg/day	182	1.00	1.00
2nd quartile: 4.26-5.42 µg/day	170	1.07 (0.70-1.64)	0.94 (0.60-1.49)
3rd quartile: 5.43-6.96 µg/day	184	1.15 (0.75-1.77)	1.13 (0.72-1.79)
4th quartile: >6.97 µg/day	191	1.75 (1.13-2.71)	1.60 (1.00-2.56)
p value, quartile 4 versus 1		0.012	0.048
Omega-3 FA	**727**		
1st quartile: ≤0.45 g/day	180	1.00	1.00
2nd quartile: 0.46-0.62 g/day	192	1.25 (0.82-1.89)	1.33 (0.85-2.06)
3rd quartile: 0.63-0.83 g/day	183	1.35 (0.89-2.07)	1.36 (0.87-2.15)
4th quartile: >0.84 g/day	172	1.64 (1.07-2.53)	1.71 (1.08-2.72)
p value, quartile 4 versus 1		0.024	0.023
Folate	**727**		
1st quartile: ≤244.88 µg/day	201	1.00	1.00
2nd quartile: 244.89-296.86 µg/day	182	1.32 (0.88-1.99)	1.35 (0.87-2.09)
3rd quartile: 296.87-365.70 µg/day	193	1.59 (1.07-2.38)	1.59 (1.03-2.45)
4th quartile: >365.71 µg/day	151	1.14 (0.74-1.75)	1.09 (0.68-1.75)

p value, quartile 4 versus 1	0.557	0.721

Multivariable adjustment for age (eleven 5-year age groups), sex, smoking pack-years, total energy intake (tertiles), supplementation (vitamin D, omega-3 FA/fish oil and folic acid), BMI (≤25 and >25), education level (high school and university) and PA (sedentary PA, moderate occasional PA, moderate regular PA and regular exercise), and use of DMARDs and GC baseline (yes or no).

p value: Comparison between 4th and 1st quartiles.

Similar results were seen in subgroup of the 653 patients who were initially treated with MTX monotherapy at baseline (OR=1.63 [95% CI 1.03-2.57] for vitamin D, OR=1.65 [95% CI 1.05-2.60] for omega-3 FA and OR=1.20 [95% CI 0.76-1.89] for folate, after adjustment for age and gender).

Dietary supplementation alone did not associate with EULAR response (OR=1.27 [95% CI 0.73-2.20] for vitamin D, OR=81 [95% CI 0.56-1.87] for omega-3 FA and OR=0.82 [95% CI 0.54-1.23] for folate, after adjustment for age and gender). In addition, patients who took supplements did not have significantly higher dietary intake of the three nutrients, compared to the patients without supplementation.

DISCUSSION

This study investigated the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. Omega-3 FA intake was significantly higher in patients with good response compared to patients with non- to moderate response. Higher vitamin D and omega-3 FA intakes were associated with good EULAR response. However, no association was found between folate intake and EULAR response. Simular results were observed in a subgroup of patients who were treated with MTX monotherapy at baseline. Dietary supplementation of vitamin D, omega-3 FA and folate alone were not associated with EULAR response.

Vitamin D, omega-3 FA, folate and EULAR response

We found that higher intake of dietary vitamin D was associated with good EULAR response. A study, performed in Philadelphia, USA, showed no association between vitamin D concentration levels and clinical response to therapy using American College of Rheumatology (ACR) response in treatment naïve RA patients,[46] however, in contrast to our study, vitamin D intake in particular was

not studied. Only 15-20% of the vitamin D in blood originates directly from the diet, the remainder is produced during sunlight exposure.[47] Vitamin D deficiency measured in blood is common in patients with RA and has been associated with disease activity and inflammatory markers.[12 48] Vitamin D deficiency in RA patients in Sweden might be caused or aggravated by limited sun exposure during the winter months as there is only enough UV radiation from the sun to produce vitamin D during 6 months each year. However, our results showed that vitamin D intake did not significantly differ between study centers by geographical locations or by the season of inclusion into EIRA. Therefore, increased vitamin D intake through either diet or supplementation might be of importance. Our results suggest that increased dietary intake of vitamin D before and/or during DMARD start may be associated with improved treatment outcome in RA patients. This finding requires confirmation.

Dietary omega-3 FA intake was associated with good EULAR response. Evidence suggests that long-chain omega-3 FA have anti-inflammatory properties and are beneficial in the treatment of autoimmune and inflammatory conditions.[49 50] Combination of MTX and omega-3 has shown a significant reduction in liver enzyme activities.[51] In line with our results, a recent study has suggested that biomarkers of omega-3 FA may predict clinical outcomes relevant to standard drug treatment of RA patients.[52] In addition to the anti-inflammatory effect of the DMARDs, omega-3 FA may have a supplementary role in reducing inflammation and/or achieving better treatment outcome in early RA.

MTX was used by the majority of the patients. We did not find that higher dietary folate intake before starting with MTX and/or other DMARDs was associated with worse treatment response. Folate fortification in food items such as flour, rice, pasta, and other grain products has been common in the last two decades in order to primarily prevent neural tube defect in unborn children,[53 54] but not in Sweden. Folic supplementation in RA patients using MTX has shown to prevent side effects of the drug.[25] Dietary folate intake during MTX treatment might have adverse effects on MTX efficacy. Folic acid fortification in foods has been associated with requirement for higher MTX dose in a small study of RA patients.[55] However, in Sweden there is no obligatory fortification of food. Results from our study, performed in a country where there is no obligatory fortification, suggest that dietary folate intake before starting with MTX and/or other DMARDs did not associate with inferior EULAR response.

This study did not show any association between dietary supplementation and EULAR response, although, several studies have reported beneficial effect of supplementation of vitamin D and omega-

3 FA/fish oil) in particular.[49 50 56 57] This could partly be explained due to higher bioavailability of the nutrients in foods rather than supplements.

Strengths and limitations

This study is to our knowledge the first to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. The study included a large number of participants that were representative of RA patients in Sweden. The FFQ used in this study was highly validated.

Serum levels of vitamin D, omega-3 FA and folate were not taken into account in this study. Dietary data from FFQ were based on estimated dietary consumption as assessed close to the start of treatment. Information on food preparation and its influence on nutrient content was not available. Under and over reporting may have occurred when completing the FFQ. This may have introduced non-differential misclassification of exposure, which would result that the odds ratios for the comparisons between the extreme groups (4th versus 1st quartile) were biased towards the null value. Dietary patterns were assumed to be unchanged during the first three months from baseline. Clinical RA manifestations, treatment history, doses, adherence and side effects of DMARDs from treatment initiation were not considered in this study due to lack of data. The majority of the patients were treated with MTX, the number of patient with other DMARDs were limited in order to perform sensitivity analysis in subgroups of other DMARDs.

Conclusions

Higher intake of dietary vitamin D and omega-3 FA during the year preceding DMARD initiation may be associated with better treatment results in early RA patients. Higher dietary folate intake was not associated with worse responses to MTX. These results, if confirmed, suggest that dietary interventions may be of interest in the management of RA, not only for reasons of optimizing general health but also for achieving optimal results with anti-rheumatic medications.

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Competing interests

We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Authors' contribution

LA was instrumental in establishing the EIRA study. LN provided data from the EIRA master file. RVV played a major role in the study design and drafting the manuscript together with CL. CL performed all the statistical analyses. RVV, AW and LA contributed to the interpretation of the results from a clinical, nutritional and epidemiological aspect, respectively. All authors approved the final manuscript.

Data sharing

No additional data available.

REFERENCES

1. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24.
2. Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73(3):492-509. doi: 10.1136/annrheumdis-2013-204573
3. Cohen S, Cannon GW, Schiff M, et al. Two-year, blinded, randomized, controlled trial of treatment of active rheumatoid arthritis with leflunomide compared with methotrexate. Utilization of Leflunomide in the Treatment of Rheumatoid Arthritis Trial Investigator Group. *Arthritis Rheum* 2001;44(9):1984-92. doi: 10.1002/1529-0131(200109)44:9<1984::AID-ART346>3.0.CO;2-B
4. Svensson B, Hafstrom I. Evidence for low-dosage prednisolone in early rheumatoid arthritis. Prevents destruction of the joints and relieves symptoms. *Lakartidningen* 2006;103(47):3704-9. [published Online First: 2007/01/11]
5. Zhang L, Brett CM, Giacomini KM. Role of organic cation transporters in drug absorption and elimination. *Annu Rev Pharmacol Toxicol* 1998;38:431-60. doi: 10.1146/annurev.pharmtox.38.1.431
6. Song GG, Bae SC, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. *Clinical Rheumatology* 2012;31(12):1733-39. doi: 10.1007/s10067-012-2080-7
7. Costenbader KH, Feskanich D, Holmes M, et al. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008;67(4):530-5. doi: 10.1136/ard.2007.072736
8. Merlino LA, Curtis J, Mikuls TR, et al. Vitamin D intake is inversely associated with rheumatoid arthritis - Results from the Iowa Women's Health Study. *Arthritis and rheumatism* 2004;50(1):72-77. doi: Doi 10.1002/Art.11434

9. Sabbagh Z, Markland J, Vatanparast H. Vitamin D Status Is Associated with Disease Activity among Rheumatology Outpatients. *Nutrients* 2013;5(7):2268-75. doi: 10.3390/nu5072268 [published Online First: 2013/06/28]
10. Kerr GS, Sabahi I, Richards JS, et al. Prevalence of Vitamin D Insufficiency/Deficiency in Rheumatoid Arthritis and Associations with Disease Severity and Activity. *Journal of Rheumatology* 2011;38(1):53-59. doi: 10.3899/jrheum.100516
11. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun Rev* 2011;11(2):84-7. doi: 10.1016/j.autrev.2011.08.003
12. Cutolo M, Straub RH. Insights into endocrine-immunological disturbances in autoimmunity and their impact on treatment. *Arthritis Res Ther* 2009;11(2) doi: ARTN 218

10.1186/ar2630
13. Luo J, Wen H, Guo H, et al. 1,25-dihydroxyvitamin D3 inhibits the RANKL pathway and impacts on the production of pathway-associated cytokines in early rheumatoid arthritis. *Biomed Res Int* 2013;2013:101805. doi: 10.1155/2013/101805
14. Almerighi C, Sinistro A, Cavazza A, et al. 1 alpha,25-Dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in Human Monocytes. *Cytokine* 2009;45(3):190-97. doi: 10.1016/j.cyto.2008.12.009
15. Chandrashekara S, Patted A. Role of vitamin D supplementation in improving disease activity in rheumatoid arthritis: An exploratory study. *Int J Rheum Dis* 2015 doi: 10.1111/1756-185X.12770
16. Wall R, Ross RP, Fitzgerald GF, et al. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010;68(5):280-9. doi: 10.1111/j.1753-4887.2010.00287.x
17. Ariza-Ariza R, Mestanza-Peralta M, Cardiel MH. Omega-3 fatty acids in rheumatoid arthritis: an overview. *Semin Arthritis Rheum* 1998;27(6):366-70.
18. Adam O, Beringer C, Kless T, et al. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol Int* 2003;23(1):27-36. doi: 10.1007/s00296-002-0234-7
19. Sperling RI. The effects of dietary n-3 polyunsaturated fatty acids on neutrophils. *Proc Nutr Soc* 1998;57(4):527-34.
20. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Semin Arthritis Rheum* 1997;27(2):85-97.
21. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002;56(8):365-79.
22. Sundrarjun T, Komindr S, Archararit N, et al. Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble tumour necrosis factor receptor p55 in active rheumatoid arthritis. *J Int Med Res* 2004;32(5):443-54.
23. Dijkmans BA. Folate supplementation and methotrexate. *Br J Rheumatol* 1995;34(12):1172-4.
24. Morgan SL, Baggott JE, Altz-Smith M. Folate status of rheumatoid arthritis patients receiving long-term, low-dose methotrexate therapy. *Arthritis Rheum* 1987;30(12):1348-56.
25. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121(11):833-41.
26. Ortiz Z, Shea B, Suarez Almazor M, et al. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *Cochrane Database Syst Rev* 2000(2):CD000951. doi: 10.1002/14651858.CD000951
27. Cronstein BN, Eberle MA, Gruber HE, et al. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci U S A* 1991;88(6):2441-5.
28. Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest* 1993;92(6):2675-82. doi: 10.1172/JCI116884
29. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacol Rev* 2005;57(2):163-72. doi: 10.1124/pr.57.2.3

30. Stolt P, Bengtsson C, Nordmark B, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Annals of the Rheumatic Diseases* 2003;62(9):835-41. doi: DOI 10.1136/ard.62.9.835
31. Messerer M, Johansson SE, Wolk A. The validity of questionnaire-based micronutrient intake estimates is increased by including dietary supplement use in Swedish men. *Journal of Nutrition* 2004;134(7):1800-05.
32. Larsson SC, Hakansson N, Giovannucci E, et al. Folate intake and pancreatic cancer incidence: A prospective study of Swedish women and men. *Journal of the National Cancer Institute* 2006;98(6):407-13. doi: 10.1093/jnci/djj094
33. Bergström L KE, Hagman U, Eriksson HB, Bruce Å. Livsmedelsdatasystemet KOST - livsmedelsverkets system för information om näringsvärden i livsmedel. *Vår Föda* 1991;43(8):439-47.
34. Gerster H. Can adults adequately convert alpha-linolenic acid (18 : 3n-3) to eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic acid (22 : 6n-3)? *International Journal for Vitamin and Nutrition Research* 1998;68(3):159-73.
35. Davis BC, Kris-Etherton PM. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. *American Journal of Clinical Nutrition* 2003;78(3):640s-46s.
36. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65(4 Suppl):1220S-28S; discussion 29S-31S.
37. Burgaz A, Akesson A, Oster A, et al. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. *American Journal of Clinical Nutrition* 2007;86(5):1399-404.
38. Wallin A, Di Giuseppe D, Burgaz A, et al. Validity of food frequency questionnaire-based estimates of long-term long-chain n-3 polyunsaturated fatty acid intake. *Eur J Nutr* 2014;53(2):549-55. doi: 10.1007/s00394-013-0563-1 [published Online First: 2013/07/28]
39. Wolk A, Ljung H, Vessby B, et al. Effect of additional questions about fat on the validity of fat estimates from a food frequency questionnaire. *European Journal of Clinical Nutrition* 1998;52(3):186-92. doi: DOI 10.1038/sj.ejcn.1600538
40. Larsson SC, Giovannucci E, Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. *J Natl Cancer Inst* 2004;96(5):396-402.
41. Kallberg H, Ding B, Padyukov L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Annals of the Rheumatic Diseases* 2011;70(3):508-11. doi: 10.1136/ard.2009.120899
42. van Gestel AM, Prevoo ML, van 't Hof MA, et al. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis and rheumatism* 1996;39(1):34-40.
43. van Gestel AM, Anderson JJ, van Riel PL, et al. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. *Journal of Rheumatology* 1999;26(3):705-11.
44. Verhoeven AC, Boers M, van Der Linden S. Responsiveness of the core set, response criteria, and utilities in early rheumatoid arthritis. *Annals of the rheumatic diseases* 2000;59(12):966-74.
45. Tetens I, Pedersen, A.G., Schwab, U., Fogelholm, M., Thorsdottir, I., Gunnarsdottir, I., Anderssen, S.A., Möltzer, H.M., Becker, W., Koivisto Hursti, U.K., Wirfält, E., editor. *Nordic Nutrition Recommendations 2012*. 5th edition ed: Nord 2014:002, 2014.
46. Baker JF, Baker DG, Toedter G, et al. Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. *Clinical and Experimental Rheumatology* 2012;30(5):658-64.
47. Reboul E. Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food Funct* 2015;6(2):356-62. doi: 10.1039/c4fo00579a

48. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmunity reviews* 2011;11(2):84-7. doi: 10.1016/j.autrev.2011.08.003 [published Online First: 2011/08/26]
49. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;83(6 Suppl):1505S-19S.
50. Stamp LK, James MJ, Cleland LG. Diet and rheumatoid arthritis: a review of the literature. *Semin Arthritis Rheum* 2005;35(2):77-94. doi: 10.1016/j.semarthrit.2005.05.001
51. Mohammad BI, Hadi NR, Thwainee AS. The effect of Omega-3 and Selenium on Methotrexate-Induced Hepatotoxicity. *Drug Metabolism Reviews* 2010;42:251-51.
52. Proudman SM, Cleland LG, Metcalf RG, et al. Plasma n-3 fatty acids and clinical outcomes in recent-onset rheumatoid arthritis. *British Journal of Nutrition* 2015;114(6):885-90. doi: 10.1017/S0007114515002718
53. Jacques PF, Selhub J, Bostom AG, et al. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *The New England journal of medicine* 1999;340(19):1449-54. doi: 10.1056/NEJM199905133401901
54. Kim YI. Folic acid supplementation and cancer risk: point. *Cancer Epidemiol Biomarkers Prev* 2008;17(9):2220-5. doi: 10.1158/1055-9965.EPI-07-2557
55. Arabelovic S, Sam G, Dallal GE, et al. Preliminary evidence shows that folic acid fortification of the food supply is associated with higher methotrexate dosing in patients with rheumatoid arthritis. *Journal of the American College of Nutrition* 2007;26(5):453-55.
56. Kostoglou-Athanassiou I, Athanassiou P, Lyraki A, et al. Vitamin D and rheumatoid arthritis. *Ther Adv Endocrinol Metab* 2012;3(6):181-7. doi: 10.1177/2042018812471070
57. Racovan M, Walitt B, Collins CE, et al. Calcium and vitamin D supplementation and incident rheumatoid arthritis: the Women's Health Initiative Calcium plus Vitamin D trial. *Rheumatol Int* 2012;32(12):3823-30. doi: 10.1007/s00296-011-2268-1

Figure 1. Overview of patient exclusions.

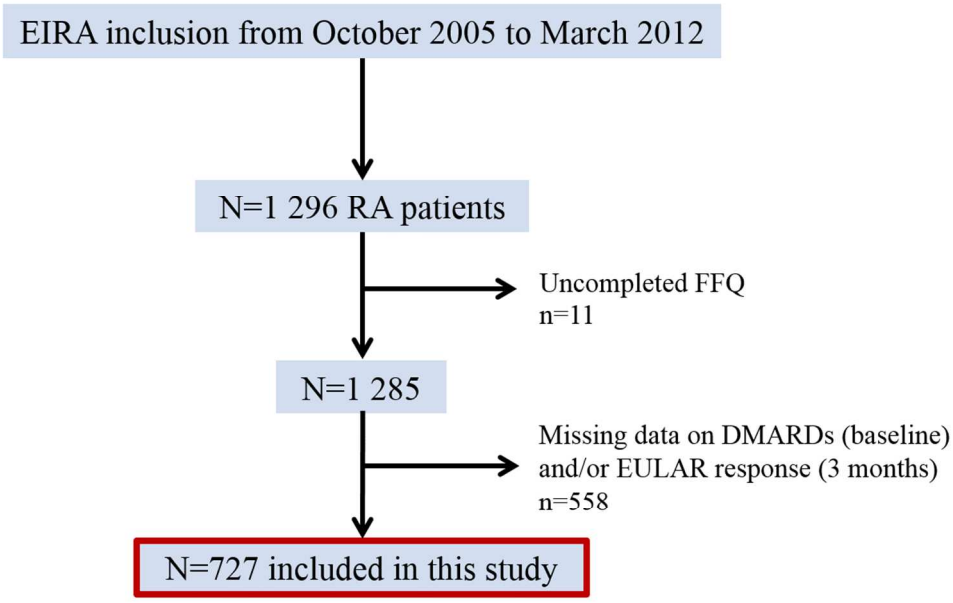

Figure 1. Overview of patient exclusions.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ (page 1-2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ (page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ (page 4-5)
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ (page 5)
Methods		
Study design	4	Present key elements of study design early in the paper ✓ (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ (page 5)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ (page 5) *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ✓ *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes ✓ (page 7), exposures ✓ (page 5-6), predictors-✓, potential confounders ✓ (page 6-7), and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ (page 7)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at 727 participants ✓ (page 5, figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ (page 7)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ (page 7)
		(*b*) Describe any methods used to examine subgroups and interactions ✓ (page 7)
		(*c*) Explain how missing data were addressed ✓ (page 5)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses ✓ (page 7)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ (page 5, figure 1)
		(b) Give reasons for non-participation at each stage ✓ (figure 1)
		(c) Consider use of a flow diagram ✓ (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ (page 8, table 1)
		(b) Indicate number of participants with missing data for each variable of interest ✓ (figure 1)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure ✓ (page 8-9, table 2)
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). ✓ (page 10-11, table 3) Make clear which confounders were adjusted for and why they were included ✓
		(*b*) Report category boundaries when continuous variables were categorized ✓ (table 3)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ✓
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ✓ (page 11)
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ (page 12-13)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. ✓ Discuss both direction and magnitude of any potential bias ✓ (page 13-14)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ (page 12, 14)
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ (page 13)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ (page 14))

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Are dietary vitamin D, omega-3 fatty acids and folate associated with treatment results in patients with early rheumatoid arthritis? Data from a Swedish population-based prospective study.

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ABSTRACT

Background: Dietary intake of vitamin D and omega-3 fatty acids (FA) may be associated with superior response to anti-rheumatic treatments. In addition, dietary folate intake may be associated with worse response to methotrexate (MTX). The aim of this study was to investigate the association between dietary vitamin D, omega-3 FA, folate and treatment results of disease modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis (RA) patients.

Methods: This prospective study was based on data from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, and included 727 early RA patients from 10 hospitals in Sweden. Data on dietary vitamin D, omega-3 FA and folate intake based on food frequency questionnaires were linked with data on European League Against Rheumatism (EULAR) response after three months of DMARD treatment. Associations between vitamin D, omega-3 FA, folate and EULAR response were analyzed with logistic regression adjusted for potential confounders.

Results: The majority of patients (89.9%) were initially treated with MTX monotherapy and more than half (56.9%) with other DMARDs and glucocorticoids. Vitamin D and omega-3 FA were associated with good EULAR response (OR=1.80 [95% CI 1.14-2.83] and OR=1.60 [95% CI 1.02-2.53], respectively). Folate was not significantly associated with EULAR response (OR=1.20 [95% CI 0.75-1.91]). Similar results were seen in a subgroup of patients who were initially treated with MTX monotherapy at baseline.

Conclusions: Higher intake of dietary vitamin D and omega-3 FA during the year preceding DMARD initiation may be associated with better treatment results in early RA patients. Dietary folate intake was not associated with worse or better response to treatment, especially to MTX. Our results suggest that some nutrients may be associated with enhanced treatment results of DMARDs.

ARTICLE SUMMARY

Strengths and limitations

- This study included a large number of participants that were representative of rheumatoid arthritis patients in Sweden.

- The food frequency questionnaire used in this study was highly validated.
- Serum levels of vitamin D, omega-3 fatty acids and folate were not taken into account in this study.
- Under and over reporting of estimated dietary intake may have occurred when completing the food frequency questionnaire.
- Clinical manifestations, treatment history, doses, adherence and side effects of anti-rheumatic treatment were not considered in this study due to lack of data.

BACKGROUND

Treatment of rheumatoid arthritis (RA) aims at providing relief from the symptoms associated with inflammation, pain and stiffness and preventing long-term disability.[1] Disease modifying anti-rheumatic drug (DMARD) can be effective in treating inflammation, delaying joint damage and improving RA patient outcomes.[2 3] Methotrexate (MTX) is the most widely used DMARD for early RA. Low dose glucocorticoids (GC) can be added to the treatment with MTX in order to achieve better outcomes.[4]

The interest in dietary factors and RA has been increasing among researchers and patients with RA over the last decade. However, there is a lack of studies that have specifically investigated how certain nutrients may associate with treatment results in newly diagnosed patients with RA. Nutrients might influence the efficacy of DMARDs through influencing the gastrointestinal absorption of the drug and pharmacokinetics in other ways, or might influence the pharmacodynamic effects of the drugs.[5]

Earlier studies in RA focused on vitamin D, omega-3 fatty acids (FA) and folate. Vitamin D deficiency has been associated with an increased risk of RA.[6] Serum vitamin D levels are decreased in patients with RA, and this was also observed in RA patients in Sweden.[6-10] Some evidence suggests that vitamin D deficiency can trigger autoimmune responses and therefore may provide an immunoregulatory effect.[11 12] The active metabolite of vitamin D ($1,25(OH)_2D$) inhibits the synthesis of several interleukins and tumor necrosis factor (TNF).[13] It also decreases the expression of pro-inflammatory surface molecules.[14] Vitamin D supplementation has been shown to decrease disease activity short-term in RA patients.[15]

Earlier data suggest that omega-3 FA is anti-inflammatory and can decrease disease activity in RA.[16-19] Omega-6 FA as well as omega-6:3 FA (ratio) have been found to be pro-inflammatory in RA.[20] Therefore, it is of importance to have a good balance between omega-6 and omega-3.[21] Increased dietary intake of omega-3 FA has been associated with decreased serum levels of TNF- and C-reactive protein (CRP) in RA.[22]

MTX is a folate antagonist.[23] Folate stores are decreased in RA patients treated with MTX.[24] Impaired folate status is also related to MTX toxicity. Gastrointestinal intolerance is one of the side effects of MTX, and folic acid and folinic acid supplementation have been shown to reduce the

mucosal and gastrointestinal side effects during low dose MTX treatment.[25 26] Of note, excessive doses of folinic acid, but not folic acid during MTX therapy for RA can impair efficacy. Although MTX is believed to work through folate antagonism, it has been hypothesized that the anti-inflammatory effect of this drug might be due to its stimulation of adenosine release that prevents inflammatory cells from attaching to connective tissues.[27-29] Folic acid fortification in foods is mandatory in some countries (i.e. US, Canada), but not in Sweden.

We hypothesized that higher dietary intake of vitamin D and omega-3 FA may be associated with superior responses to anti-rheumatic treatments, whereas higher dietary folate intake might be associated with worse response to MTX. The aim of this study was to investigate the association between dietary intake of vitamin D, omega-3 FA, folate and treatment results of DMARDs in patients with early RA.

MATERIAL AND METHODS

Study participants

This study included initially 1,296 newly diagnosed RA patients (disease duration ≤12 months) from a population-based prospective case-control study called Epidemiological Investigation of Rheumatoid Arthritis (EIRA). EIRA was initiated in May 1996 and the study design has previously been described.[30] Participants of EIRA were asked to complete a food frequency questionnaire (FFQ) at inclusion/baseline.[31 32] EIRA has been linked to the Swedish Rheumatology Quality register (SRQ) in order to provide clinical data. The study period for this report was from October 2005 to March 2012. Patients who did not fully complete the FFQ and/or had missing data on DMARD use at baseline as well as treatment results (European League Against Rheumatism (EULAR) response) after three months were excluded. After exclusions, 727 patients remained for analyses. (Figure 1.) This study was approved by the Regional Ethical Review Board at Karolinska institutet, Stockholm, Sweden.

Dietary assessment

EIRA participants were asked to complete a FFQ at baseline. This self-administered, semi-quantitative FFQ included questions regarding frequency intake of 123 food items and beverages during the previous year before baseline. Pre-specified food frequency intake ranged over eight

categories from *Never* to *≥3 times per day*. For frequently consumed foods, open questions were used and the participants could fill in number of slices, cups, glasses etc. Dietary nutrient intake of vitamin D (µg/day), omega-3 FA (g/day) and folate (µg/day) were calculated by multiplying the average frequency of consumption of each food item by the nutrient content, the nutrient content values were obtained from the Swedish Food Administration Database.[33] Total omega-3 FA intake was calculated based on the most common FA of omega-3 ((alpha-linolenic acid, C18:3/10) + eicosapentaenoic acid, C20:5 + docosapentaenoic acid, C22:5 + docosahexaenoic acid, C22:6).[34 35] All dietary nutrients were energy-adjusted to the mean energy intake during the precious year from baseline in the study population using the residual method.[36] This dietary assessment method and the validation of this FFQ have been described previously.[31 32] The FFQ included also questions regarding dietary supplement use of vitamin D, omega-3 FA/fish oil as well as folic acid (yes or no) during the previous year before baseline.

The estimated FFQ-based intakes of vitamin D-rich foods were validated in comparison to 4 x 1-week weighed dietary records among 129 persons. Pearson's correlations (*r*) between reported dietary vitamin D intake and vitamin D–fortified reduced-fat dairy products, vitamin D–fortified margarines and fatty fish were 0.60–0.70, 0.30-0.70 and 0.50, respectively (Wolk, A. et al., unpublished observations, 1992). Furthermore, serum concentrations of 25(OH)D among 116 persons have been significantly correlated with the FFQ-based vitamin D intake of fatty fish (r = 0.21, p = 0.02) and vitamin D-fortified reduced-fat dairy products (r = 0.20, p = 0.04).[37] Estimate of FFQ-based omega-3 FA intake has been validated in comparison to adipose tissue composition in 239 persons and in comparison to 4 x 1-week weighed dietary records in 184 persons. Pearson's correlation between the estimated intake of omega-3 FA and adipose tissue was 0.41[38] and 0.40 with dietary records.[39] Pearson's correlation was 0.50 between the estimated intake of folate and 4 x 1-week weighed dietary records of folate intake (n=129).[40] The validity of a FFQ can differ between different populations.

Additional assessments

In addition to the FFQ, the EIRA participants were asked to report their age, weight, height, smoking habits, education and physical activity (PA) level. The EIRA questionnaire included questions regarding smoking status (never, former or current smoker) as well as smoking duration and intensity (pack-year). Pack-years were categorized into 0–9, 10–19 and 20+ pack-years; one pack-year was equal to 20 smoked cigarettes per day during one year.[41] Education level was categorized into high

school and university degree. The participants reported the amount of PA performed during the previous year before inclusion in the EIRA study. PA was categorized into four groups: sedentary PA, moderate occasional PA, moderate regular PA and regular exercise.

Treatment result assessment

Treatment results were assessed using EULAR response criteria, which is validated tool for assessing treatment efficacy in RA.[42-44] It includes non-, moderate and good response to treatment. EULAR response was determined based on changes in 28-joint disease activity score (DAS28) from baseline/treatment initiation up to three months as well as the endpoint DAS28 at three months. EULAR response was studied primarily in all patients with any DMARD at baseline, and then specifically in all patients with MTX monotherapy at baseline. In this study, non- and moderate EULAR response were merged into one category to increase stratified power.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 23. Baseline characteristics were compared between non-/moderate responders and good responders after three months with Mann-Whitney U test or Kruskal-Wallis test for continuous variables (mean and standard deviation (SD)) as well as Pearson's chi-square test for proportions (%). Further analyses included comparisons of dietary intake of vitamin D, omega-3 FA and folate between different subgroups of patients based on age, sex, smoking, supplement use, BMI, education, PA, geographical locations and season (spring/summer versus fall/winter) at EIRA inclusion, with Mann-Whitney U test and Kruskal-Wallis test.

Associations between dietary intake of vitamin D, omega-3 FA, folate and EULAR response, respectively, were analyzed with logistic regression. All multivariate analyses were adjusted for potential confounders such as age, sex, smoking, total energy intake, supplement use (vitamin D, omega-3 FA/fish oil and folic acid), BMI (weight (Kg)/[height (m)]^2), education, PA, DAS28 at baseline and anti-rheumatic treatment at baseline. Dietary intakes of vitamin D, omega-3 FA and folate were divided into quartiles based on intake of all the EIRA participants including the controls. The first quartile was the referent group. Age was categorized into eleven age groups of five-year range each, total energy intake into quartiles, supplement use into yes and no, education into high school degree and university degree, and PA into sedentary to occasional moderate PA and moderate

regular PA to regular exercise. BMI and DAS28 at baseline were used as continuous variables. Use of anti-rheumatic treatment was categorized into yes and no for each treatment. Smoking adjustment was based on pack-years. The analyses were primarily including the total study sample regardless of treatment (n=727). The same analyses were also performed separately on patients with only MTX monotherapy (n=653). Further sensitivity analyses were performed where EULAR response of three separate groups (non, moderate, good) was used as the outcome in an ordinal regression. In addition, association between omega-3/fish oil supplementation and EULAR response were analyzed.

RESULTS

Patient characteristics

This study included 727 RA patients. Baseline characteristics for the total study sample are presented in table 1. The proportion of current smokers was 31.9% and obtained university degree 25.2% (no one had an education level below high school degree). The proportions of patients who had performed moderate regular PA to regular exercise was 30.7%.

Table 1. Clinical baseline characteristics.

	N=727
Female, %	72.6
Age (years), mean ± SD	52.5 ± 13.1
BMI, (Kg/m²), mean ± SD	25.7 ± 4.6
Symptom duration (days), mean ± SD	302.3 ± 419.1
Rheumatoid factor, positive, %	51.4
ACPA positive, %	66.2
DAS28, mean ± SD	5.2 ± 1.3
HAQ, mean ± SD	1.0 ± 0.6
CRP (mg/L), mean ± SD	22.6 ± 29.8
Pain (VAS 0-100 mm), mean ± SD	53.5 ± 24.7
Patients' global assessment (VAS 0-100 mm), mean ± SD	51.0 ± 24.4
Physicians' global assessment (5-point scale), mean ± SD	2.2 ± 0.7
SJC, mean ± SD	9.2 ± 5.4
TJC, mean ± SD	8.2 ± 5.9

ACPA, anti–citrullinated protein antibody; BMI, body mass index; CRP, C-reactive protein; DAS28, 28-joint disease activity score; HAQ, health assessment questionnaire; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale

After three months, 140 patients (19.3%) had non-EULAR response, 259 patients (35.6%) had moderate EULAR response and 328 patients (45.1%) had good EULAR response. Good responders, in comparison to non- to moderate responders, had significantly lower BMI (25.1 ± 4.4 versus 26.2 ± 4.8 Kg/m^2, p=0.005) and lower baseline TJC (TJC: 7.5 ± 6.3 versus 8.8 ± 6.3, p=0.019). The remaining baseline characteristics as well as smoking status did not differ significantly between non- to moderate and good responders.

Treatment use

The majority of the patients (89.9%) were initially treated with MTX monotherapy, 5.9% with sulfasalazine (SSZ) and 2.3% with triple therapy (MTX, SSZ, hydroxychloroquine (HCQ)). More than half of the patients (56.9%) combined their DMARD treatment with GC. Similar pattern of treatment use was seen at the three month follow-up. (Table 2) The comparison of treatment patterns between the different EULAR response groups after three months showed that triple therapy at baseline was more common in good responders than non- to moderate responders (3.7% versus 1.7%, p=0.046), and combined therapy of MTX and SSZ at three months was more common in non- to moderate responders (4.3% versus 1.0%, p=0.009).

Table 2. Treatment use at baseline and at three months follow-up.

	Baseline		3 months	
Treatment	**N (%)**	**GC use n (%)**	**N (%)**	**GC use n (%)**
MTX *	653 (89.9)	373 (90.1)	579 (79.6)	351 (85.8)
SSZ	43 (5.9)	18 (4.3)	31 (4.3)	18 (4.4)
MTX + SSZ + HCQ	17 (2.3)	15 (3.6)	31 (4.3)	18 (4.4)
HCQ	8 (1.1)	3 (0.7)	9 (1.2)	5 (1.2)
LFM	2 (0.3))	1 (0.2)	0 (0)	0 (0)
AZA	1 (0.1)	1 (0.2)	0 (0)	0 (0)
MTX + SSZ	1 (0.1)	1 (0.2)	19 (2.6)	9 (2.2)
HCQ + SSZ	1 (0.1)	0 (0)	2 (0.3)	2 (0.5)
HCQ + AZA	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.2)
GC	414 (56.9)	-	432 (59.4)	-

Missing data	0 (0)	-	46 (6.3)	-

AZA, azathioprine; GC, glucocorticoids; HCQ, hydroxychloroquine; LFM, leuflunomide; MTX, methotrexate; SSZ, sulfasalazine.

* Oral treatment

Nutrient intake and treatment results

The mean (standard deviation) of the energy intake was 1939 (693) kcal, the median (interquartile range) was 1838 (1480-2266). The mean intake of vitamin D, omega-3 FA and folate as well as supplement use in the whole study sample as well as in different EULAR response groups are presenteerd in table 3. Mean omega-3 FA intake at baseline was significantly higher in good responders. No difference between responders and non-responders was seen for vitamin D and folate intake. Dietary supplement use did not differ significantly across EULAR response groups. The intakes of both vitamin D and omega-3 were below the recommended daily intake (RDI) and the intake of folate was borderline to the RDI according to Nordic Nutrition Recommendations 2012.[45]

Table 3. The mean intake of vitamin D, omega-3 FA, folate and dietary supplement use during the previous year from study inclusion in the whole study sample as well as in different EULAR response groups.

		EULAR response at three months		
Nutrient intake	**Total study sample (N=727)**	**Non-/Moderate (n=399)**	**Good (n=328)**	**p value ***
Vitamin D				
Dietary intake, µg/day, mean ± SD	5.86 ± 2.30 [RDI: 10-20]	5.68 ± 2.15	6.07 ± 2.45	0.062
Supplementation, n (%)	57 (7.8)	29 (7.3)	28 (8.5)	0.580
Omega-3 FA				
Dietary intake, g/day, mean ± SD	0.68 ± 0.35 [RDI: ≥2.15 **]	0.65 ± 0.30	0.71 ± 0.39	0.040
Supplementation, n (%)	142 (19.5)	85 (21.3)	57 (17.4)	0.222
Folate				
Dietary intake, µg/day, mean ± SD	308.39 ± 107.09 [RDI: 300-400]	308.28 ± 115.00	308.52 ± 101.57	0.417
Supplementation, n (%) ***	113 (15.5)	67 (16.8)	46 (14.0)	0.355

RDI, Recommended daily intake accoring to Nordic Nutrition Recommendations 2012.[45] The recommendations are age and gender specific. Women and men ≥75 years are recommended a daily intake of 20 µg of vitamin D. Both women and men are recommended a daily intake of omega-3 fatty acids that equals 1 energy percent (E%) or more of the total daily energy intake. Women in fertile age are recommended a daily intake of 400 µg of folate, other women as well as men are recommended 300 µg.

* Mean intakes and proportions of supplementation between non-/moderate and good EULAR response were compared with Mann-Whitney U test and Pearson's chi-square test, respectively.

** RDI for this particular study sample equals ≥1 E% of the total energy intake of the study sample (1939 kcal).

*** Folate supplementation use *before* treatment start. All patients who eventually started MTX treatment were also recieving folate supplements.

Further, vitamin D intake was found to be significantly higher in males and patients with higher BMI (>25). Omega-3 and folate intakes were higher in patients with a university degree and higher PA, but lower in smokers. In addition, nutrient intakes between the different study centers did not significantly differ due to their geographical locations or the season at EIRA inclusion. (Data not shown.)

Dietary vitamin D and omega-3 FA intake were associated with good EULAR response, after adjustment for age, sex, smoking, total energy intake, supplementation, BMI, education, PA, DAS28 at baseline, and use of DMARDs and GC at baseline. However, dietary folate intake did not significantly associate with EULAR response. (Table 4) Additional analysis showed that omega-6:3 FA did not associate with EULAR response (OR=0.73 [95% CI 0.47-1.14]). Further adjustments for season of EIRA inclusion did not change the OR's markedly (results not shown).

Table 4. Association between dietary intake of vitamin D, omega-3 FA, folate and EULAR response after three months.

Nutrient intake	N	OR (95 % CI) Age and sex adj	OR (95 % CI) Multivariable adj
Vitamin D	**727**		
1st quartile: ≤4.25 µg/day	182	1.00	1.00
2nd quartile: 4.26-5.42 µg/day	170	1.07 (0.70-1.64)	0.94 (0.59-1.50)
3rd quartile: 5.43-6.96 µg/day	184	1.15 (0.75-1.77)	1.11 (0.70-1.76)
4th quartile: >6.97 µg/day	191	1.75 (1.13-2.71)	1.61 (1.01-2.57)
p value, quartile 4 versus 1		0.012	0.048
Omega-3 FA	**727**		
1st quartile: ≤0.45 g/day	180	1.00	1.00
2nd quartile: 0.46-0.62 g/day	192	1.25 (0.82-1.89)	1.28 (0.82-2.00)
3rd quartile: 0.63-0.83 g/day	183	1.35 (0.89-2.07)	1.35 (0.85-2.13)
4th quartile: >0.84 g/day	172	1.64 (1.07-2.53)	1.68 (1.05-2.68)
p value, quartile 4 versus 1		0.024	0.030
Folate	**727**		
1st quartile: ≤244.88 µg/day	201	1.00	1.00

2nd quartile: 244.89-296.86 µg/day	182	1.32 (0.88-1.99)	1.40 (0.90-2.16)
3rd quartile: 296.87-365.70 µg/day	193	1.59 (1.07-2.38)	1.66 (1.07-2.58)
4th quartile: >365.71 µg/day	151	1.14 (0.74-1.75)	1.11 (0.69-1.79)
p value, quartile 4 versus 1		0.557	0.672

Multivariable adjustment for age (eleven 5-year age groups), sex, smoking pack-years, total energy intake (tertiles), supplementation (vitamin D, omega-3 FA/fish oil and folic acid), BMI (continuous), education level (high school and university) and PA (sedentary PA, moderate occasional PA, moderate regular PA and regular exercise), DAS28 at baseline (continuous) and use of DMARDs and GC baseline (yes or no).

p value: Comparison between 4th and 1st quartiles.

Similar results were seen in subgroup of the 653 patients who were initially treated with MTX monotherapy at baseline (OR=1.63 [95% CI 1.03-2.57] for vitamin D, OR=1.65 [95% CI 1.05-2.60] for omega-3 FA and OR=1.20 [95% CI 0.76-1.89] for folate, after adjustment for age and gender).

Further sensitivity analyses when using EULAR response of three separate groups as the outcome (non, moderate, good) in an ordinal regression did not show stronger associations as in logistic regression, after adjusting for age, sex, smoking, total energy intake, supplementation, BMI, education, PA, DAS28 at baseline, and use of DMARDs and GC at baseline (OR=1.14 [95% CI 0.99-1.30] for vitamin D, OR=1.30 [95% CI 1.03-1.34] for omega-3 FA and OR=1.06 [95% 0.93-1.22] for folate).

Dietary supplementation alone did not associate with EULAR response (OR=1.27 [95% CI 0.73-2.20] for vitamin D, OR=81 [95% CI 0.56-1.87] for omega-3 FA and OR=0.82 [95% CI 0.54-1.23] for folate, after adjustment for age and gender). In addition, patients who took supplements did not have significantly higher dietary intake of the three nutrients, compared to the patients without supplementation.

DISCUSSION

This study investigated the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. Omega-3 FA intake was significantly higher in patients with good response compared to patients with non- to moderate response. Higher vitamin D and omega-3 FA intakes were associated with good EULAR response. However, no association was found between folate intake and EULAR response. Simular results

were observed in a subgroup of patients who were treated with MTX monotherapy at baseline. Dietary supplementation of vitamin D, omega-3 FA and folate alone were not associated with EULAR response.

Vitamin D, omega-3 FA, folate and EULAR response

We found that higher intake of dietary vitamin D was associated with good EULAR response. A study, performed in Philadelphia, USA, showed no association between vitamin D concentration levels and clinical response to therapy using American College of Rheumatology (ACR) response in treatment naïve RA patients,[46] however, in contrast to our study, vitamin D intake in particular was not studied. Only 15-20% of the vitamin D in blood originates directly from the diet, the remainder is produced during sunlight exposure.[47] Vitamin D deficiency measured in blood is common in patients with RA and has been associated with disease activity and inflammatory markers.[12 48] Vitamin D deficiency in RA patients in Sweden might be caused or aggravated by limited sun exposure during the winter months as there is only enough UV radiation from the sun to produce vitamin D during 6 months each year. However, our results showed that vitamin D intake did not significantly differ between study centers by geographical locations or by the season of inclusion into EIRA. Therefore, increased vitamin D intake through either diet or supplementation might be of importance. Our results suggest that increased dietary intake of vitamin D before and/or during DMARD start may be associated with improved treatment outcome in RA patients. This finding requires confirmation.

Dietary omega-3 FA intake was associated with good EULAR response. Evidence suggests that long-chain omega-3 FA have anti-inflammatory properties and are beneficial in the treatment of autoimmune and inflammatory conditions.[49 50] Combination of MTX and omega-3 has shown a significant reduction in liver enzyme activities.[51] In line with our results, a recent study has suggested that biomarkers of omega-3 FA may predict clinical outcomes relevant to standard drug treatment of RA patients.[52] In addition to the anti-inflammatory effect of the DMARDs, omega-3 FA may have a supplementary role in reducing inflammation and/or achieving better treatment outcome in early RA.

MTX was used by the majority of the patients. We did not find that higher dietary folate intake before starting with MTX and/or other DMARDs was associated with worse treatment response. Folate fortification in food items such as flour, rice, pasta, and other grain products has been common in the last two decades in order to primarily prevent neural tube defect in unborn children,[53]

[54] but not in Sweden. Folic supplementation in RA patients using MTX has shown to prevent side effects of the drug.[25] Dietary folate intake during MTX treatment might have adverse effects on MTX efficacy. Folic acid fortification in foods has been associated with requirement for higher MTX dose in a small study of RA patients.[55] However, in Sweden there is no obligatory fortification of food. Results from our study, performed in a country where there is no obligatory fortification, suggest that dietary folate intake before starting with MTX and/or other DMARDs did not associate with inferior EULAR response.

This study did not show any association between dietary supplementation and EULAR response, although, several studies have reported beneficial effect of supplementation of vitamin D and omega-3 FA/fish oil) in particular.[49 50 56 57] This could partly be explained due to higher bioavailability of the nutrients in foods rather than supplements.

Strengths and limitations

This study is to our knowledge the first to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. The study included a large number of participants that were representative of RA patients in Sweden. The FFQ used in this study was highly validated. The results of our study apply to Swedish patients with newly diagnosed RA. It is very likely that they also apply to patients with newly diagnosed RA in other European countries and North America. It is also likely that they apply to patients with established RA as well as some other autoimmune inflammatory conditions.

Serum levels of vitamin D, omega-3 FA and folate were not taken into account in this study. Dietary data from FFQ were based on estimated dietary consumption as assessed close to the start of treatment. Information on food preparation and its influence on nutrient content was not available. Under and over reporting may have occurred when completing the FFQ. This may have introduced non-differential misclassification of exposure, which would result that the odds ratios for the comparisons between the extreme groups (4^{th} versus 1^{st} quartile) were biased towards the null value. Dietary patterns were assumed to be unchanged during the first three months from baseline. Clinical RA manifestations, treatment history, doses, adherence and side effects of DMARDs from treatment initiation were not considered in this study due to lack of data. The majority of the patients were treated with MTX, the number of patient with other DMARDs were limited in order to perform sensitivity analysis in subgroups of other DMARDs.

Conclusions

Higher intake of dietary vitamin D and omega-3 FA during the year preceding DMARD initiation may be associated with better treatment results in early RA patients. Higher dietary folate intake was not associated with worse responses to MTX. These results, if confirmed, suggest that dietary interventions may be of interest in the management of RA, not only for reasons of optimizing general health but also for achieving optimal results with anti-rheumatic medications.

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Competing interests

We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Authors' contribution

LA was instrumental in establishing the EIRA study. LN provided data from the EIRA master file. RVV played a major role in the study design and drafting the manuscript together with CL. CL performed all the statistical analyses. RVV, AW and LA contributed to the interpretation of the results from a clinical, nutritional and epidemiological aspect, respectively. All authors approved the final manuscript.

Data sharing

No additional data available.

REFERENCES

1. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315-24.

2. Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;**73**:492-509. doi: 10.1136/annrheumdis-2013-204573
3. Cohen S, Cannon GW, Schiff M, et al. Two-year, blinded, randomized, controlled trial of treatment of active rheumatoid arthritis with leflunomide compared with methotrexate. Utilization of Leflunomide in the Treatment of Rheumatoid Arthritis Trial Investigator Group. *Arthritis Rheum* 2001;**44**:1984-92. doi: 10.1002/1529-0131(200109)44:9<1984::AID-ART346>3.0.CO;2-B
4. Svensson B, Hafstrom I. Evidence for low-dosage prednisolone in early rheumatoid arthritis. Prevents destruction of the joints and relieves symptoms. *Lakartidningen* 2006;**103**:3704-9. [published Online First: 2007/01/11]
5. Zhang L, Brett CM, Giacomini KM. Role of organic cation transporters in drug absorption and elimination. *Annu Rev Pharmacol Toxicol* 1998;**38**:431-60. doi: 10.1146/annurev.pharmtox.38.1.431
6. Song GG, Bae SC, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. *Clinical Rheumatology* 2012;**31**:1733-39. doi: 10.1007/s10067-012-2080-7
7. Costenbader KH, Feskanich D, Holmes M, et al. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008;**67**:530-5. doi: 10.1136/ard.2007.072736
8. Merlino LA, Curtis J, Mikuls TR, et al. Vitamin D intake is inversely associated with rheumatoid arthritis - Results from the Iowa Women's Health Study. *Arthritis and rheumatism* 2004;**50**:72-77. doi: Doi 10.1002/Art.11434
9. Sabbagh Z, Markland J, Vatanparast H. Vitamin D Status Is Associated with Disease Activity among Rheumatology Outpatients. *Nutrients* 2013;**5**:2268-75. doi: 10.3390/nu5072268
10. Kerr GS, Sabahi I, Richards JS, et al. Prevalence of Vitamin D Insufficiency/Deficiency in Rheumatoid Arthritis and Associations with Disease Severity and Activity. *Journal of Rheumatology* 2011;**38**:53-59. doi: 10.3899/jrheum.100516
11. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun Rev* 2011;**11**:84-7. doi: 10.1016/j.autrev.2011.08.003
12. Cutolo M, Straub RH. Insights into endocrine-immunological disturbances in autoimmunity and their impact on treatment. *Arthritis Res Ther* 2009;**11** doi: ARTN 21810.1186/ar2630
13. Luo J, Wen H, Guo H, et al. 1,25-dihydroxyvitamin D3 inhibits the RANKL pathway and impacts on the production of pathway-associated cytokines in early rheumatoid arthritis. *Biomed Res Int* 2013;**2013**:101805. doi: 10.1155/2013/101805
14. Almerighi C, Sinistro A, Cavazza A, et al. 1 alpha,25-Dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in Human Monocytes. *Cytokine* 2009;**45**:190-97. doi: 10.1016/j.cyto.2008.12.009
15. Chandrashekara S, Patted A. Role of vitamin D supplementation in improving disease activity in rheumatoid arthritis: An exploratory study. *Int J Rheum Dis* 2015 doi: 10.1111/1756-185X.12770
16. Wall R, Ross RP, Fitzgerald GF, et al. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010;**68**:280-9. doi: 10.1111/j.1753-4887.2010.00287.x
17. Ariza-Ariza R, Mestanza-Peralta M, Cardiel MH. Omega-3 fatty acids in rheumatoid arthritis: an overview. *Semin Arthritis Rheum* 1998;**27**:366-70.
18. Adam O, Beringer C, Kless T, et al. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol Int* 2003;**23**:27-36. doi: 10.1007/s00296-002-0234-7
19. Sperling RI. The effects of dietary n-3 polyunsaturated fatty acids on neutrophils. *Proc Nutr Soc* 1998;**57**:527-34.
20. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Semin Arthritis Rheum* 1997;**27**:85-97.
21. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002;**56**:365-79.
22. Sundrarjun T, Komindr S, Archararit N, et al. Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble tumour necrosis factor receptor p55 in active rheumatoid arthritis. *J Int Med Res* 2004;**32**:443-54.

23. Dijkmans BA. Folate supplementation and methotrexate. *Br J Rheumatol* 1995;**34**:1172-4.
24. Morgan SL, Baggott JE, Altz-Smith M. Folate status of rheumatoid arthritis patients receiving long-term, low-dose methotrexate therapy. *Arthritis Rheum* 1987;**30**:1348-56.
25. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;**121**:833-41.
26. Ortiz Z, Shea B, Suarez Almazor M, et al. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *Cochrane Database Syst Rev* 2000;CD000951. doi: 10.1002/14651858.CD000951
27. Cronstein BN, Eberle MA, Gruber HE, et al. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci U S A* 1991;**88**:2441-5.
28. Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest* 1993;**92**:2675-82. doi: 10.1172/JCI116884
29. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacol Rev* 2005;**57**:163-72. doi: 10.1124/pr.57.2.3
30. Stolt P, Bengtsson C, Nordmark B, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Annals of the Rheumatic Diseases* 2003;**62**:835-41. doi: DOI 10.1136/ard.62.9.835
31. Messerer M, Johansson SE, Wolk A. The validity of questionnaire-based micronutrient intake estimates is increased by including dietary supplement use in Swedish men. *Journal of Nutrition* 2004;**134**:1800-05.
32. Larsson SC, Hakansson N, Giovannucci E, et al. Folate intake and pancreatic cancer incidence: A prospective study of Swedish women and men. *Journal of the National Cancer Institute* 2006;**98**:407-13. doi: 10.1093/jnci/djj094
33. Bergström L KE, Hagman U, Eriksson HB, Bruce Å. Livsmedelsdatasystemet KOST - livsmedelsverkets system för information om näringsvärden i livsmedel. *Vår Föda* 1991;**43**:439-47.
34. Gerster H. Can adults adequately convert alpha-linolenic acid (18 : 3n-3) to eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic acid (22 : 6n-3)? *International Journal for Vitamin and Nutrition Research* 1998;**68**:159-73.
35. Davis BC, Kris-Etherton PM. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. *American Journal of Clinical Nutrition* 2003;**78**:640s-46s.
36. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;**65**:1220S-28S; discussion 29S-31S.
37. Burgaz A, Akesson A, Oster A, et al. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. *American Journal of Clinical Nutrition* 2007;**86**:1399-404.
38. Wallin A, Di Giuseppe D, Burgaz A, et al. Validity of food frequency questionnaire-based estimates of long-term long-chain n-3 polyunsaturated fatty acid intake. *Eur J Nutr* 2014;**53**:549-55. doi: 10.1007/s00394-013-0563-1
39. Wolk A, Ljung H, Vessby B, et al. Effect of additional questions about fat on the validity of fat estimates from a food frequency questionnaire. *European Journal of Clinical Nutrition* 1998;**52**:186-92. doi: DOI 10.1038/sj.ejcn.1600538
40. Larsson SC, Giovannucci E, Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. *J Natl Cancer Inst* 2004;**96**:396-402.
41. Kallberg H, Ding B, Padyukov L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Annals of the Rheumatic Diseases* 2011;**70**:508-11. doi: 10.1136/ard.2009.120899
42. van Gestel AM, Prevoo ML, van 't Hof MA, et al. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis and rheumatism* 1996;**39**:34-40.

43. van Gestel AM, Anderson JJ, van Riel PL, et al. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. *J Rheumatol* 1999;**26**:705-11.
44. Verhoeven AC, Boers M, van Der Linden S. Responsiveness of the core set, response criteria, and utilities in early rheumatoid arthritis. *Annals of the rheumatic diseases* 2000;**59**:966-74.
45. Tetens I, Pedersen AG, Schwab U, et al. Nordic Nutrition Recommendations 2012. 5th edition: *Nord* 2014;**002**.
46. Baker JF, Baker DG, Toedter G, et al. Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. *Clinical and Experimental Rheumatology* 2012;30(5):658-64.
47. Reboul E. Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food Funct* 2015;**6**:356-62. doi: 10.1039/c4fo00579a
48. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmunity reviews* 2011;**11**:84-7. doi: 10.1016/j.autrev.2011.08.003 [published Online First: 2011/08/26]
49. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;**83**:1505S-19S.
50. Stamp LK, James MJ, Cleland LG. Diet and rheumatoid arthritis: a review of the literature. *Semin Arthritis Rheum* 2005;**35**:77-94. doi: 10.1016/j.semarthrit.2005.05.001
51. Mohammad BI, Hadi NR, Thwainee AS. The effect of Omega-3 and Selenium on Methotrexate-Induced Hepatotoxicity. *Drug Metabolism Reviews* 2010;**42**:251-51.
52. Proudman SM, Cleland LG, Metcalf RG, et al. Plasma n-3 fatty acids and clinical outcomes in recent-onset rheumatoid arthritis. *British Journal of Nutrition* 2015;**114**:885-90. doi: 10.1017/S0007114515002718
53. Jacques PF, Selhub J, Bostom AG, et al. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *The New England journal of medicine* 1999;**340**:1449-54. doi: 10.1056/NEJM199905133401901
54. Kim YI. Folic acid supplementation and cancer risk: point. *Cancer Epidemiol Biomarkers Prev* 2008;**17**:2220-5. doi: 10.1158/1055-9965.EPI-07-2557
55. Arabelovic S, Sam G, Dallal GE, et al. Preliminary evidence shows that folic acid fortification of the food supply is associated with higher methotrexate dosing in patients with rheumatoid arthritis. *Journal of the American College of Nutrition* 2007;**26**:453-55.
56. Kostoglou-Athanassiou I, Athanassiou P, Lyraki A, et al. Vitamin D and rheumatoid arthritis. *Ther Adv Endocrinol Metab* 2012;**3**:181-7. doi: 10.1177/2042018812471070
57. Racovan M, Walitt B, Collins CE, et al. Calcium and vitamin D supplementation and incident rheumatoid arthritis: the Women's Health Initiative Calcium plus Vitamin D trial. *Rheumatol Int* 2012;**32**:3823-30. doi: 10.1007/s00296-011-2268-1

Figure 1. Overview of patient exclusions.

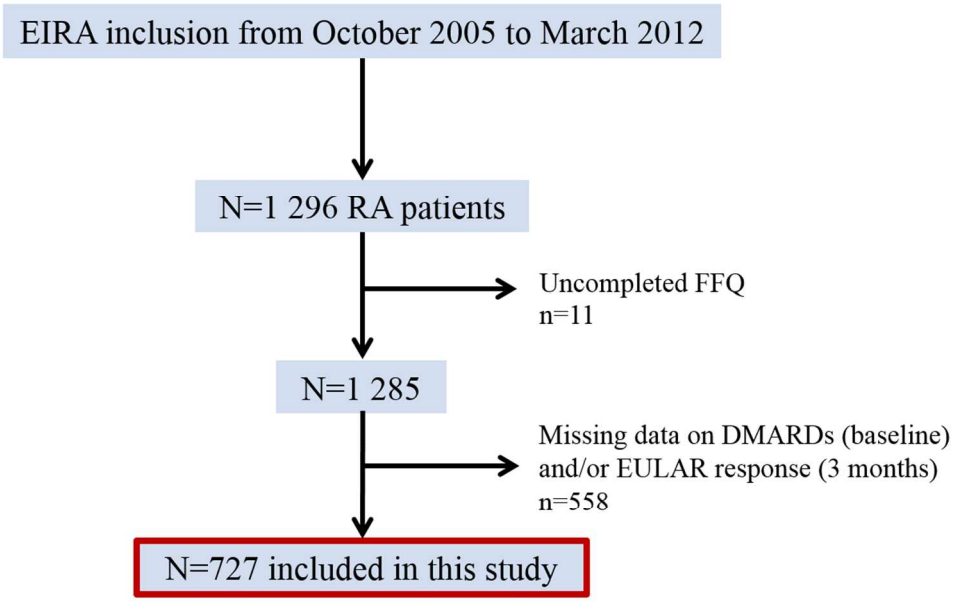

Figure 1. Overview of patient exclusions.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ (page 1, 2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ (page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ (page 4-5)
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ (page 5)
Methods		
Study design	4	Present key elements of study design early in the paper ✓ (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ (page 5)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ (page 5) *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes ✓ (page 7), exposures ✓ (page 5-6), predictors, potential confounders ✓ (page 6-7), and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ (page 5-7)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at 727 participants ✓ (page 5, figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ (page 7)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ (page 7)
		(*b*) Describe any methods used to examine subgroups and interactions ✓ (page 7)
		(*c*) Explain how missing data were addressed ✓ (page 5)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses ✓ (page 7-8)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ (page 5, figure 1)
		(b) Give reasons for non-participation at each stage ✓ (figure 1)
		(c) Consider use of a flow diagram ✓ (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ (page 8, table 1)
		(b) Indicate number of participants with missing data for each variable of interest ✓ (figure 1)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time ✓ (page 7)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). ✓ (page 11-12, table 4) Make clear which confounders were adjusted for and why they were included ✓
		(*b*) Report category boundaries when continuous variables were categorized ✓ (page 7, table 4)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ✓ (page 12)
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ (page 13-14)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ (page 13-14)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ (page, 13-14)
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ (page 14)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ (page 14-15)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.