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Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD☆

Jordi de Batlle[a,b,c,d], Jaume Sauleda[e,f,g], Eva Balcells[b,e], Federico P. Gómez[e,h], Michelle Méndez[a,b,c], Esther Rodriguez[i], Esther Barreiro[b,d,e,j], Jaume J. Ferrer[e,i], Isabelle Romieu[k], Joaquim Gea[b,d,e,j], Josep M. Antó[a,b,c,d], Judith Garcia-Aymerich[a,b,c,d,*] and the PAC-COPD Study Group

[a]*Centre for Research in Environmental Epidemiology, (CREAL), Barcelona, Spain*
[b]*Municipal Institute of Medical Research, (IMIM-Hospital del Mar), Barcelona, Spain*
[c]*CIBER Epidemiología y Salud Pública, (CIBERESP), Barcelona, Spain*
[d]*Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain*
[e]*CIBER de Enfermedades Respiratorias (CIBERES), Bunyola, Spain*
[f]*Servei de Pneumologia, Hospital Universitari Son Dureta, Palma de Mallorca, Spain*
[g]*Fundació Caubet-Cimera, Bunyola, Spain*
[h]*Servei de Pneumologia, Hospital Clínic-Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[i]*Servei de Pneumologia, Hospital General Universitari Vall d'Hebron, Barcelona, Spain*
[j]*Pulmonology department, URMAR, Barcelona, Spain*
[k]*Centro de investigación en salud poblacional, Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico*

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Abstract

Dietary intake of polyunsaturated fatty acids, including omega-3 and omega-6, could modulate chronic obstructive pulmonary disease (COPD) persistent inflammation. We aimed to assess the relationship between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD. A total of 250 clinically stable COPD patients were included. Dietary data of the last 2 years were assessed using a validated food frequency questionnaire (122 items), which provided levels of three omega-3 fatty acids: docosahexaenoic acid, eicosapentaenoic acid and α-linolenic acid (ALA); and two omega-6 fatty acids: linoleic acid and arachidonic acid (AA). Inflammatory markers [C-reactive protein (CRP), interleukin (IL)-6, IL-8 and tumor necrosis factor alpha (TNFα)] were measured in serum. Fatty acids and inflammatory markers were dichotomised according to their median values, and their association was assessed using multivariate logistic regression. Higher intake of ALA (an anti-inflammatory omega-3 fatty acid) was associated with lower TNFα concentrations [adjusted odds ratio (OR)=0.46; *P*=.049]. Higher AA intake (a proinflammatory omega-6 fatty acid) was related to higher IL-6 (OR=1.96; *P*=.034) and CRP (OR=1.95; *P*=.039) concentrations. Therefore, this study provides the first evidence of an association between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD patients.

Keywords: Food intake; Inflammation; Public health; Pulmonary disease, chronic obstructive

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* Corresponding author. Centre for Research in Environmental Epidemiology (CREAL), 08003 Barcelona, Catalonia, Spain. Tel.: +34 93 214 73 50; fax: +34 93 214 73 02.

E-mail address: jgarcia@creal.cat (J. Garcia-Aymerich).

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide and is expected to become the fourth leading cause of mortality by 2030 [1]. It is characterized by a complex chronic inflammatory condition usually associated with smoking-induced inflammation and oxidative stress [2]. This persistent inflammatory condition is located not only in the lungs [3] but also in extrapulmonary organs and tissues [4]. Levels of systemic inflammatory markers increase during COPD exacerbations [5], suggesting that inflammatory processes play a key role in COPD evolution [6]. A longitudinal population-based study has reported lower lung function decline in subjects with decreasing levels of C-reactive protein (CRP), an inflammatory marker, when compared with subjects with stable or increasing levels, suggesting that reducing the levels of circulating inflammatory markers could be an effective way of reducing lung function decline [7]. Finally, CRP serum levels have been related to mortality in COPD patients [8,9].

It has been hypothesized that dietary intake of polyunsaturated fatty acids (PUFAs), including omega-3 and omega-6 fatty acids, could modulate persistent inflammation in COPD [10–12], although this hypothesis has never been tested so far. It is known that omega-3 fatty acids mostly promote anti-inflammatory activities [13]. In contrast, omega-6 fatty acids are the most relevant precursors of proinflammatory eicosanoids and, therefore, mostly mediate proinflammatory activities [14]. We hypothesized that COPD patients with higher omega-3 and lower omega-6 intakes would have lower levels of circulating inflammatory mediators.

Therefore, this study aims to assess the association between dietary intakes of omega-3 and omega-6 fatty acids and several serum inflammatory markers, specifically CRP, interleukin (IL)-6, IL-8 and tumor necrosis factor alpha (TNFα), in COPD patients in the framework of the 'Phenotype and Course of COPD Project' (PAC-COPD) [15].

2. Subjects and methods

2.1. Study population

This study is a cross-sectional analysis of the PAC-COPD. Briefly, the sample includes COPD patients recruited during their first hospital admission at 9 universitary hospitals in Spain between January 2004 and March 2006 with a confirmed diagnosis of COPD [postbronchodilator forced expiratory volume in the first second to forced vital capacity ratio (FEV_1/FVC)≤0.70] [16] and in a clinically stable condition at least 3 months after discharge. Detailed information on PAC-COPD recruitment, methods and results is available elsewhere [17]. The protocol was approved by the Ethics Committees of all the participating hospitals, and written informed consent was obtained from all the COPD patients.

Of the 342 patients included in the PAC-COPD cohort, a total of 250 had available information on dietary PUFAs and serum inflammatory markers. No differences regarding sociodemographic characteristics, comorbidities, dyspnea or lung function parameters were found between PAC-COPD patients who provided dietary information and those who did not, as previously published [18]. All epidemiological and clinical measures as well as blood samples were obtained during clinical stability at least 3 months after recruitment.

2.2. Dietary assessment

A previously validated 122-item food frequency questionnaire [19] asking for dietary habits in the last 2 years was administered by trained interviewers. Reported information was converted into a daily intake frequency of each food, which was in turn converted into the daily intake in grams per day for each food. A food composition table from the US Department of Agriculture [20] was used to estimate intakes of three omega-3 fatty acids: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid (ALA); and two omega-6 fatty acids: linoleic acid (LA) and arachidonic acid (AA). Additionally, the following ratios between omega-3 and omega-6 fatty acids were computed: ALA/LA, EPA/AA and DHA/AA. More details about the development and validation of the questionnaire have been previously published [19]. Additionally, our group tested the reproducibility of the questionnaire when telephonically administered in a subsample of 18 subjects. Briefly, moderate to high correlations were found between the first and second questionnaire administration, and no statistically significant differences in means of intakes of most food groups, macronutrient and micronutrients were found [18].

2.3. Systemic inflammation

Blood samples were obtained after fasting overnight and, after 30 min of blood withdrawing, centrifuged at 2000–3000 rpm for 10 min. Serum was separated and stored in cryotubes at −80°C. Serum levels of high-sensitivity CRP were determined by nephelometry, and those of IL-6, IL-8 and TNFα were determined by high-sensitivity enzyme-linked immunosorbent assay kit (Biosource, Camarillo, CA, USA). All analyses were performed in duplicate centrally at Hospital Universitari Son Dureta (Palma Mallorca, Spain). The lower limits of detection of these assays were 0.16 mg/L, 0.104 pg/ml, 0.10 pg/ml and 0.09 pg/ml for CRP, IL-6, IL-8 and TNFα, respectively. Intraassay variation was always <10%, and reported values correspond to the average of the two determinations.

2.4. Clinical and functional assessment

Information regarding sociodemographic characteristics, pharmacological treatment, respiratory symptoms and lifestyle was obtained using a standardized epidemiological questionnaire. Nutritional status was assessed through body mass index (BMI). Postbronchodilator spirometry (FEV_1, FVC and FEV_1/FVC ratio), and arterial oxygen (PaO_2) and carbon dioxide partial pressures were also measured. The Charlson index of comorbidity [21] was obtained by an expert pulmonologist from medical records and personal anamnesis and exploration. Detailed information on the methods is described elsewhere [15,17].

2.5. Statistical analysis

Sociodemographic and clinical characteristics, intakes of omega-3 and omega-6 fatty acids, and inflammatory markers were described by mean (S.D.), median (P25–P75) or number (%), as appropriate according to the distribution of each variable. Given their skewed distribution, inflammatory marker concentrations were dichotomised according to their median values (TNFα: 0.238 pg/ml, IL-6: 1.004 pg/ml, IL-8: 4.296 pg/ml and CRP: 0.37 mg/L). Levels of omega-3 and omega-6 fatty acid intakes according to inflammatory markers categories (above or below corresponding median values) were compared using Student's *t* test.

The association between PUFA intake and high levels (above the median) of inflammatory markers was estimated using logistic regression models. In order to improve the interpretability of the results, PUFA variables were also dichotomised at their median values (corresponding medians were as follows: DHA: 0.42 g/day, EPA: 0.21 g/day, ALA: 1.22 g/day, LA: 11.21 g/day, AA: 0.18 g/day, ALA/LA: 0.108, EPA/AA: 1.152, DHA/AA: 2.358). Lower intakes were always used as the reference category. The following confounders were considered and included in the final models if they were related to both the exposure and the outcome, or modified (>10% change in coefficient) the estimates for the variables of interest in each model: age, gender, BMI, FEV_1, smoking status, reported physical activity, total caloric intake, inhaled corticosteroid treatment, statin treatment and the Charlson index of comorbidity. Finally, a multivariate model for each inflammatory marker was built including all five fatty acids and confounders. Similarly, a multivariate model for each inflammatory marker was built including all three ratios and confounders. Effect modification by smoking status was assessed by means of both stratification of final models and inclusion of interaction terms. The goodness of fit of all the models was assessed using Hosmer–Lemeshow test [22]. As a sensitivity analysis, all analyses were repeated excluding women (7% of total subjects) and using exposure variables as continuous. Data analysis was conducted using Stata 8.2 (StataCorp, College Station, TX, USA).

3. Results

Table 1 shows the main characteristics of the patients. Ninety-three percent of participants were males with a mean age of 68 years. Most subjects had moderate to severe COPD (distribution in COPD severity stages: 4% mild, 54% moderate, 35% severe and 7% very severe).

In the bivariate analysis, higher intake of ALA (omega-3, anti-inflammatory) was related to lower TNFα levels (1.30 ALA g/day in the low-TNFα category vs. 1.21 g/day in the high-TNFα category, $P=.03$). Regarding omega-6 (proinflammatory), higher intakes of LA and AA were, respectively, associated with higher CRP and IL-6 levels (LA: 11.31 g/day in the low- vs. 12.35 g/day in the high-CRP group, $P=.03$; AA: 0.185 g/day in the low- vs. 0.202 g/day in the high-IL-6 group, $P=.05$). Remaining comparisons did not provide statistically significant differences.

Tables 2 and 3 show crude and adjusted associations between fatty acids intake and each of the inflammatory markers. Being in the

higher category of ALA intake was associated with significantly lower TNFα serum concentrations [adjusted odds ratio (OR)=0.46; P=.049]. Being in the higher category of AA intake was associated with higher serum concentrations of IL-6 (adjusted OR=1.96; P=.034). Elevated intake of AA was associated with a higher concentration of serum CRP (adjusted OR=1.95; P=.039). Regarding the omega-3/omega-6 ratios (Table 3), DHA/AA ratio was associated with TNFα serum concentrations (adjusted OR=3.02; P=.045). No effect modification by smoking status was observed. Sensitivity analysis excluding women or using exposure variables as continuous yielded very similar results.

4. Discussion

Our study with 250 clinically stable COPD patients showed for the first time that dietary intake of omega-3 and omega-6 fatty acids relates to the level of serum inflammatory markers. Specifically, high dietary intake of ALA (an anti-inflammatory omega-3 fatty acid) was associated with reduced risk of high levels of serum TNFα, while dietary intake of AA (a proinflammatory omega-6 fatty acid) was related to increased risk of elevated IL-6 and CRP.

Our finding of an inverse association between ALA intake and TNFα is consistent with previous studies both in healthy populations and among subjects with cardiovascular disease, which showed a negative correlation between omega-3 fatty acid intake and several proinflammatory biomarkers, including CRP, IL-6 and TNFα [13], involved in key inflammatory processes such as NF-κβ activation and MAPK pathways (TNFα), as well as in acute phase response (IL-6). Regarding omega-6 fatty acids, our results are also in agreement with the common assumption that omega-6 fatty acids may be proinflammatory [14], although it has been noted that the relationship between dietary omega-6 fatty acids and proinflammatory mediators is rather complex and not easily predictable [23]. A 25-year prospective study conducted in the Netherlands found an increased risk of incidence of chronic lung diseases associated with LA intake [relative risk of 1.55, 95% confidence interval (CI)=1.11–2.16], although it failed to find any associations with omega-3 fatty acids [24]. Interestingly, there are cross-sectional studies showing that omega-6 fatty acid proinflammatory activities occur only when the intake of omega-3 fatty acids is low [25].

Biochemical evidence about the production of pro- and anti-inflammatory mediators derived from different types of PUFA supports the biological plausibility of our findings [14,23,26]. Eicosapentaenoic acid and DHA, as well as ALA via conversion into EPA or DHA, have several ways of mediating anti-inflammatory activities. One pathway involves the inhibition of AA metabolism, as these omega-3 fatty acids can compete with AA as a constituent of lipidic membranes or directly compete as substrates for cyclooxygenases and lipoxygenases leading to synthesis of less bioactive mediators than those derived from AA [14,26]. Additionally, EPA and

Table 1
Description of sociodemographic and clinical data, polyunsaturated omega-3 and omega-6 fatty acids, and levels of serum inflammatory markers in 250 COPD patients

Male [n (%)]	234 (93.6)
Age (years) [*m* (S.D.)]	68 (8)
Primary or higher education [*n* (%)]	145 (58)
Active worker [*n* (%)]	43 (17.2)
Low socioeconomic status[a] [*n* (%)]	190 (82.25)
BMI (kg/m^2) [*n* (%)]	
<20	4 (1.6)
≥20 & <25	46 (18.4)
≥25 & <30	106 (42.4)
≥30	94 (37.6)
Positive skin prick test [*n* (%)]	30 (12)
>1 Comorbidity (Charlson index) [*n* (%)]	137 (54.8)
Dyspnea score (MMRC, score 0–5) median (P25–P75)	2 (2–3)
Postbronchodilator FEV1 (% predicted) [*m* (S.D.)]	53 (16)
PaO_2 (mmHg) [*m* (S.D.)]	75 (11)
Inhaled corticosteroid treatment [*n* (%)]	165 (66)
Statin treatment [*n* (%)]	38 (15)
Current smoker [*n* (%)]	78 (31)
Regular physical activity (kcal/week) [*m* (S.D.)]	6708 (4976)
Dietary intake	
Daily energy intake (kcal/day) [*m* (S.D.)]	2026 (614)
DHA (g/day) [*m* (S.D.)]	0.49 (0.28)
EPA (g/day) [*m* (S.D.)]	0.23 (0.13)
ALA (g/day) [*m* (S.D.)]	1.26 (0.39)
LA (g/day) [*m* (S.D.)]	11.9 (4.34)
AA (g/day) [*m* (S.D.)]	0.2 (0.09)
Ratio ALA/LA [*m* (S.D.)]	0.11 (0.02)
Ratio EPA/AA [*m* (S.D.)]	1.22 (0.56)
Ratio DHA/AA [*m* (S.D.)]	2.53 (1.14)
Serum inflammatory markers	
TNFα (pg/ml) [median (P25–P75)]	0.238 (0.045–1.018)
IL-6 (pg/ml) [median (P25–P75)]	1.004 (0.545–1.957)
IL-8 (pg/ml) [median (P25–P75)]	4.296 (3.241–5.752)
CRP (mg/L) [median (P25–P75)]	0.370 (0.164–0.661)

Abbreviation: MMRC, Modified Medical Research Council dyspnea scale.
[a] Skilled or unskilled manual workers were classified as low socioeconomic status.

Table 2
Crude and adjusted associations between PUFAs and serum inflammatory markers in a sample of 250 COPD patients

	TNFα>0.238 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Omega-3 (anti-inflammatory):				
DHA>0.42 g/day	1.52	0.92–2.50	2.58	0.69–9.63
EPA>0.21 g/day	1.38	0.84–2.27	0.68	0.18–2.51
ALA>1.22 g/day	0.68	0.41–1.12	0.46	0.21–0.99
Omega-6 (proinflammatory):				
LA>11.21 g/day	0.94	0.57–1.54	1.56	0.71–3.43
AA>0.18 g/day	1.14	0.69–1.87	0.99	0.53–1.82
	IL-6>1.004 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Omega-3 (anti-inflammatory):				
DHA>0.42 g/day	0.97	0.59–1.59	0.83	0.23–2.97
EPA>0.21 g/day	1.00	0.61–1.64	0.92	0.26–3.29
ALA>1.22 g/day	1.07	0.65–1.75	1.24	0.58–2.65
Omega-6 (proinflammatory):				
LA>11.21 g/day	1.21	0.74–1.99	1.29	0.60–2.80
AA>0.18 g/day	1.67	1.02–2.76	1.96	1.05–3.64
	IL-8>4.296 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Omega-3 (anti-inflammatory):				
DHA>0.42 g/day	1.17	0.71–1.93	1.76	0.49–6.25
EPA>0.21 g/day	1.14	0.69–1.87	0.58	0.17–2.07
ALA>1.22 g/day	0.94	0.57–1.54	0.75	0.35–1.60
Omega-6 (proinflammatory):				
LA>11.21 g/day	1.14	0.69–1.87	1.25	0.58–2.70
AA>0.18 g/day	1.47	0.89–2.42	1.56	0.85–2.88
	CRP>0.37 mg/L			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Omega-3 (anti-inflammatory):				
DHA>0.42 g/day	0.97	0.59–1.59	1.71	0.45–6.46
EPA>0.21 g/day	0.88	0.54–1.44	0.40	0.11–1.53
ALA>1.22 g/day	1.57	0.95–2.58	2.00	0.93–4.33
Omega-6 (proinflammatory):				
LA>11.21 g/day	1.67	1.02–2.76	1.86	0.85–4.05
AA>0.18 g/day	1.67	1.02–2.76	1.95	1.03–3.66

[a] An adjusted model has been built for each inflammatory marker, including all five PUFAs and BMI, total caloric intake and smoking status.

Table 3
Crude and adjusted associations between PUFA intake ratios and serum inflammatory markers in a sample of 250 COPD patients

	TNFα>0.238 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Ratio ALA/LA>0.1084	0.83	0.50–1.36	0.76	0.45–1.27
Ratio EPA/AA>1.152	1.25	0.76–2.06	0.50	0.17–1.46
Ratio DHA/AA>2.358	1.62	0.98–2.67	3.02	1.03–8.91
	IL-6>1.004 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Ratio ALA/LA>0.1084	0.88	0.54–1.44	0.92	0.55–1.54
Ratio EPA/AA>1.152	0.70	0.43–1.56	0.43	0.15–1.20
Ratio DHA/AA>2.358	0.85	0.52–1.40	1.85	0.66–5.17
	IL-8>4.296 pg/ml			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Ratio ALA/LA>0.1084	1.07	0.65–1.75	1.04	0.62–1.73
Ratio EPA/AA>1.152	0.85	0.52–1.40	0.66	0.25–1.76
Ratio DHA/AA>2.358	0.91	0.55–1.49	1.32	0.49–3.54
	CRP>0.37 mg/L			
	Crude OR	95% CI	Adjusted OR[a]	95% CI
Ratio ALA/LA>0.1084	0.83	0.50–1.36	0.87	0.52–1.44
Ratio EPA/AA>1.152	0.80	0.49–1.31	0.90	0.34–2.36
Ratio DHA/AA>2.358	0.80	0.49–1.31	0.91	0.34–2.41

[a] An adjusted model has been built for each inflammatory marker, including all three PUFA ratios and BMI, total caloric intake and smoking status.

DHA could reduce NF-κβ DNA-binding proinflammatory activities, leading to reduced cytokine expression [23]. Alternatively, AA, as well as LA via conversion into AA, is the most relevant precursor of eicosanoids (prostaglandins, prostacyclins, thromboxanes and leukotrienes) that have predominantly proinflammatory activities [14,26]. Although mediators such as lipoxins and resolvins may derive from the AA cascade and have anti-inflammatory activities, these are minor paths in the cascade [23,26].

The lack of associations between PUFA intake and IL-8 may be explained by the specific functions of this cytokine. Interleukin-8 is a potent chemoattractant that is needed to recruit and activate neutrophils. Interleukin-8 has been found to be increased in bronchoalveolar lavage of smokers with emphysema [27]. Therefore, IL-8 could be considered a local, rather than systemic, marker of inflammation, and in consequence, finding an association between PUFA intake and IL-8 serum levels would have been unexpected, although it is plausible that this association could arise when measuring IL-8 levels in the airways rather than the serum.

It has been proposed that the ratios between omega-3 and omega-6 fatty acid could be more appropriate for assessing health effects than individual fatty acids levels [26]. Our study found estimates coherent with our hypotheses in seven out of eight of the associations between ALA/LA and EPA/AA ratios and inflammatory markers, although none of them achieved statistical significance. Results with DHA/AA ratio were in the opposite direction than would have been expected and were statistically significant for TNFα.

In our cohort, the main source of omega-3 fatty acids was fish, mainly fresh sardines and tuna, while the main sources of omega-6 fatty acids were vegetable oils, poultry, eggs and baked goods. Therefore, our findings are consistent with previous literature suggesting an association between foods rich in PUFA, such as fatty fish, and respiratory function, COPD symptoms or the prevalence of other chronic lung diseases [10–12]. This manuscript, along with previous literature suggesting that reducing the levels of circulating inflammatory markers could be an effective way of reducing lung function decline [7], provides data about potential mechanisms for these associations.

Limitations to the current study include (a) measurement error in the estimation of dietary fatty acids intake due to the use of a food frequency questionnaire; (b) reduced variability in PUFA levels in our sample, as mean daily intake of PUFA was high (99% of patients had intakes above the Spanish recommendations [18]); and (c) the lack of repeated data about inflammatory markers, which could have short-term variability [28]. All previous limitations have most likely resulted in a reduced statistical power (calculated *a posteriori* no greater than 60%), thus yielding a considerable risk of false-negative results. The cross-sectional study design makes impossible to establish the direction of the observed associations. However, it seems unlikely that dietary habits would change as a result of serum inflammatory marker levels and much more likely that the latter may have been affected by habitual diet. Finally, it could be argued that statistical methods for multiple testing were needed. However, authors did not apply these methods as has been suggested when *a priori* clear hypotheses exist [29]. Importantly, in addition to *P* values, we emphasized the consistency in magnitude and direction of associations across multiple inflammatory markers, as well as used both individual fatty acids and fatty acid ratios, in evaluating and interpreting our results.

In conclusion, this study provides the first evidence of an association between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD patients. However, given the abovementioned limitations of the present study, these findings need further replication in larger samples of COPD patients with wider variability in dietary habits. Future research should consider both local and systemic inflammatory markers, thus allowing a comprehensive picture of the effects of PUFA intake in COPD.

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