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Effect of amino acid-based formula added to four-food elimination in adult eosinophilic esophagitis patients: A randomized clinical trial

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Abstract

Background: Elimination of key foods restricts dietary options in eosinophilic esophagitis (EoE) patients. Addition of amino acid-based formula (AAF) to an elimination diet might facilitate adherence and, therefore, enhance efficacy of dietary management.

Aim: To evaluate whether addition of AAF to a four-food elimination diet (FFED) is more effective than FFED alone in decreasing eosinophilia, endoscopic signs, and clinical outcomes.

Methods: This randomized controlled trial enrolled 41 adult patients with active EoE ($\geq$15 eosinophils (eos) per high power field (hpf)) at baseline biopsy. Subjects were randomized (1:1 ratio) to groups given a FFED or FFED with addition of AAF providing 30% of their daily energy needs (FFED + AAF). Histological disease activity, endoscopic signs, symptoms, and disease-related quality of life (EoEQoL) were measured at baseline and after 6 weeks of intervention.

Results: Patients (60% male, age 34.5 (interquartile range (IQR) 29–42.8 years)) were randomized to FFED ($n = 20$) or FFED + AAF ($n = 21$); 40 participants completed the diet. Complete histological remission (<15 eos/hpf) was achieved in 48% of FFED + AAF subjects ($n = 21$) vs. 25% of FFED subjects ($n = 20$), respectively ($p = 0.204$). Peak eosinophil counts (PEC) decreased significantly in both groups between baseline and week 6, but the change in PEC between groups was not different ($p = 0.130$). A significant but similar endoscopic and symptomatic reduction was observed in both groups (all; $p<0.05$). Total EoEQoL scores significantly improved in the FFED + AAF group between baseline and week 6 ($p = 0.007$), and not in the FFED group.

Abbreviations: AAF, amino acid-based formula; BMI, body mass index; EGD, esophagogastroduodenoscopy; EoE, eosinophilic esophagitis; Eos, eosinophils; EREFS, Endoscopic Reference score; FFED, four-food elimination diet; GI, gastrointestinal; hpf, high power field; IQR, interquartile range; Kg, kilograms; PEC, peak eosinophil count; PPI, proton pump inhibitor; QoL, quality of life; SD, standard deviation; SDI, Straumann Dysphagia Instrument; SFED, six-food elimination diet; Th2, T-helper type 2.

Conclusion: The addition of AAF to a FFED did not lead to a larger decrease in PEC between baseline and 6 weeks, but may result in a significant improvement of QoL in adult EoE patients NL6014 (NTR6778).

KEYWORDS
elemental diet, elimination diet, eosinophilic esophagitis, esophageal eosinophilia and allergy, quality of life

1 | INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic T-helper type 2 (Th2) immune-mediated disorder of the esophagus characterized by symptoms of esophageal dysfunction (i.e., dysphagia and/or food impaction) and eosinophil-predominant inflammation.[1,2] After its first description in the early 1990s, the worldwide EoE incidence and prevalence have surged to rates that outpace increased disease detection.[3–6] Food allergens have been suggested to play a causal role in EoE pathogenesis after primary reports of documented disease remission in children being treated with amino acid-based formula (AAF).[7] The current management of EoE involves targeting the esophageal inflammation with medical therapy (i.e., proton pump inhibitors (PPIs) or swallowed topical steroids) and dietary elimination of culprit foods.[8] EoE generally flares after cessation of induction treatment.[9–11] Hence, maintenance therapy is needed since long-standing eosinophilic inflammation is associated with esophageal narrowing and stricture formation often requiring dilation.[12–15] The rationale of non-pharmacological therapy is linked to EoE pathogenesis, with dietary treatment being indicated as a potential safe and drug-free solution for the long term.[16–19] Elemental diets (i.e., complete removal of food allergens by exclusive use of AAF) have proven to be highly efficacious (85%–95% disease remission rates) in EoE patients of all ages.[7,20–24] However, adherence is challenged by its poor palatability (i.e., absence of solid foods, monotonous taste) and impaired socialization. Therefore, the six-food elimination diet (SFED) has become a more preferred approach with consistently reported remission rates of 70% after restriction of milk, wheat/gluten, egg, soy, peanut/tree nuts, fish, and seafood.[16,18,25] Efficacy of elimination diets parallels the number of excluded foods, yet rigorous diet restrictions with risk of inadequate food intake (e.g., nutritional deficiencies or low calories) and subsequent need for multiple re-endoscopies impede patients' acceptability in daily life.[26,27] As such, there has been extensive interest in more efficient empiric diets to induce disease remission and lower diet costs and quality-of-life (QoL) burdens of treatment.[28,29] Elimination of four foods including wheat/gluten, milk, egg, and either soy or legumes (FFED) is less restrictive, but also less effective with remission rates in children and adults between 54% and 64%.[24,30] Patients' motivation and acceptance yields a key factor of successful dietary treatment in order to increase adherence and minimize impact on QoL. Aside from the hypoallergenic properties of AAF, which may decrease the risk of diet errors (i.e., mistakes of food label reading or

KEY POINTS
- Elimination of key foods restricts dietary options, hence a combined strategy of empiric elimination of causative foods with amino acid-based formula (AAF) added to a diet may thus improve EoE patients' adherence and acceptance along with efficacy of dietary management.
- A combination dietary intervention (AAF added to a four-food elimination diet) did not lead to a larger decrease in peak eosinophil counts between baseline and 6 weeks intervention, but may result in a significant improvement of QoL in adult patients with EoE.

allergen cross-contamination), recent insights suggested it to have immune-modulating effects itself.[31–34] Hence, a combined strategy of empiric elimination of causative foods with AAF added to the diet may thus improve patients' adherence and acceptance along with efficacy of dietary management. The aim of this study was therefore to determine whether AAF added to a FFED is more effective than a standard FFED in decreasing esophageal eosinophilia, improving endoscopic signs, and clinical- and nutritional outcomes in adult EoE patients.

2 | METHODS

2.1 | Study patients

In this single-center, open-label, randomized controlled trial, all patients were included from the outpatient clinic of the Amsterdam UMC motility center between December 2017 and January 2020. Adult patients were eligible for enrollment if EoE was diagnosed per consensus guidelines, defined as having symptoms of esophageal dysfunction (Straumann Dysphagia Instrument (SDI) score of ≥1) and ≥15 eosinophils (eos) per microscopic high power field (hpf) on baseline biopsy.[9] Exclusion criteria were severe comorbidity scored as the American Society of Anesthesiologists (ASA) Physical Classification System class IV or higher, a recent history of gastrointestinal (GI) cancer or major GI surgery, and the inability to stop anti-inflammatory drugs (i.e., topical or systemic steroids, leukotriene inhibitors, or monoclonal antibodies). The study protocol was approved by the

Medical Ethics Committee of our institution and prospectively registered in the Dutch trial registry NL6014 (NTR6778). All participants provided written informed consent before taking part and were given a unique study ID to ensure anonymity.

2.2 | Study design

After signed informed consent at visit 1, patients consulted a dietician specialized in allergies for extensive nutritional evaluation. To guarantee sufficient intake and to improve diet adherence, patients subsequently received a personalized nutritional advice with restriction of gluten, milk, soy, and eggs (FFED). The amount of prescribed AAF added to the FFED in the intervention group was 30% of patients' daily caloric requirements based on body mass index and weekly physical activity. The AAF was consumed over 3 moments per day. After conformation of eligibility by baseline upper endoscopy, patients were randomized in a 1:1 fashion to the treatment arms (FFED or FFED + AAF) using a blocked randomization protocol with sealed envelopes. All participants underwent an esophagogastroduodenoscopy (EGD) at baseline and after 6 weeks of dietary intervention. Histologic, endoscopic, and clinical outcomes and nutritional outcomes were evaluated between week 1 and week 6. Side effects, patients' adherence, weight loss, and AAF intake were carefully monitored by a dietician and a physician during the 6 weeks of intervention. The study design overview is presented in Table S1.

2.2.1 | Study product

An amino acid-based, hypoallergenic powdered formula (Neocate Junior, Nutricia, Utrecht, the Netherlands), unflavored, strawberry and vanilla flavor, was used in this clinical trial. This formula was selected by the study team because of its relatively good taste compared to similar formulas. To increase adherence to the prescribed AAF intake, patients were able to taste all three formulas during a test round to indicate their preferred flavor(s).

2.3 | Study endpoints and procedures

2.3.1 | Primary endpoint

The primary outcome of this trial was the change in peak eosinophil count (PEC), measured as the maximum number of eos/hpf.

2.3.2 | Secondary endpoints

In addition, the difference between groups in complete histological remission rates were evaluated, which was achieved if the reduction in absolute number of eos/hpf decreased to <15. Other secondary pre-specified endpoints were endoscopic signs, clinical and nutritional outcomes, including diet feasibility and adherence, and weight loss.

TABLE 1 | Baseline characteristics of all patients (n = 41) that were eligible for randomization (ITT-cohort)

Characteristics	ITT-cohort	
	FFED (n = 20)	FFED + AAF (n = 21)
Male gender, n(%)	12 (60)	13 (62)
Age, years, median (IQR)	32 (27.5–43)	36 (29–42)
Race, Caucasian, n(%)	19 (95)	20 (95)
History of allergic disease, n(%)	17 (85)	18 (86)
Allergic rhinitis	14 (70)	14 (67)
Asthma	5 (25)	7 (33)
Atopic dermatitis	5 (25)	8 (38)
Food allergy	7 (35)	6 (29)
Angioedema	1 (5)	2 (10)
Oral Allergy Syndrome	6 (30)	8 (38)
PPIs at baseline, n(%)	8 (40)	9 (43)
Prior use of topical steroids, n(%)	10 (50)	9 (43)
Esophageal stricture dilation, n(%)	1 (5)	2 (10)
Previous endoscopic intervention with food bolus extraction, n (%)	8 (40)	10 (48)
Diagnostic delay*, median (IQR)	5 (1–8.8)	2 (1–9.5)
BMI (kg/m2), median (IQR)	24.1 (22.4–28.4)	23.7 (22.2–26.6)

Abbreviations: BMI, Body Mass Index; FFED, Four Food Elimination Diet; FFED + AAF, Four Food Elimination Diet with addition of amino acid-based formula; ITT, Intention-to-treat; PPIs, Proton Pump Inhibitors.

*Time interval between first reported EoE symptoms and year of diagnosis.

2.3.3 | Histological outcomes

Six biopsies taken from the distal, mid, and proximal esophagus were directly fixed in formalin and subsequently embedded in paraffin. After 24 h, the biopsies were sectioned at 5 μm thickness and stained with hematoxylin and eosin and tryptase. To determine eligibility for enrolment, all biopsies were directly analyzed in the Amsterdam UMC pathology department to determine PEC as per standardized protocol. In a low-power view setting, the area of most densely populated eosinophilia in the esophageal biopsy specimen was identified. A x400 magnification was used in order to determine the PEC per hpf (an area of 0.24 mm^2).

2.3.4 | Endoscopic outcomes

During EGD, images of the esophagus were recorded for evaluation of endoscopic signs and were incorporated in a slideshow (Microsoft PowerPoint 2016; Microsoft Inc.,). All images were blinded and scored according to the Endoscopic Reference Score (EREFS) by a single gastroenterologist with expertise in EoE to minimize the risk of inter-observer bias.[35] All endoscopic features were sub-classified as inflammatory (white exudates, edema, and linear furrows) and fibrotic (rings and strictures) signs.[13]

2.3.5 | Clinical outcomes

Symptoms of dysphagia were evaluated by means of the Straumann Dysphagia Instrument (SDI) measure.[36] This measure evaluates dysphagia frequency and intensity.[37] Furthermore, diet restrictions are known to impact QoL in EoE patients.[38] Therefore, disease-specific QoL was assessed by the validated Adult Eosinophilic Esophagitis Quality of Life (EoEQoL) survey. Overall scores range from 0 to 96, with higher scores indicating better QoL. The total EoEQoL index score includes the weighted average of all QoL subscales.

2.3.6 | Nutritional outcomes

To evaluate the effort needed to maintain the diet (i.e., feasibility), participants were asked to respond to the statement: "The diet is difficult to maintain for me" (0–4 = strongly agree–disagree). Participants were also asked to rate their diet adherence on a 10-point scale (0–10 = low–high) at week 6. In addition, at weeks 2 and 4, diet adherence was monitored via telephone and/or e-mail contact by the dietician/physician. Body mass index (BMI), nutritional intake (i.e., 3 days food diaries), diet adherence, and energy intake were evaluated at baseline and after 6 weeks of dietary intervention. The total consumption of AAF for each participant was calculated by the amount of returned empty and full study product cans at week 6. AAF consumption was also monitored via telephone and/or e-mail contact during the study period. Individual adherence to the prescribed intake of AAF (i.e., AAF adherence rate (%)) was defined as the total amount of consumed AAF (kilograms (kg)) as percentage of the total prescribed AAF (kg) over the period of 6 weeks.

2.4 | Sample size calculation

A single-arm study of Molina Infante et al. showed that a cohort of 52 adult EoE patients was sufficient to demonstrate a significant effect of a standard FFED.[39] A decrease of mean PEC per hpf from 55 with an estimated standard deviation (SD) of 30–24 (with a difference of 31) was observed. The SD after treatment was not reported in this study. We used the reported mean PEC (eos/hpf) after treatment for responders (<15 eos/hpf) 2 (0–8) and non-responders 45 (26–141) to estimate a SD of 1.96 (responders) and 29.64 (non-responders), respectively. The estimated pooled SD after treatment with a standard FFED was 20. Since no data were available on this new approach, the estimated improvement was partly based on efficacy rates of the elemental dietary treatment. We based the SD of our FFED + AAF group on a study of Peterson et al., evaluating the effect of an exclusive elemental diet treatment on EoE.[20] In this study, the PEC (eos/hpf) after treatment decreased from 54 (SD 32) to 10 (SD 12) (with a difference of 44). Since the SD of the standard FFED group and FFED + AAF group were based on different populations, we assumed that an estimated SD of 15 would be appropriate.

Therefore, a sample size of 20 patients per treatment arm was calculated to provide 80% power to detect a clinically meaningful treatment effect, with an expected difference of 13 in mean change in PEC after treatment between the standard FFED group and FFED + AAF group, and with 5% significance and an assumed SD of 15.

2.5 | Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 25.0) (SPSS). Descriptive statistics were used to summarize all characteristics of the study groups. Categorical variables are described as percentages and continuous variables are expressed as mean ($\pm$ standard deviation (SD)) or median (interquartile range (IQR)). Change in PEC was analyzed by fitting a linear least squares model with treatment group and baseline PEC value as covariates. Categorical analyses between or within treatment groups were performed on secondary endpoints by using the Fisher's exact test and McNemar's test. Comparisons of additional endpoints between groups and between pre- and post-treatment were performed by using the Wilcoxon signed rank test and Mann–Whitney U-test, as appropriate, in case of continuous data. The primary and secondary outcomes were evaluated in both the intention to treat (ITT) and per-protocol (PP) data sets. A two-sided p-value of <0.05 was considered significant.

3 | RESULTS

3.1 | Inclusions and patient characteristics

Fifty-three EoE patients with clinical active disease were invited for an intake visit at the outpatient clinic. A total of 52 patients were eligible for screening and underwent an EGD with biopsies at baseline, after which 11 patients were excluded due to the absence of active disease at histological assessment (<15 eos/hpf). Eventually, 41 patients met all eligibility requirements and were randomized to the FFED (n = 20) group and FFED + AAF (n = 21) group and were analyzed according to ITT. A protocol violation was reported in one participant as a result of non-adherence to the diet at week 1 in the FFED group with subsequent disqualification of the trial. In addition, a protocol violation was reported due to non-adherence (0% intake) to the AAF intake in one patient between baseline and week 6 in the FFED + AAF-group. Considering no other protocol deviations or violations were reported, this patient was switched to the FFED group in the PP analysis. In total, 40/40 EoE participants (FFED (n = 20) and FFED + AAF (n = 20)) completed the diet after 6 weeks according to the protocol and were entered for final PP analysis (Figure 1).

Baseline characteristics of the ITT cohort were well balanced, with a male predominance in both groups. No significant differences were found on gender, age, race, presence of atopy, previous use of PPIs, and BMI between both treatment groups. Most of the participants had ≥2 additional atopic comorbidities (Table 1). Observations were similar in the PP cohort and these patient characteristics are presented in the Table S2.

3.2 | Histological outcomes

3.2.1 | Primary endpoint: Peak eosinophil count

A significant decrease in the median PEC from baseline to 6 weeks was observed in both groups of ITT population (FFED + AAF: from 50 (IQR 45–100) to 22 (IQR 3.5–38); $p = 0.001$, and (FFED: from 56.5 (IQR 41.3–78.8) to 25 (IQR 12–50); $p = 0.011$, respectively) (Table 2, Figure 2A, Figure S1A,B). Primary endpoint analysis showed no difference in the change in the median PEC from baseline to 6 weeks between the two groups, FFED + AAF and FFED (–41.5 (SD 37) vs. –26.9 (SD 39)), respectively ($p = 0.127$) (Table 2, Figure 2A). Comparing FFED + AAF vs. FFED at week 6 showed lower peak eosinophil levels in the participants treated with the combination of FFED + AAF (22 (IQR 3.5–38) vs. 25 (IQR 12–50), respectively ($p = 0.158$)) (Table 2, Figure 2A). Similar results were observed in the PP cohort (Table S3A, Figure S2A,B).

3.2.2 | Histological remission rates

Forty-eight percent of the FFED + AAF subjects in PP population showed complete histological remission (<15 eosinophils per hpf) at

week 6 vs. 26% of the FFED subjects ($p = 0.204$). Partial histological remission (≥50% reduction in pre-treatment PEC) was achieved in 24% of the FFED + AAF subjects vs. 25% of the FFED subjects after 6 weeks (p = 1.000) (Table 3, Figure 2B). In addition, the proportions of FFED + AAF subjects with peak eosinophil levels of ≤10 eos/hpf and ≤5 eos/hpf compared to the FFED subjects at week 6 were 43% vs. 20% ($p = 0.186$) and 43% vs. 10% ($p = 0.034$), respectively. In FFED + AAF subjects, 14% had PEC of ≤1 eos/hpf at week 6 vs. 0% of the FFED subjects ($p = 0.233$) (Table 3). Similar results were observed in the PP cohort (Table S3B).

3.3 | Endoscopic outcomes

The total EREFS score significantly changed in both groups of the ITT population after the diet (FFED + AAF: from 4 (IQR 3–5) to 3 (IQR 1.5–4); $p = 0.002$, and FFED: from 4 (IQR 3.3–5) to 4 (IQR 1–4); $p = 0.026$), respectively). No difference in the change in the total EREFS score from baseline to 6 weeks was observed between the FFED + AAF group and FFED group (–1 (IQR 2–0) vs. –1 (IQR 2–0)), respectively ($p = 0.687$) (Table 4, Figure 3A). In addition, inter-group ITT analysis showed a significant improvement of the inflammatory sub-scores in both groups after intervention, whereas the fibrotic sub-score only significantly improved in the FFED + AAF subjects ($p = 0.013$) and not in the FFED subjects ($p = 0.109$) (Table 4). Results of the PP analysis are presented in Table S4. All individual components of the EREFS classification improved after treatment and pre-/post-treatment outcomes in the ITT population were similar between both groups (all; $p > 0.05$). Similar observations were seen in the PP population.

3.4 | Symptom outcomes

3.4.1 | Dysphagia

The SDI score decreased significantly from baseline to week 6 in both groups of the ITT population (FFED + AAF: from 5 (IQR 3.5–6) to 3 (IQR 0.5–3.5); $p = 0.001$, and FFED: from 5 (IQR 3.8–7) to 2 (IQR 0–4); $p = 0.001$). No difference in the change in the total SDI score from baseline to 6 weeks was observed between the FFED + AAF-group and FFED group (–2 (IQR –4 to –2) vs. –2.5 (IQR –4.3 to –1), respectively ($p = 0.829$)) (Table 5, Figure 3B). Similar results were observed in the PP population (Table S5).

3.4.2 | Disease-specific quality of life

ITT analysis showed that the disease-specific QoL (EoEQoL) scores only significantly improved in the FFED + AAF group (3 (IQR 2.4–3.2) - 3 (IQR 2.6–3.4); $p = 0.007$), whereas no significant improvement was observed in the FFED group after 6 weeks treatment (2.5 (IQR 1.8–3.3) to 2.8 (IQR 2.2–3.5); $p = 0.378$). No difference in the
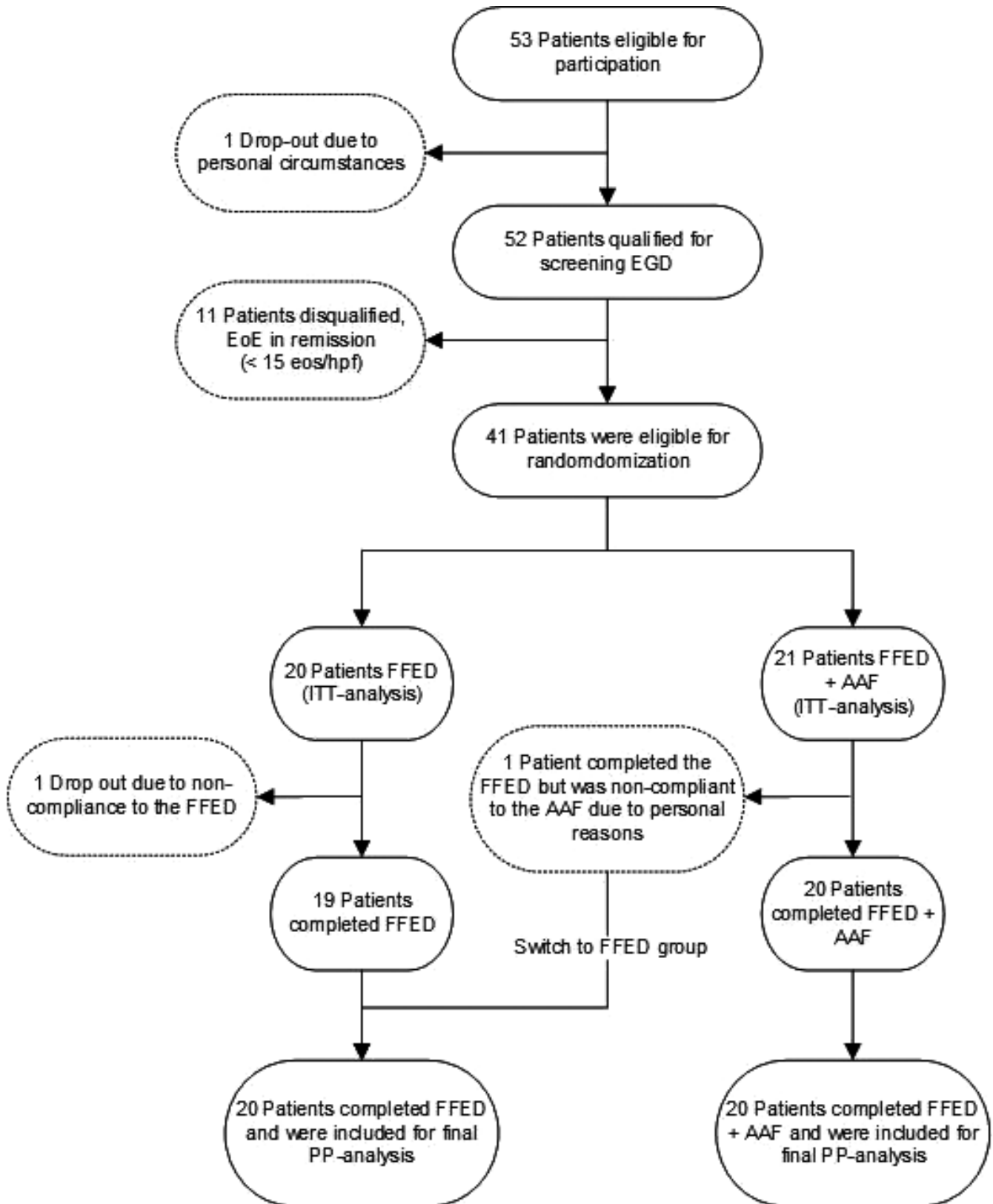
FIGURE 1 | Flow chart demonstrating the number of patients who were eligible for participation, randomization, and ITT analysis. In addition, all patients who discontinued the trial or were switched to the FFED group for final PP analyses are presented. FFED, Four-food elimination diet; ITT, Intention to treat; PP, Per protocol

TABLE 2 | Histological features before and after treatment in both groups

Histological outcomes	ITT-cohort				
	FFED (n = 20)	FFED + AAF (n = 21)	p value	B	SE B
Peak Eosinophil Count					
Baseline, *eos/hpf*, median (IQR)	56.5 (41.3–78.8)	50 (45–100)	0.969[a]		
Post-treatment, *eos/hpf*, median (IQR)	25 (12–50)	22 (3.5–38)	0.158[a]		
P value (paired pre/post treatment)	0.011[b] *	0.001[b] *			
Absolute change in peak eosinophil count from baseline to wk 6, eos/hpf, mean (SD)	−26.2 (39.9)	−40 (36)	0.130[c]	−16	10.3

Abbreviations: B, Unstandardized beta; FFED + AAF, Four Food Elimination Diet with addition of amino acid-based formula; FFED, Four Food Elimination Diet; IQR, Interquartile range; ITT, Intention-to-treat; SD, Standard Deviation, SE B, Standard Error for the unstandardized beta.

[a]*p* value FFED vs. FFED + AAF (Mann-Whitney *U*-test).

[b]*p* value baseline vs. after-treatment (Wilcoxon signed rank test).

[c]Unadjusted *p*-value for the effect between treatment groups from linear least squares model with treatment group and baseline peak eosinophil count value as covariates.

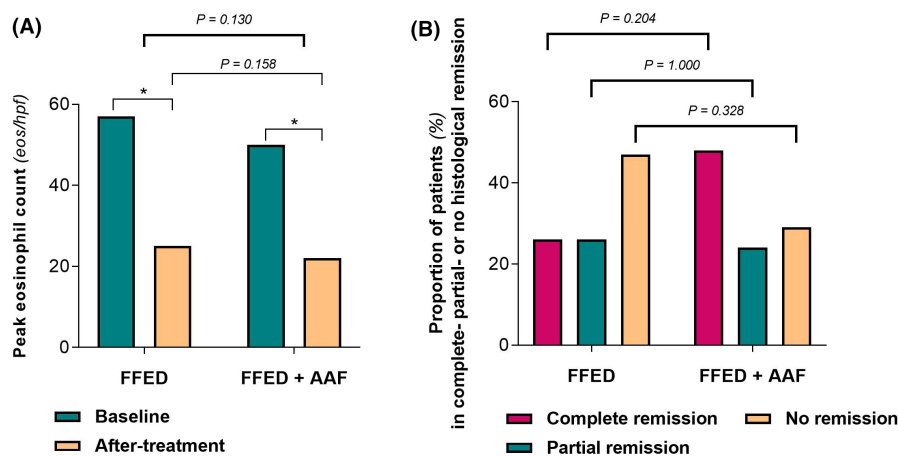*p*-value (two-sided) of <0.05, indicating a significant outcome.

FIGURE 2 | Histological outcome measures (ITT cohort): (A) peak eosinophil count (eos/hpf) pre-/post-treatment and between groups; and (B) post-treatment proportion (%) of patients in complete-/partial-/no histological remission between groups. Complete histological remission = Rate of patients with complete histological remission at week 6 (<15 *eos/hpf*), partial histological remission = Rate of patients with partial histological remission at week 6 (≥50% reduction in pre-treatment *eos/hpf*), and no histological remission = Rate of patients with no histological remission at week 6., eos, Eosinophils; hpf, High power field; ITT, Intention to treat

change in the total EoEQoL score from baseline to 6 weeks was observed between the FFED+AAF group and FFED group (0.1 (IQR 0.04–0.56) vs. 0 (IQR -0.08–0.40), respectively (*p* = 0.298)) (Table 5, Figure 3C). Similar observations were seen in the PP population (Table S5). Comparison of the EoEQoL sub-scores in the ITT population showed only a significant improvement in the change in the social impact score at week 6 in the FFED + AAF subjects and not in the FFED subjects (0.3 (IQR 0.1–1) vs. 0 (IQR -0.3–0.3), respectively) (*p* = 0.012) (Table S6). The change in the EoEQoL sub-scores: eating/diet impact, emotional impact, disease anxiety, and swallowing anxiety after 6 weeks of intervention were similar between both groups (Table S6). In addition, improvements from baseline to 6 weeks in the total EoEQoL score and sub-scores of social impact, disease anxiety, and swallowing anxiety were significant in the FFED + AAF group (all; *p* < 0.05), whereas no significant improvements of the total EoEQoL score and sub-scores were noted in the FFED group

(Table S6). Furthermore, post-treatment EoEQoL eating/diet impact sub-scores (4 items and 10 items) did not differ significantly between the FFED + AAF group and FFED group (4 items: 2.3 (IQR 2.0–2.8) vs. 2 (IQR 0.8–2.8), respectively (*p* = 0.544) and 10 items: 2.5 (IQR 1.6–2.8) vs. 2.2 (IQR 1.4–2.7), respectively) (*p* = 0.361) (Table S6). Similar results were observed in the PP population.

3.5 | Nutritional outcomes

3.5.1 | Weight loss, diet feasibility, diet adherence and AAF intake

The median BMI (kg/m^2) in the ITT population decreased significantly from 24 (IQR 22.3–26.7) to 23.8 (IQR 21.5–26) after FFED + AAF (*p* = 0.001) and from 24 (IQR 22.4–28.2) to 23.3 (IQR

Histological outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	p value
Histological remission rates			
Rate of patients with complete histological remission at wk 6 (<15 *eos/hpf*), n (%)	5 (25)	10 (48)	0.204[a]
Rate of patients with partial histological remission at wk 6 (≥50% reduction of pre-treatment *eos/hpf*), n (%)	5 (25)	5 (24)	1.000[a]
Rate of patients with no histological remission at wk 6, n(%)	9 (45)	6 (29)	0.328[a]
Histological response thresholds			
Rate of patients with histological remission at wk 6 (≤10 *eos/hpf*), n (%)	4 (20)	9 (43)	0.186[a]
Rate of patients with histological remission at wk 6 (≤5 *eos/hpf*), n (%)	2 (10)	9 (43)	0.034[a,*]
Rate of patients with histological remission at wk 6 (≤1 *eos/hpf*), n (%)	0 (0)	3 (14)	0.233[a]

Abbreviations: FFED + AAF, Four Food Elimination Diet with addition of amino acid-based formula; FFED, Four Food Elimination Diet; IQR, Interquartile range; ITT, Intention-to-treat.

[a] *p* value FFED vs. FFED + AAF (Fisher's exact test).

[*] *p*-value (two-sided) of <0.05, indicating a significant outcome.

22–27.3) after FFED ($p < 0.001$). No difference in the change in BMI (kg/m^2) from baseline to 6 weeks was observed between the FFED + AAF group and the FFED group (−0.6 (IQR −1.2 to −0.1) vs. −0.8 (IQR −1.5 to −0.3, respectively) ($p = 0.472$) (Table 6). Furthermore, secondary endpoints on self-reported feasibility of and adherence to the dietary intervention were similar between groups (Table 6). Results were similar in the PP population. No additional protocol deviations were reported by the dietician or physician regarding patients' adherence to the diet at week 6 in both groups. The median adherence rate of AAF intake at week 6 was 84% (IQR 69–97) in the ITT population and 87% (IQR 72–98) in the PP population (Table S7, Figure S3).

3.6 | Adverse events

No serious adverse events occurred during the study period. One adverse event was reported in the FFED + AAF group (i.e., emergency room visit due to severe abdominal pain after eating a kiwi) but was not related to the intervention or study product.

4 | DISCUSSION

In this randomized controlled trial, we determined whether AAF added to a FFED was more effective than FFED alone in dietary treatment of adult EoE patients. Although our primary outcome was not significantly different between the two groups, lower peak eosinophil levels were seen in the FFED + AAF subjects compared to the FFED subjects (PP population) after 6 weeks of treatment (17 (IQR 3.3–35.5) vs. 26.5 (IQR 14-48.8); $p = 0.098$). Moreover, a higher proportion of PP subjects in the FFED + AAF group achieved complete histological remission at week 6 compared to the FFED group (50% vs. 25%; $p = 0.191$). Disease-related QoL scores significantly improved between baseline and week 6 in subjects treated with the FFED + AAF and not in the FFED group. These findings could suggest that a combined approach of FFED and AAF may have benefits above FFED alone.

Significant intra-group improvements of histological, endoscopic, and symptomatic outcomes were seen in both the FFED + AAF group and FFED group, which affirms previous reports on FFED efficacy in adult EoE patients.[24] Improvements in the intervention group were not statistically superior to those seen in the FFED group between baseline and week 6, yet this trial was not powered to show differences between groups in these pre-specified secondary outcomes.

Considering the levels of post-treatment eosinophilia and other histological endpoints, it is possible that the absence of a significantly different primary outcome might have resulted from a low power (type II error). Since no data are available on this combined dietary approach, the estimated improvement of the intervention group was based on a study of Peterson et al., in which AAF intake comprised 100% of patients' caloric intake.[20] Therefore, expected post-treatment differences between FFED + AAF and FFED subjects used in our power calculations may have been overestimated (large effect size) resulting in a sample size with too low power.

The overall observed (complete-) remission rate of 38% (PP-cohort) is remarkably lower compared to a study by Molina Infante

TABLE 4 | Endoscopic features before and after treatment in both groups

Endoscopic outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	p value
EREFS			
Total EREFS score			
Baseline, median (IQR)	4 (3–5)	4 (3–5)	0.685[a]
Post-treatment, median (IQR)	4 (1–4)	3 (1.5–4)	0.689[a]
p value (paired pre/post treatment)	0.026[b] *	0.002[b] *	
Change in total EREFS score from baseline to wk 6, median (IQR)	−1 (−2–0)	−1 (−2–0)	0.687[a]
Inflammatory score			
Baseline, median (IQR)	2 (2–3)	3 (2–3)	0.469[a]
Post-treatment, median (IQR)	2 (1–2)	2 (1–2)	0.567[a]
p value (paired pre/post treatment)	0.07[b],*	0.017[b],*	
Change in inflammatory score from baseline to wk 6, median (IQR)	0 (−1.75–0)	−1 (−1–0)	0.779[a]
Fibrostenotic score			
Baseline, median (IQR)	2 (0.25–3)	2 (1–2)	0.547[a]
Post-treatment, median (IQR)	1 (1–2)	1 (1–2)	0.933[a]
p value (paired pre/post treatment)	0.109[b]	0.013[b],*	
Change in fibrostenotic score from baseline to wk 6, median (IQR)	0 (−1–0)	0 (−1–0)	0.341[a]

Note: Endoscopic features are scored according to the EREFS classification and sub-classified as (i) inflammatory signs including white exudates, edema and linear furrows (ii) fibrostenotic signs including rings and strictures.

Abbreviations: FFED + AAF, Four Food Elimination Diet with addition of amino acid-based formula; FFED, Four Food Elimination Diet; IQR, Interquartile range; ITT, Intention-to-treat.

[a]p value FFED vs. FFED + AAF (Mann-Whitney U-test).

[b]p value baseline vs. after-treatment (Wilcoxon signed rank test).

*p-value (two-sided) of <0.05, indicating a significant outcome.
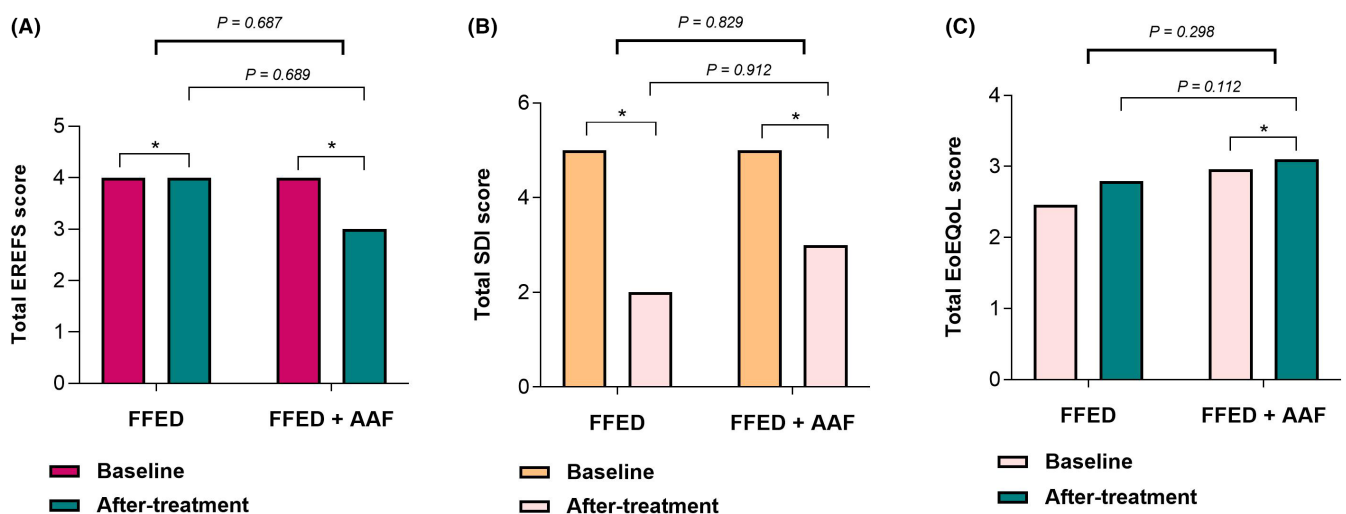

FIGURE 3 | Endoscopic and Symptom outcome measures (ITT-cohort): (A) EREFS pre-/post-treatment and between groups; (B) SDI-PRO measure score pre-/post-treatment and between groups; and (C) EoEQoL pre-/post-treatment and between groups. EREFS, Endoscopic Reference score; ITT, Intention to treat; SDI, Straumann Dysphagia Instrument

Symptom outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	p value
Dysphagia symptoms			
SDI score			
Baseline, median (IQR)	5 (3.75–7)	5 (3.5–6)	0.343[a]
Post-treatment, median (IQR)	2 (0–4)	3 (0.5–3.5)	0.912[a]
p value (paired pre/post treatment)	0.001[b,*]	0.001[b,*]	
Change in total SDI score from baseline to wk 6, median (IQR)	−2.5 (−4.25−−1)	−2 (−4−−2)	0.829[a]
Disease specific Quality of Life			
Total EoEQoL score			
Baseline, median (IQR)	2.46 (1.82–3.29)	2.96 (2.42–3.15)	0.345[a]
Post-treatment, median (IQR)	2.79 (2.21–3.5)	3.1 (2.6–3.44)	0.112[a]
p value (paired pre/post treatment)	0.378[b]	0.007[b,*]	
Change in total EoE-QoL score from baseline to wk 6, median (IQR)	0 (−0.08–0.4)	0.1 (0.04–0.56)	0.298[a]

Abbreviations: EoEQoL, Adult Eosinophilic Esophagitis Quality of Life survey (24 items, *weighted average)*; FFED +AAF, Four Food Elimination Diet with addition of amino acid-based formula; FFED, Four Food Elimination Diet; IQR, Interquartile range; ITT, Intention-to-treatRDQ, Reflux Disease Questionnaire. RDQ score includes heartburn and regurgitation; SDI, Straumann Dysphagia Instrument.

[a]p value FFED vs. FFED + AAF (Mann-Whitney *U*-test).

[b]p value baseline vs. after-treatment (Wilcoxon signed rank test).

*p-value (two-sided) of <0.05, indicating a significant outcome.

Nutritional outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	p value
Weight loss			
BMI (kg/m²)			
Baseline, median (IQR)	24 (22.4–28.3)	23.7 (22.2–26.7)	0.540[a]
Post-treatment, median (IQR)	23.6 (22–27.5)	23.4 (21.5–26)	0.645[a]
p value (paired pre/post treatment)	<0.001[b,*]	0.001[b,*]	
Change in BMI (kg/m²), median (IQR)	−0.9 (−1.48−−0.3)	−0.58 (−1.2–0)	0.248[a]
Weight loss (kg), median (IQR)	3 (1–5)	2 (0–4)	0.255[a]
Feasibility score			
Post-treatment, median (IQR)	3 (1–3)	3 (1.3–3)	0.872[a]
Self-reported adherence rate (%)			
Post-treatment, median (IQR)	90 (90–100)	90 (90–100)	0.867[a]

Abbreviations: BMI, Body Mass IndexFFED + AAF, Four Food Elimination Diet with addition of amino acid-based formula; FFED, Four Food Elimination Diet; IQR, Interquartile range; ITT, Intention-to-treat.

[a]p value FFED vs. FFED + AAF (Mann-Whitney *U*-test).

[b]p value baseline vs. after-treatment (Wilcoxon signed rank test).

*p-value (two-sided) of <0.05, indicating a significant outcome.

et al., in which complete histological remission (<15 eos/hpf) was reported in 54% EoE patients after 6 weeks FFED.[39] The use of a more extensive food elimination approach in the study of Molina Infante et al., including gluten, milk, egg, and all kind of legumes (e.g., soy, lentil, peanut) alternatively to only soy, may explain the observed differences in remission rates.[18,27,40] Although a prospective approach was used in the study by Molina Infante et al., our randomized controlled design with comprehensive monitoring

of participants may have resulted in a lower risk of selection bias. There are more data of lower than expected results in a recent large multicenter trial in both pediatric and adult EoE, suggesting a potential bias in previous cohort studies as one of the explanations for the observed discrepancies in results. Comparison of 1FED (milk) to FFED in children showed similar histologic improvements and remission rates (~40%) to our study.[41] In adults, 1FED (milk) to SFED showed that histological response (<15 eos/hpf) was similar between groups (34% vs. 40%).[42]

With regards to the overall high proportion of participants (25%) in partial histological remission (≥50% reduction in pre-treatment PEC) at week 6, it could also be argued that the intervention period was too short to determine efficacy of the diet. In addition to this, considering that both treatment arms eliminated the same potential food triggers, there is still the conceptual issue that both groups had the same probability of having culprit foods in the diet that were not eliminated in the FFED. This may be also a reason for the absence of a more evident response in the FFED + AAF group.

For dietary treatment to be effective, patients should adhere to it as much as possible; therefore, their motivation and acceptance of the impact of a diet is key. During the study, a significant improvement of the EoEQoL score was observed in the FFED + AAF group, whereas the FFED group showed no change in this score.[21,38,43] In addition, intra-group comparison showed a significant improvement of the "social impact" domain (e.g., "I feel frustrated when people think I cause my own choking episodes by eating too fast or taking too big bites") in patients treated with the FFED + AAF combination. It could be hypothesized that the option of using AAF to reach the required daily intake facilitated participation of patients in normal social life, instead of it being perceived as a limitation. Several participants stated to have benefited the AAF, since they felt it was feasible to decrease their daily solid food intake while still maintaining adequate nutrient intake and a healthy body weight. In addition, they considered the AAF as feasible snack while underway from home. Self-reported diet feasibility scores were similar between groups, indicating that this combined diet (i.e., palatability of the AAF included) is acceptable and well tolerated.

Aside from its hypoallergenic properties, the specially designed AAF includes multiple macro- and micronutrients. Hence, the risk of potential nutritional deficiencies that are common when eliminating key foods might be reduced. Vitamin B1, B2, B6, folic acid, and vitamin D intake was significantly higher in the FFED + AAF group compared to the FFED group (data not shown). In addition, the beneficial effects of this combined approach may be further supported by AAF itself, which is suggested to have immune-modulating properties.[31–34,43–51]

Taken together, it seems that this combined dietary approach of AAF added to a FFED is acceptable for patients and keeps them motivated. Hence, this may increase diet adherence and thus long-term efficacy of the strategy. These observations provide also future directions for a "combined dietary approach" as long-term therapy, which has also been suggested as maintenance approach for inflammatory bowel disease patients.[52]

Our study design has a few methodological limitations. Firstly, we did not adjust for adherence of AAF intake which may have affected our results. However, based on exploratory subgroup analysis of individual adherence rates, we judged the overall AAF intake of 84% at group level sufficiently high (Table S7, Figure S3A,3B). In addition, some patients in a normal setting will also not adhere to the prescribed AAF intake, therefore our results provide a more "real-life" estimate of the effect size. Secondly, we did not include a placebo formula in this trial, so we were not able to determine whether the potential benefit of AAF is related to the lack of placebo, potential immune-modulating properties, and/or increased diet adherence. However, it was previously observed that the addition of a placebo does not affect esophageal eosinophilic inflammation in EoE.[53,54] Finally, histological assessment (i.e., determination of PEC) was performed as per standardized protocol by multiple blinded pathologists (Amsterdam UMC pathology department) instead of central reading, which may have increased the risk of observer bias. The risk of observer bias on endoscopic outcomes was reduced by our blinded endoscopic scoring strategy and the use of the validated EREFS. Despite these limitations, our study adds to the existing literature being the first adult EoE combination-dietary intervention trial with a randomized controlled study design. Another strength of our study lies in the extensive patient monitoring within the study timeframe, thereby increasing diet adherence (e.g., less risk of diet errors and improved patients' motivation) and adherence to AAF intake. Another strength is the use of multiple outcome measures (i.e., endoscopic, symptoms, QoL, and nutrition related).

In summary, the addition of AAF to a FFED did not lead to a larger decrease in PEC between baseline and 6 weeks, but may result in a significant improvement of QoL in adult patients with EoE. Thus, further investigation within a larger sample seems warranted.

AUTHOR CONTRIBUTION

DISCLOSURES

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

1. Furuta GT, Liacouras CA, Collins MH, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. sponsored by the american gastroenterological association (AGA) Institute and North American society of pediatric gastroenterol. *Gastroenterology*. 2007;133:1342-1363.

2. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol*. 2011;128:3-20.

3. Attwood S, Smyrk T, Demeester T, Jones J. Eosophageal eosinophilia with dysphagia. a distinct clincopathological syndrome. *J Dig Dis*. 1993;38:109-116.

4. Navarro P, Arias Á, Arias-González L, Laserna-Mendieta EJ, Ruiz-Ponce M, Lucendo AJ, et al. Systematic review with meta-analysis: the growing incidence and prevalence of eosinophilic oesophagitis in children and adults in population-based studies. *Aliment Pharmacol Ther*. 2019;49:1116-1125.

5. Dellon ES, Erichsen R, Baron JA, et al. The increasing incidence and prevalence of eosinophilic oesophagitis outpaces changes in endoscopic and biopsy practice: national population-based estimates from Denmark. *Aliment Pharmacol Ther*. 2015;41:662-670.

6. Warners MJ, de Rooij W, van Rhijn BD, et al. Incidence of eosinophilic esophagitis in the Netherlands continues to rise: 20-Year results from a nationwide pathology database. *Neurogastroenterol Motil*. 2018;30:1-7.

7. Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: Improvement with an amino acid-based formula. *Gastroenterology*. 1995;109:1503-1512.

8. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated international consensus diagnostic criteria for eosinophilic esophagitis: proceedings of the AGREE conference. *Gastroenterology*. 2018;155(4):1022-1033. 10.1053/j.gastro.2018.07.009

9. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated international consensus diagnostic criteria for eosinophilic esophagitis: proceedings of the AGREE conference. *Gastroenterology*. (The American Gastroenterological Association, 2018). 2018;155(4):1022-1033. 10.1053/j.gastro.2018.07.009

10. Greuter T, Bussmann C, Safroneeva E, et al. Long-term treatment of eosinophilic esophagitis with swallowed topical corticosteroids: development and evaluation of a therapeutic concept. *Am J Gastroenterol*. 2017;112:1527-1535.

11. Schaefer ET, Fitzgerald JF, Molleston JP, et al. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. *Clin Gastroenterol Hepatol*. 2008;6:165-173.

12. Hirano I, Aceves SS. Clinical implications and pathogenesis of esophageal remodeling in eosinophilic esophagitis. *Gastroenterol Clin North Am*. 2014;43:297-316.

13. Schoepfer AM, Safroneeva E, Bussmann C, et al. Delay in diagnosis of eosinophilic esophagitis increases risk for stricture formation in a time-dependent manner. *Gastroenterology*. 2013;145:1230-1236.

14. Straumann A, Bussmann C, Zuber M, Vannini S, Simon HU, Schoepfer A, et al. Eosinophilic esophagitis: analysis of food impaction and perforation in 251 adolescent and adult patients. *Clin Gastroenterol Hepatol*. 2008;6:598-600.

15. Warners MJ, Oude Nijhuis RAB, De Wijkerslooth LRH, Smout AJPM, Bredenoord AJ. The natural course of eosinophilic esophagitis and long-term consequences of undiagnosed disease in a large cohort. *Am J Gastroenterol*. 2018;113:836-844.

16. Kagalwalla AF, Shah A, Li BUK, et al. Identification of specific foods responsible for inflammation in children with eosinophilic esophagitis successfully treated with empiric elimination diet. *J Pediatr Gastroenterol Nutr*. 2011;53:145-149.

17. Gonsalves N, Doerfler B, Hirano I. Long term maintenance therapy with dietary restriction in adults with eosinophilic esophagitis. *Gastroenterology*. 2011;140:S180-S181.

18. Lucendo AJ, Arias Á, González-Cervera J, et al. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: a prospective study on the food cause of the disease. *J Allergy Clin Immunol*. 2013;131:797-804.

19. Reed CC, Fan C, Koutlas NT, Shaheen NJ, Dellon ES. Food elimination diets are effective for long-term treatment of adults with eosinophilic oesophagitis. *Aliment Pharmacol Ther*. 2017;46:836-844.

20. Peterson KA, Byrne KR, Vinson LA, et al. Elemental diet induces histologic response in adult eosinophilic esophagitis. *Am J Gastroenterol*. 2013;108:759-766.

21. Warners MJ, Vlieg-Boerstra BJ, Verheij J, et al. Elemental diet decreases inflammation and improves symptoms in adult eosinophilic oesophagitis patients. *Aliment Pharmacol Ther*. 2017;45:777-787.

22. Liacouras CA, Spergel JM, Ruchelli E, et al. Eosinophilic esophagitis: a 10-year experience in 381 children. *Clin Gastroenterol Hepatol*. 2005;3:1198-1206.

23. Olson AA, Evans MD, Johansson MW, et al. Role of food and aeroallergen sensitization in eosinophilic esophagitis in adults. *Ann Allergy, Asthma Immunol*. 2016;117:387-393.

24. Arias Á, González-Cervera J, Tenias JM, Lucendo AJ. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and meta-analysis. *Gastroenterology*. 2014;146:1639-1648.

25. Gonsalves N, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology*. 2012;142:1451-1459.

26. Molina-Infante J, Arias Á, Alcedo J, et al. Step-up empiric elimination diet for pediatric and adult eosinophilic esophagitis: the 2-4-6 study. *J Allergy Clin Immunol*. 2018;141:1365-1372.

27. Gonsalves N, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology*. 2012;142:1451-1455.

28. Mukkada V, Falk GW, Eichinger CS, King D, Todorova L, Shaheen NJ, et al. Health-related quality of life and costs associated with eosinophilic esophagitis: a systematic review. *Clin Gastroenterol Hepatol*. 2018;16:495-503.

29. Cotton CC, Erim D, Eluri S, Palmer SH, et al. Cost utility analysis of topical steroids compared with dietary elimination for treatment of eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. 2017;15:841-849.

30. Kagalwalla AF, Wechsler JB, Amsden K, et al. Efficacy of a 4-food elimination diet for children with eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. 2017;15:1698-1707.

31. Tsune I, Ikejima K, Hirose M, et al. Dietary glycine prevents chemical-induced experimental colitis in the rat. *Gastroenterology*. 2003;125:775-785.

32. Hasegawa S, Ichiyama T, Sonaka I, et al. Cysteine, histidine and glycine exhibit anti-inflammatory effects in human coronary arterial endothelial cells. *Clin Exp Immunol*. 2012;167:269-274.

33. Jones HE, Hartog A, Stephenson H, et al. Amino acid-based formula affects the gastrointestinal cytokine milieu of children with non-IgE mediated cow's milk allergy. *Clin Transl Allergy*. 2015;5:P41.

34. Hartog A, Bastiaans J, Buurman N, et al. Anti-inflammatory features of the amino acid-based formula Neocate. *Clin Transl Allergy*. 2015;5:P151.

35. Hirano I, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR, et al. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. *Gut*. 2013;62:489-495.

36. Straumann A, Conus S, Degen L, et al. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology*. 2010;139:1526-1537.

37. Hirano I, Dellon ES, Hamilton JD, et al. Efficacy of dupilumab in a phase 2 randomized trial of adults with active eosinophilic esophagitis. *Gastroenterology*. 2019;158:111-122.

38. Lucendo AJ, Arias-González L, Molina-Infante J, Arias Á. Determinant factors of quality of life in adult patients with eosinophilic esophagitis. *United Eur Gastroenterol J*. 2018;6:38-45.

39. Molina-Infante J, Arias A, Barrio J, Rodríguez-Sánchez J, Sanchez-Cazalilla M, Lucendo AJ, et al. Four-food group elimination diet for adult eosinophilic esophagitis: a prospective multicenter study. *J Allergy Clin Immunol*. 2014;134:1093-1099.

40. Kagalwalla AF, Sentongo TA, Ritz S, et al. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. 2006;4:1097-1102.

41. Kliewer K, Aceves SS, Atkins D, et al. 817 – Efficacy of 1-food and 4-food elimination diets for pediatric eosinophilic esophagitis in a randomized multi-site study. *Gastroenterology*. 2019;156:S-172-S-173.

42. Kliewer K, Gonsalves N, Dellon ES, et al. 535 Efficacy of one-food versus six-food elimination diet for treatment of eosinophilic esophagitis in adults: results from the multicenter randomized controlled sofeed trial. *Gastroenterology*. 2021;160:S-109-S-110.

43. Gonsalves N, Doerfler B, Schwartz S, et al. 877 Prospective trial of four food elimination diet demonstrates comparable effectiveness in the treatment of adult and pediatric eosinophilic esophagitis. *Gastroenterology*. 2013;144:S-154.

44. Andou A, Hisamatsu T, Okamoto S, et al. Dietary histidine ameliorates murine colitis by inhibition of proinflammatory cytokine production from macrophages. *Gastroenterology*. 2009;136:564-574.

45. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients*. 2015;7:3011-3021.

46. Brown CC, Noelle RJ. Seeing through the dark: new insights into the immune regulatory functions of vitamin A. *Eur J Immunol*. 2015;45:1287-1295.

47. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab*. 2007;51:301-323.

48. De Rosa V, Galgani M, Santopaolo M, Colamatteo A, Laccetti R, Matarese G Nutritional control of immunity: balancing the metabolic requirements with an appropriate immune function. *Semin Immunol*. 2015;27:300-309.

49. Miyoshi Y, Tanabe S, Suzuki T. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. *Am J Physiol - Gastrointest Liver Physiol*. 2016;311:G105-G116.

50. Cassani B, Villablanca EJ, De Calisto J, Wang S, Mora JR. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. *Mol Aspects Med*. 2012;33:63-76.

51. Spencer SP, Belkaid Y. Dietary and commensal derived nutrients: Shaping mucosal and systemic immunity. *Curr Opin Immunol*. 2012;24:379-384.

52. Takagi S, Utsunomiya K, Kuriyama S, et al. Quality of life of patients and medical cost of 'half elemental diet' as maintenance therapy for Crohn's disease: secondary outcomes of a randomised controlled trial. *Dig Liver Dis*. 2009;41:390-394.

53. Lucendo AJ, Miehlke S, Schlag C, et al. Efficacy of budesonide orodispersible tablets as induction therapy for eosinophilic esophagitis in a randomized placebo-controlled trial. *Gastroenterology*. 2019;157:74-86.

54. Miehlke S, Hruz P, Vieth M, et al. A randomised, double-blind trial comparing budesonide formulations and dosages for short-term treatment of eosinophilic oesophagitis. *Gut*. 2016;65:390-399.

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