

³Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden

⁴Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

Correspondence

Inger Kull, Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden.

Email: inger.kull@ki.se

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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The relationship of habitual diet with esophageal inflammation and integrity in eosinophilic esophagitis

To the Editor,

The prevalence of eosinophilic esophagitis (EoE), affecting both adults and children, has strongly increased in recent years for reasons currently unknown. It is characterized by inflammation of the esophagus, dysphagia, and food impaction, and is a relatively new chronic immune-mediated manifestation within the spectrum of allergic diseases. We hypothesized that the habitual diet composition is a risk factor of the development and/or persistence of EoE, as nutrition can, either favorably or unfavorably, influence mucosal integrity and tolerance, local and systemic immune function, the oral and intestinal microbiome (and therefore probably also the esophageal microbiome),¹ and epigenetics. Immunomodulatory effects are known for certain fibers, fats, proteins, iron, zinc, copper, selenium, vitamins A, C, D, B6, and B12, omega 3 and 6 fatty acids and folate.²⁻⁵ Thus, nutrition may have an allergy-protective or allergy-provoking effect. However, no studies have been published yet on the habitual dietary intake in EoE patients. This study is the first reporting the relationship between habitual dietary intake in adult EoE patients, before starting any (elimination) diet other than self-imposed dietary measures, and esophageal inflammation and mucosal integrity. Our *aims* were to assess in adult EoE patients (a) the relationships of the habitual dietary intake with esophageal eosinophilic inflammation and mucosal integrity, and (b) the dietary intake in those patients with active disease compared to those in remission.

Our study had a cross-sectional design and was carried out in adult patients, previously diagnosed with EoE (≥ 15 eosinophils/high power field (HPF) and symptoms of esophageal dysfunction) after inclusion in two different studies in 2013-2015 (Trialregister.nl NTR4052 and NTR4892) in the Academic Medical Center, Amsterdam, the Netherlands. We used the data collected at baseline^{6,7} in 34 patients, including five patients who did not receive the intended treatment because of histologic remission assessed at baseline, after inclusion. Until this baseline assessment, the included EoE patients of these studies had no dietary intervention other than self-imposed dietary measures. All patients stopped the intake of steroids, leukotriene inhibitors, monoclonal antibodies anticoagulants, NSAIDs at least 8 weeks prior to the study. If necessary, continuation of proton pump inhibition in stable dosage was allowed. At baseline, habitual dietary intake, esophageal inflammation and mucosal integrity were assessed. No dietary analyses were performed during or after dietary intervention.

Esophageal inflammation was measured by peak eosinophil counts/HPF, and integrity by Electrical Tissue Impedance Spectroscopy (ETIS), transepithelial resistance (TER), and transepithelial flux of fluorescein and rhodamine. See Boxes S1 and S2, for more details on these measurements.

Patient characteristics are described in Table 1. Table 2 shows that in the multiple regression analyses the following nutrients or food groups had a statistically significant negative (favorable)

TABLE 1 Patient characteristics of EoE patients (n = 34) at baseline

	n/N (%), or median; IQR
Gender; males (n/N; %)	26/34 (77)
Age in years: median; IQR (n = 34)	45.2; 29.0-49.3
BMI (kg/m ²), median; IQR (n = 34)	24.7; 21.0-26.6
Atopic disease in % (n = 34)	27/34 (79)
- Atopic dermatitis	7/34 (21)
- Asthma	14/34 (41)
- Allergic rhinitis	17/34 (50)
- Food allergy	21/34 (62)
- of which oral allergy symptoms	17/34 (50)
Food avoidance in % (n = 34)	
Food groups avoided because of OAS, food allergy or EoE	19/34 (56)
Food groups avoided because of OAS	12/34 (35)
- one or more types of fruit	2/34 (6)
- one or more types of nuts/peanut/seeds/legumes	2/34 (6)
- one or more types of fruits/vegetables and nuts/peanut/ seeds/legumes	8/34 (24)
Food groups avoided because of other food allergy	7/34 (21)
- one or more types of fruits/vegetables	3/34 (9)
- one or more types of nuts/peanut/seeds	3/34 (9)
- fish	1/34 (3)
- buckwheat	1/34 (3)
- cow's milk	1/34 (3)
Food groups avoided because of EoE (dysphagia, impaction or dyspepsia)	12/34 (35)
- one or more types of nuts/peanut/seeds	7/34 (21)
- one or more types of fruits/vegetables	7/34 (21)
- alcoholic beverages	5/34 (15)
- dairy	3/34 (9)
- meat	3/34 (9)
- fish	2/34 (6)
- egg	2/34 (6)
- gluten	2/34 (6)
- bread	1/34 (3)
Supplements % (n = 34)	
- taken on a regular base	8/34 (23)
- taken on an irregular base	2/34 (6)
- no supplements	20/34 (59)
- unknown or unclear	4/34 (12)
Baseline measures of eosophageal inflammation and mucosal integrity	
In vivo	
- peak eosinophil count/HPF: median;IQR (n = 34)	40; 29-80
- ETIS, Ω m: median; IQR (n = 19)	4736; 2417-8070

(Continues)

TABLE 1 (Continued)

	n/N (%), or median; IQR
In vitro	
- TER, Ω cm ² : median; IQR (n = 21)	74; 52-117
- Fluorescein flux ^a : median; IQ (n = 21)	489; 34-1283
- Rhodamine flux ^a :median; IQR (n = 21)	65; 0-407

EoE, eosinophilic esophagitis; ETIS, electrical tissue impedance spectroscopy; IQR, interquartile range; TER, transepithelial resistance.

^aExpressed as μ mol/cm²/h.

relationship with eosinophil counts: the amount of dietary fiber ($\beta = -0.443$; $P = 0.044$), iron ($\beta = -0.656$; $P = 0.001$), rice/pasta instead of potatoes ($\beta = -0.361$; $P = 0.028$), and soy products ($\beta = -0.450$; $P = 0.007$). In contrast, the amount of dietary phosphorus ($\beta = 0.659$; $P = 0.001$) had a significantly positive (unfavorable) relationship with eosinophil counts. As compared to patients with active disease (≥ 15 eosinophils/HPF) (n = 29), patients in remission consumed statistically significantly more fiber ($P = 0.012$), magnesium ($P = 0.024$), folate ($P = 0.002$), vegetables ($P = 0.025$), yoghurt ($P = 0.021$), and soy ($P = 0.037$) (Mann-Whitney test). Additionally, patients in disease remission consumed statistically significantly less vitamin B12 ($P = 0.006$) and meat ($P = 0.034$) than patients with active disease. Median amounts of these nutrients/foods consumed by the two groups of patients are presented in Table S1. It is unlikely that differences in disease activity between the two groups could be explained by food avoidance in the group in remission, because in this group dietary elimination was very limited.

Multiple regression analyses also revealed that, in patients with active disease, the intake of sunflower oil and/or stir fry oil was negatively (ie, unfavorably) related to values of TER ($\beta = -0.499$; $P = 0.019$). The consumption of buttermilk/LGG yoghurt drink had a statistically negative (ie, favorable) relationship with values of fluorescein flux ($\beta = -0.716$; $P = 0.002$), while the amount of added fat was positively (unfavorably) related to fluorescein flux ($\beta = 0.624$; $P = 0.012$). Moreover, the amount of dairy was negatively (favorably) related to rhodamine flux ($\beta = -0.566$; $P = 0.015$), while the amount of sunflower oil or stir fry oil ($\beta = 0.735$; $P = <0.001$) was positively (unfavorably) related to rhodamine flux.

Our findings show that dietary factors may either favorably or unfavorably impact inflammation and mucosal integrity of the esophagus. With respect to these health outcomes, high intake of dietary fiber, iron, fermented dairy (buttermilk, LGG yoghurt drink), dairy, pasta/rice, and soy may be favorable, while high intake of omega-6 rich oil (sunflower oil/stir fry oil), total added fat, and phosphorus (abundant in animal-based foods) may be unfavorable.

Remarkably, different nutrients/foods were significantly related to different outcomes. However, different types of fat or fatty acids (total added fat, linoleic acid, and sunflower oil/stir fry oil) all showed unfavorable relationships with one or more in vitro permeability tests (TER, Fluorescein, and Rhodamine flux).

Generally, we found weak or moderate relationships with the outcome measures. However, the relationship of all ingredients of

TABLE 2 Relationship assessed by multivariable regression analyses between nutrients and food groups, adjusted for age, gender and energy intake (in habitual diet) and number of eosinophils, ETIS in vivo, TER and influx of fluorescein and rhodamine in vitro (only results with P values < 0.1 are presented)

	Standardized beta (SE)	P-value	Adjusted explained variance	Nature of the relationship
Relationship between nutrients or food groups and number of eosinophils (n = 34)				
Nutrients				
Protein (g)	0.468 (0.17)	0.066	0.209	Unfavorable
Fiber (g)	-0.443 (0.17)	0.044*	0.227	Favorable
Iron (mg)	-0.656 (0.17)	0.001*	0.408	Favorable
Magnesium (mg)	-0.404 (0.17)	0.055	0.217	Favorable
Phosphorus (mg)	0.659 (0.17)	0.001*	0.404	Unfavorable
Vitamin B12 (mg)	0.329 (0.17)	0.054	0.218	Unfavorable
Food (groups)				
Pasta and rice	-0.361 (0.17)	0.028*	0.248	Favorable
Meat and meat products	0.347 (0.17)	0.075	0.203	Unfavorable
Soy products	-0.450 (0.17)	0.007*	0.310	Favorable
Relationship between nutrients or food groups and mucosal integrity in vivo (ETIS)(n = 19)				
Nutrient				
Retinol Activity Equivalent (RAE)	0.496 (0.23)	0.059	0.153	Favorable
Relationship between nutrient or food groups and mucosal integrity in vitro (TER) (n = 21)				
Nutrient				
Potassium	0.571 (0.22)	0.077	0.266	Favorable
Food (groups)				
Bread	-0.589 (0.22)	0.059	0.286	Unfavorable
Total added fat (margarine, butter, oil, any fat used for cooking)	-0.391 (0.22)	0.093	0.251	Unfavorable
Sunflower oil, stir fry oil	-0.499 (0.22)	0.019*	0.370	Unfavorable
Relationship between nutrient or food groups and mucosal permeability/integrity in vitro (fluorescein) (n = 21)				
Nutrient				
Linoleic acid	0.405 (0.22)	0.090	0.059	Unfavorable
Food (groups)				
Buttermilk/LGG yoghurt drink	-0.716 (0.22)	0.002*	0.417	Favorable
Breakfast cereals and other grain products	0.503 (0.22)	0.099	0.050	Unfavorable
Bread	0.603 (0.22)	0.089	0.060	Unfavorable
Total added fat (margarine, butter, oil, any fat used for cooking)	0.624 (0.22)	0.012*	0.248	Unfavorable
Sunflower oil, stir fry oil	0.430 (0.22)	0.083	0.067	Unfavorable
Relationship between nutrient or food groups and mucosal permeability/integrity in vitro (rhodamine) (n = 21)				
Food (groups)				
Dairy products	-0.566 (0.22)	0.015*	0.280	Favorable
Milk	-0.486 (0.22)	0.051	0.178	Favorable
Breakfast cereals and other grain products	0.493 (0.22)	0.093	0.124	Unfavorable
Pasta and rice	0.424 (0.22)	0.060	0.164	Unfavorable
Total added fat (margarine, butter, oil, any fat used for cooking)	0.484 (0.22)	0.051	0.178	Unfavorable
Sunflower oil, stir fry oil	0.735 (0.22)	<0.001*	0.532	Unfavorable

*The bold values indicate the statistical significance of P values < 0.05 .

the total diet together may be stronger related to these outcomes, because of cumulative, synergistic, or interactive effects.⁸

Our study design does not allow for conclusions on causal relationships and the results should be interpreted with caution. However, our hypothesis that the habitual diet composition is a risk factor for the development and/or maintenance of EoE seems promising because the found relationships (or trends) are in line with general concepts of a healthy (immune-enhancing and anti-inflammatory) diet, or, in contrast, unhealthy (immune-suppressive and pro-inflammatory) diet:

1. Fiber, iron, and vitamin A are known for their beneficial effects on the intestinal microbiome, the maintenance of mucosal integrity²⁻⁵ and their anti-inflammatory effects in asthma;⁹
2. Omega-6 fatty acids (in sunflower oil/stir fry oil), linoleic acid, and high total fat intake are known for their pro-inflammatory and (epi)genetic effects;^{5,10}
3. Consumption of buttermilk/ LGG drink yoghurt and dairy all fit in the concept of a healthy diet, rich in nutrient dense and fermented foods;^{4,5}
4. High intakes of protein, meat/meat products, vitamin B12, and phosphorus are provided by animal-based foods, that might be therefore be determined as unfavorable, but not meaning that, for example, protein and vitamin B12 per se are unhealthy, whereas high intakes of dietary fiber, magnesium, potassium, and soy products are provided by plant-based foods that might be considered as favorable related to EoE pathogenesis.^{4,5}

It seems contradictory that we found protective effects for soy and dairy, while soy and dairy are common triggers in EoE. We hypothesize that the protective effects of dairy might be contributed to fermented dairy and/or full fat dairy (rich in vitamin A) as long as the disease has not yet developed. The protective effect of soy could be explained by the plant-based origin of soy. Once EoE has developed and permeability has increased, T-cell mediated reactions are induced to these commonly consumed foods.

Strengths of our study are the well-characterized study population and the use of a detailed, standardized way of diet history taking. Limitations of this study are the cross-sectional design, the relatively small study population. In addition, the study design did not allow us to identify the culprit food allergens in all patients because study NTR4052 was stopped preliminary. Because of the hypothesis-generating character of our study, we did not correct for multiple testing. However, when verifying the direction (favorable or unfavorable) of the non-significant effects of the nutrients/foods in Table 2 for the other outcome variables, the directions of 71% of relationships point at the same direction, yielding the most consistent results for high intake of protein and meat/meat products (unfavorable) and fiber (favorable) (see Table S2). Finally, we were not able to calculate the quantitative intake of dietary supplements, because of the inconsistent use of amounts and types of supplements by patients.

If our hypothesis will be confirmed in prospective or intervention studies, there may be a role for an immune-enhancing and anti-inflammatory diet, to be encouraged and facilitated from a very young age, in the prevention and, in addition to elimination diets, also in the treatment of EoE.

CONFLICTS OF INTEREST

Marleen van Ampting, Lucien Harthoorn, and Simone Eussen are employed by Nutricia Research BV, but declare that they were not affected by this affiliation in performing this study. The other authors declare that they do not have any conflicts of interest that may be inherent in this submission.

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ORCID

Marlou L. A. de Kroon  <https://orcid.org/0000-0003-1730-4994>

Marlou L. A. de Kroon¹ 
 Marijn J. Warners²
 Marleen T. J. van Ampting³
 Lucien F. Harthoorn³
 Arjan J. Bredenoord²
 Mylene van Doorn⁴
 Melanie Kok²
 Bram D. van Rhijn⁵
 Simone R. B. M. Eussen³
 Berber J. Vlieg-Boerstra⁶

¹Department of Health Sciences, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

²Department of Gastroenterology & Hepatology, Academic Medical Center, Amsterdam, The Netherlands

³Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands

⁴Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

⁵Department of Dermatology & Allergology, University Medical Center, Utrecht, The Netherlands

⁶Department of Paediatrics, OLVG, Amsterdam, The Netherlands

Correspondence

Berber J. Vlieg-Boerstra, Department of Paediatrics, OLVG, Amsterdam, The Netherlands.

Email: b.vlieg-boerstra@olv.nl

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Three-dimensional structure of the wheat β -amylase Tri a 17, a clinically relevant food allergen

To the Editor,

Wheat is one of the most important staple foods worldwide and has been recognized as a potent food allergen source. Allergies are on the rise in western countries and the prevalence of food allergy has reached approximately 7% in children in the United States,¹ making improved diagnostics an important goal. Not all known potential allergens of wheat have been characterized so far and the prevalence of true wheat allergy is difficult to determine due to IgE cross-reactivity with grass pollen allergens.²

The wheat β -amylase (Tri a 17) has been found to bind IgE of wheat allergic patients,³ but its structure and allergenic activity have not been studied. To evaluate its possible clinical relevance and to shed some light on its biochemical properties, we elucidated the three-dimensional structure, measured the enzymatic activity, IgE-binding capacity, and allergenic activity of the recombinant enzyme.

The β -amylases of crop plants like wheat, soy, or barley have been under investigation for decades and the presence of two general forms has been described. One protein isoform found in all parts of the plant, termed ubiquitous β -amylase, and one variant specific to the endosperm, featuring an elongated C-terminal tail region are known. To date, only the ubiquitous form of wheat β -amylase has been sequenced.⁴

We screened a wheat seed cDNA library with serum IgE antibodies from patients suffering from wheat-induced food allergy and identified an IgE-reactive cDNA clone which was homologous to the

barley endosperm β -amylase at its C-terminus and to the sequence of wheat ubiquitous β -amylase at its N-terminus.

We then expressed a recombinant protein consisting of the ubiquitous wheat β -amylase sequence fused with the recovered IgE-reactive C-terminus (named Tri a 17_clone) and the unaltered ubiquitous β -amylase (termed Tri a 17_inactive) both as inclusion bodies in *Escherichia coli*. A refolding attempt yielded soluble, yet misfolded and aggregated protein. However, we were able to optimize the expression conditions to obtain natively folded amylase (named Tri a 17_active) (Figure S1 and S2 and the Methods section in this article's Online Repository).

The far-UV CD spectra of the inactive proteins indicate the presence of β -sheets and random coil signal, while Tri a 17_active exhibits the high α -helical content of a TIM-barrel (Figure 1B).

Purified Tri a 17_active crystallized readily and the structure was solved by X-ray crystallography to a resolution of 2 Å (PDB: 6GER). The structure superimposes well with the previously published structure of barley β -amylase (rmsd = 0.62 Å when 442 of 486 C α atoms are aligned with the PDB structure 2XFF⁵), which was used as the template for molecular replacement.

The crystal structure of Tri a 17_active shows the same (β/α)₈-barrel architecture found for other plant and bacterial β -amylases (Figure 1A).

Figure 1C shows the variation in the relative enzymatic activity of the β -amylase with respect to pH. The enzyme shows a tolerance

Online supporting information

Box 1. Description of the data obtained in EoE patients after inclusion, at baseline

Habitual dietary intake in EoE patients

All patients filled in a 3-day food diary (2 working days and 1 weekend day). Patients were asked to record amounts of foods used in grams or household portion sizes, as well as to record types and brand names of foods consumed in detail. Patients also recorded the use of dietary supplements (type, yes, no, infrequently). The food diaries were reviewed by a dietician with expertise in food allergy, using a checklist for completeness. Patients were contacted in case of unclear or missing data. Portion sizes were coded using Evry software (1). The average of the 3-day diaries (without supplements) was calculated, using the Dutch NEVO-online Food Composition Database (2) and Evry software (1) for energy (kCal) and 32 nutrients (Box 2). Additionally, all foods used were allocated to 38 food groups, based on the Dutch National Food Consumption Survey (DNFCS) (3) (Box 2). Information on atopic status and food avoidance was collected by history.

Biological parameters in EoE patients

After inclusion in one of the studies, endoscopies were performed with biopsy sampling at different levels of the esophagus at baseline. The degree of inflammation was expressed as peak eosinophil counts/HPF. Active disease was defined as a peak eosinophil count of ≥ 15 (4). In patients participating in Trial NTR4892, also the following tests were performed: (a) Electrical Tissue Impedance Spectroscopy (ETIS), a validated marker for mucosal integrity, to measure esophageal mucosal integrity *in vivo* (5) (b) transepithelial resistance (TER) to measure esophageal mucosal integrity *in vitro*, (c) transepithelial flux of fluorescein (0.3 kDa) and rhodamine (40 kDa, size similar to common food allergens) in Ussing chambers, to measure mucosal permeability *in vitro* (4). Decreased values of ETIS and TER and, in contrast, increased values of fluorescein and rhodamine flux point at an impaired mucosal integrity. More details and the results of these studies are described elsewhere (4,6).

Box 2. Nutrients calculated on the basis of 3-day food diaries and distribution of food groups used. Distribution of food groups is based on the Dutch National Food Consumption Survey (DNFCS) (3).

Energy and nutrients calculated:

Energy (kCal), protein (g and energy percentage), fat (g and energy percentage), saturated fat (g and energy percentage), carbohydrates (g and energy percentage), linoleic acid (g), α -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (g), dietary fiber (g), alcohol (g), calcium (mg), iron (mg), sodium (mg), potassium (mg), magnesium (mcg), zinc (mg), selenium (mcg), iodine (mg), copper (mg), phosphorus (mg), retinol activity equivalent (RAE = retinol + precursor carotenoids) (mcg), vitamins A (mcg), B1 (mg), B2 (mg), B3 (mg), B6 (mg), folic acid (in mcg; folate in mcg and folate equivalents), B12 (mcg), C (mg), D (mg) and E (mg).

Distribution of food groups calculated:

Ingested amount of potatoes and other turnips in grams/day

Ingested amount of vegetables in grams/day

Ingested amount of legumes in grams/day

Ingested amount of fruit, nuts and olives in grams/day

Ingested amount of fruit in grams/day

Ingested amount of dairy products in grams/day

Ingested amount of milk in grams/day

Ingested amount of skimmed milk in grams/day

Ingested amount of semi-skimmed/whole milk in grams/day

Ingested amount of yogurt in grams/day

Ingested amount of low-fat yogurt in grams/day

Ingested amount of semi-fat/full-fat yogurt in grams/day

Ingested amount of cheese in grams/day

Ingested amount of breakfast cereals and other grain products in grams/day

Ingested amount of bread in grams/day

Ingested amount of white bread in grams/day

Ingested amount of multigrain/whole wheat bread in grams/day

Ingested amounts of wheat bread in grams/day

Ingested amount of breakfast cereals in grams/day

Ingested amount of pasta and rice in grams/day

Ingested amount of meat and meat products in grams/day

Ingested amount of fish and seafood in grams/day

Ingested amount of egg and egg products in grams/day

Ingested amount of added fat (margarine, butter, oil, cooking fat) in grams/day

Ingested amount of low-fat margarine and margarine in grams/day
Ingested amount of butter in grams/day
Ingested amount of vegetable oil in grams/day
Ingested amount of olive oil in grams/day
Ingested amount of other oils than olive oil in grams/day
Ingested amount of baking and roasting products in grams/day
Ingested amount of sugar and sweets in grams/day
Ingested amount of pastries, cakes and biscuits in grams/day
Ingested amount of non-alcoholic beverages in ml/day
Ingested amount of alcoholic beverages in ml/day
Ingested amount of spices and sauces in grams/day
Ingested amount of soup and broths in grams/day
Ingested amount of other products in grams/day
Ingested amount of soy products in grams/day

Table S1. Median amounts (IQRs 25%; 75%) of nutrients/foods consumed by EoE-patients in remission and patients with active disease that significantly differed between these groups. [#]

Nutrients/foods (per day)	Patients		P value*
	in remission (<15 Eos/HPF) (N = 5)	with active disease (≥ 15 Eos/HPF) (N = 29)	
Fibre (g)	27 (24-27)	19 (14 – 24)	0.012
Magnesium (mg)	404 (356-465)	323 (231 – 377)	0.024
Folate (mcg)	305 (287–372)	227 (176 – 295)	0.002
Vegetables(g)	200 (153-327)	133 (97 – 182)	0.025
Vitamin B12 (mg)	2.2 (2.0-2.7)	4.2 (2.9-5.4)	0.034
Yoghurt (g)	83 (25-225)	0 (0 – 42)	0.021
Soy products (g)	0 (0-248)	0 (n.a.)**	0.037
Meat and meat products (g)	20 (0-99)	117 (117-139)	0.006

[#] The nutrients/foods for which no significant difference was shown between these two groups are not presented in the table.

* *Mann-Whitney-test*

** *Only 2 patients with active disease consumed soy products*

Table S2. Relationships with all outcome measures (Eos, ETIS, TER, FLUOR and RHO) for variables included in table 2

	Standardized Beta (SE)	p-value	Adjusted explained variance	Nature of the relationship	Standardized Beta (SE)	p-value	Adjusted explained variance	Nature of the relationship	Standardized Beta (SE)	p-value	Adjusted explained variance	Nature of the relationship
	<i>Eos (n = 34)</i>				<i>ETIS (n = 19)</i>				<i>TER (n = 21)</i>			
Nutrients												
Protein (g)	<i>0.468 (0.17)</i>	<i>0.066</i>	<i>0.209</i>	<i>unfavorable</i>	-0.059 (0.23)	0.863	-0.100	<i>unfavorable</i>	-0.308 (0.22)	0.318	0.158	<i>unfavorable</i>
Linoleic acid (g)	-0.105 (0.17)	0.586	0.118	Favorable	0.031 (0.23)	0.905	-0.102	favourable	0.031 (0.22)	0.888	0.103	favorable
Fiber (g)	<i>-0.443 (0.17)</i>	<i>0.044*</i>	<i>0.227</i>	<i>favorable</i>	0.041 (0.23)	0.898	-0.101	<i>favorable</i>	0.247 (0.22)	0.367	0.148	<i>favorable</i>
Iron (mg)	<i>-0.656 (0.17)</i>	<i>0.001*</i>	<i>0.408</i>	<i>favorable</i>	-0.154 (0.23)	0.627	-0.084	unfavorable	0.248 (0.22)	0.364	0.148	<i>favorable</i>
Potassium (mg)	0.083 (0.17)	0.798	0.111	unfavorable	0.383 (0.23)	0.383	-0.035	<i>favorable</i>	<i>0.571 (0.22)</i>	<i>0.077</i>	<i>0.266</i>	<i>favorable</i>
Magnesium (mg)	<i>-0.404 (0.17)</i>	<i>0.055</i>	<i>0.217</i>	<i>favorable</i>	0.177 (0.23)	0.553	-0.074	<i>favorable</i>	0.359 (0.22)	0.146	0.216	<i>favorable</i>
Phosphorus (mg)	<i>0.659 (0.17)</i>	<i>0.001*</i>	<i>0.404</i>	<i>unfavorable</i>	-0.028 (0.23)	0.929	-0.102	<i>unfavorable</i>	0.013 (0.22)	0.965	0.102	favorable
Retinol Activity Equivalent (RAE)	-0.052 (0.17)	0.774	0.112	<i>favorable</i>	<i>0.496 (0.23)</i>	<i>0.059</i>	<i>0.153</i>	<i>favorable</i>	0.104 (0.22)	0.656	0.113	<i>favorable</i>
Vitamin B12 (mg)	<i>0.329 (0.17)</i>	<i>0.054</i>	<i>0.218</i>	<i>unfavorable</i>	0.008 (0.23)	0.976	-0.103	Favorable	-0.063 (0.22)	0.775	0.107	<i>unfavorable</i>
Food (groups)												
Dairy products	0.152 (0.17)	0.396	0.131	unfavorable	-0.252 (0.23)	0.333	-0.029	unfavorable	0.070 (0.22)	0.767	0.107	<i>favorable</i>
Milk	0.211 (0.17)	0.238	0.152	unfavorable	-0.377(0.23)	0.148	0.055	unfavorable	-0.027 (0.22)	0.911	0.103	unfavorable
Buttermilk/ LGG yoghurt drink	0.060 (0.17)	0.734	0.120	unfavorable	-0.313 (0.23)	0.245	0.021	unfavorable	0.091 (0.22)	0.704	0.086	<i>favorable</i>
Breakfast cereals and Other grain products	-0.233 (0.17)	0.248	0.150	favorable	-0.020 (0.23)	0.950	-0.102	<i>unfavorable</i>	-0.438 (0.22)	0.107	0.240	<i>unfavorable</i>
Bread	0.032 (0.17)	0.878	0.110	<i>unfavorable</i>	0.077 (0.23)	0.835	-0.099	favorable	<i>-0.589 (0.22)</i>	<i>0.059</i>	<i>0.286</i>	<i>unfavorable</i>
Pasta and rice	<i>-0.361 (0.17)</i>	<i>0.028*</i>	<i>0.248</i>	<i>favorable</i>	-0.101 (0.23)	0.699	-0.091	<i>unfavorable</i>	-0.438 (0.22)	0.107	0.240	<i>unfavorable</i>
Meat and meat products	<i>0.347 (0.17)</i>	<i>0.075</i>	<i>0.203</i>	<i>unfavorable</i>	-0.143 (0.23)	0.609	-0.082	<i>unfavorable</i>	-0.319 (0.17)	0.164	0.207	<i>unfavorable</i>
Total added fat (margarine, butter, oil, any fat used for cooking)	-0.245 (0.17)	0.173	0.165	favorable	0.040 (0.23)	0.148	0.101	favorable	<i>-0.391 (0.22)</i>	<i>0.093</i>	<i>0.251</i>	<i>unfavorable</i>
Sunflower oil, stir fry oil	0.070 (0.17)	0.690	0.114	<i>unfavorable</i>	0.380 (0.23)	0.142	0.060	favorable	<i>-0.499 (0.22)</i>	<i>0.019*</i>	<i>0.370</i>	<i>unfavorable</i>
Soy products	<i>-0.450 (0.17)</i>	<i>0.007*</i>	<i>0.310</i>	<i>favorable</i>	0.251 (0.23)	0.335	-0.029	<i>favorable</i>	0.199 (0.17)	0.369	0.147	<i>favorable</i>

	Standardized Beta (SE)	p-value	Adjusted explained variance	Nature of the relationship	Standardized Beta (SE)	p-value	Adjusted explained variance	Nature of the relationship	Nr of consisten effects (favorable/ unfavorable) with outcomes (p < 0.01) in table 1
	Fluorescein (n = 21)				rhodamine(n = 21)				
Nutrients									
Protein (g)	0.157 (0.22)	0.654	-0.118	unfavorable	0.130 (0.22)	0.730	-0.041	unfavorable	5 unfavorable
Linoleic acid	0.405 (0.22)	0.090	0.059	unfavorable	0.127 (0.22)	0.596	-0.032	unfavorable	2 unfavorable
Fiber (g)	-0.180 (0.22)	0.560	-0.108	favorable	-0.018 (0.22)	0.952	-0.050	favorable	5 favorable
Iron (mg)	0.118 (0.22)	0.691	-0.121	unfavorable	0.216 (0.22)	0.447	-0.012	unfavorable	2 favorable
Potassium (mg)	-0.422 (0.22)	0.259	-0.043	favorable	-0.506 (0.22)	0.155	0.078	favorable	4 favorable
Magnesium (mg)	0.143 (0.22)	0.617	-0.114	unfavorable	-0.013 (0.22)	0.962	-0.050	favorable	4 favorable
Phosphorus (mg)	-0.437 (0.22)	0.170	-0.003	favorable	-0.201 (0.22)	0.523	-0.023	favorable	2 unfavorable
Retinol Activity Equivalent (RAE)	0.130 (0.22)	0.622	-0.115	unfavorable	0.156 (0.22)	0.538	-0.025	unfavorable	3 favorable
Vitamin B12 (mg)	0.196 (0.22)	0.426	-0.087	unfavorable	0.007 (0.22)	0.977	-0.050	unfavorable	4 unfavorable
Food (groups)									
Dairy products	-0.307 (0.22)	0.239	-0.036	favorable	-0.566 (0.22)	0.015*	0.280	favorable	3 favorable
Milk	-0.177 (0.22)	0.516	-0.102	favorable	-0.486 (0.22)	0.051	0.178	favorable	2 favorable
Buttermilk/ LGG yoghurt drink	-0.716 (0.22)	0.002*	0.417	favorable	-0.378 (0.22)	0.128	0.085	favorable	3 favorable
Breakfast cereals and porridge	0.503 (0.22)	0.099	0.050	unfavorable	0.493 (0.22)	0.093	0.124	unfavorable	4 unfavorable
Bread	0.603 (0.22)	0.089	0.060	unfavorable	0.290 (0.22)	0.412	-0.006	unfavorable	4 unfavorable
Pasta and rice	0.133 (0.22)	0.590	-0.112	unfavorable	0.424 (0.22)	0.060	0.164	unfavorable	1 favorable 4 unfavorable
Meat and meat products	0.090 (0.22)	0.735	-0.124	unfavorable	0.067 (0.22)	0.793	-0.046	unfavorable	5 unfavorable
Total added fat (margarine, butter, oil, Any fat used for cooking)	0.624 (0.22)	0.012*	0.248	unfavorable	0.484 (0.22)	0.051	0.178	unfavorable	3 unfavorable
Sunflower oil, stir fry oil	0.430 (0.17)	0.083	0.067	unfavorable	0.735 (0.22)	<0.001*	0.532	unfavorable	4 unfavorable
Soy products	0.003 (0.22)	0.990	-0.133	unfavorable	-0.233 (0.22)	0.329	0.012	favorable	4 favorable

Bold and italics are results as presented in table 2. Bold : relationships which are consistent (favorable or unfavorable) with the relationships with p < 0.01 from table 2.

References online supporting information

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