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RESEARCH ARTICLE

The intake of grain fibers modulates cytokine levels in blood

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Abstract

Dietary fiber may modulate the environment of the intestinal lumen, alter the intestinal microflora populations, and influence the immune response and disease risk. Epidemiological investigations have suggested that higher fiber intake is associated with lower overall mortality, in particular from cardiovascular and digestive tract diseases. Here a panel of 17 cytokines and chemokines were measured in plasma of 88 cancer-free subjects sampled within the Italian EPIC-Italy cohort. A statistically significant inverse association (p-trend = 0.01) was observed for cereal fiber and cytokines included in the main factor in factor analysis (IL-1β, IL-4, IL-5, IL-6, IL-13, and TNF-α), which alone explained 35.5% of variance. Our study suggests that fiber intake, especially cereal fiber, may be associated with a decreased level of pro-inflammatory cytokines.

Keywords: fiber, inflammation, cytokine, chemokine, EPIC-Italy

Introduction

A recent cohort study suggested that a high intake of fibers in food protects from early death from several causes (Park et al., 2011). Dietary fiber from grains, in particular, was significantly inversely related to total and cause-specific death in both men and women. Such observation had been made before, but on a smaller scale (Brown et al., 1999; Pereira et al., 2004; Schulze et al., 2007; Whelton et al., 2005). These studies attributed the beneficial effects of fibers almost entirely to grain-derived fibers. However, it remains unclear, how grain fiber intake influences mortality.

One of the most promising areas of research on the action of fibers refers to their effects on the gut microbiome– the complement of microorganisms resident in the gastrointestinal system, and their interacting genomes– which is intimately involved in normal physiological function, and can modulate pharmacological, toxicological and disease processes (Kinross et al., 2011). The gut microfloral population strongly influenced by dietary habits (such as fiber intake), is related to obesity (an important determinant of mortality), and is amenable to preventive intervention (Cani and Delzenne, 2009; Roberfroid et al., 2010; Smith and Tucker, 2011). Diet may have a significant effect on the composition of gut microbiota. Early studies showed that specific diets, such as high-fat diets, can modulate the gut microbiome efficiently and rapidly (Tilg, 2010). A recent study using mass spectrometry-based metabolomics has demonstrated a large effect of the gut microbiome on mammalian blood metabolites

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and suggested that gut microflora might have a direct impact on the metabolic capacity of the host (Wikoff et al., 2009). In addition, the gut microflora has been shown to have local (e.g. on the colonic mucosa) and systemic immunological effects, modulating the immune response in experimental animals (Schrenk, 2009). We hypothesize that the apparent beneficial effects of grain fiber intake may be related to their action on the gut microflora, and consequently on the immune system.

Here we describe an exploration of the associations between total fiber intake, and subtypes of fibers, and a panel of 17 pro-or anti-inflammatory markers in 88 volunteers for whom baseline dietary and lifestyle data are available.

Methods

The parent study was based on 176 adults (106 women, 70 men), who participated in a case-control study nested in the Italian European Prospective Investigation into Cancer and Nutrition (EPIC-Italy). The original aim was to explore the association between plasma cytokine and chemokine levels and the risk of non-Hodgkin lymphomas (NHL) (Saberi Hosnijeh et al., 2010b). The current analyses utilized the control subjects from the study.

The design of the EPIC cohort has been described previously (Riboli et al., 2002). Informed consent forms were filled at each local center, and the study was approved by the Institutional Review Board at the International Agency for Research on Cancer (IARC), and the local ethics committees. EPIC-Italy recruited 47,749 volunteers (15,171 men, 32,578 women, aged 35–65 years) during 1993–1998 from five different administrative centers covered by cancer registries, including Varese (12,083 men and women volunteers) and Turin (10,604) in the Northern part of the country, Florence (13,597) in Central Italy, and Ragusa (6,403) and Naples (5,062 women only) in Southern Italy.

Lifestyle questionnaires included questions on dietary items, education, occupation, previous illness, alcohol and tobacco consumption, and physical activity. Dietary intake assessment was carried out by extensive centre-specific dietary questionnaires, aiming to provide high compliance rates, and to detect variations in dietary habits. Estimation of fiber intake has been described previously (Cust et al., 2009). In brief, dietary fiber was defined by the AOAC (Association of Official Agricultural Chemists) gravimetric method for Total Dietary Fiber (DeVries and Rader, 2005), and includes soluble and insoluble forms (including lignin) of non-starch polysaccharides (NSP) and resistant starch. The AOAC method is the reference labeling method in Europe. For fruits and vegetables, but not for potatoes and other tubers, the AOAC and NSP (Englyst method) values were assumed to be comparable.

Laboratory assay

Sample collection and laboratory assays have been described previously (Saberi Hosnijeh et al., 2010a; Saberi Hosnijeh et al., 2010b;). Briefly, citrate plasma samples (50 μl) were used to measure 11 cytokines, i.e. Interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, and TNF-α, four chemokines, i.e. IL-8, RANTES, Eotaxin and IP-10, and two adhesion molecules (ICAM and VCAM). We used the Luminex multi-analyte profiling technology (Lab-MAP™) according to the protocol described by Jager et al (de Jager et al., 2003) except that an overnight incubation at 4°C was used (Saberi Hosnijeh et al., 2010b). Capture and detection antibodies and recombinant proteins were purchased from different commercial sources as described previously (de Jager et al., 2005). As a reporter; streptavidine-PE (BD Bioscience Pharmingen, San Diego, CA, USA) was used. The median time interval between sample collection and freezing was four hours. Mean fluorescence intensities were calculated from duplicates for each sample. Standard curves were derived from recombinant protein standards with 12-fold dilutions. All laboratory personnel were blinded with regard to the case-control status.

Due to the case-control study design of the parent study, all samples were run in duplicate with matched case-control sets assayed in the same batch. Quality control sets (low and high concentration cytokines quality control samples) were run in duplicate with the case-control sets in each batch. The median intra-batch coefficient of variation for all cytokines based on these quality control duplicate sets was 6.7% (range: 4.3–30) and the median inter-batch coefficient of variation was 30.7% (range: 9.6–110). The lower limits of detection were 0.24 pg/ml for IL-4; 0.61pg/ml for IL-12; 1.22 pg/ml for IL-1β, IL-2, IL-5, IL-6, IL-8, IL-10, IL-13, IFN-γ and TNF-α; 2.44 pg/ml for IL-1α, RANTES and Eotaxin; 4.88 pg/ml for IP10; and 73.24 pg/ml for ICAM and VCAM (Saberi Hosnijeh et al., 2010b).

Statistical analysis

The associations between levels of plasma cytokines and chemokines, and fiber intake were analyzed using multiple linear regression models. The dependent variables were the natural log-transformed cytokine or chemokine concentration values. Intake of fibers from total, cereal, fruit, and vegetable fibers were each divided into tertiles, and were included as ordinal variables. All models were adjusted for age at recruitment (years), sex, centers (categorical), education (none or primary school and above), smoking status (never, former, current, and unknown), alcohol drinking at baseline (g/day), body mass index (BMI, kg/m^2), physical activity (low or medium, and high or very high), and total energy intake (kcal/day). To assess the independent associations between plasma cytokine and chemokine concentration values, and a specific source of fibers, the models were mutually adjusted for other fiber sources. The normality assumption was tested by graphic examination of the residual distribution. The adjusted means were calculated by exponentiating the natural-log transformed means adjusted for the other effects in the model. Tests for trend were performed by

modeling the median values of each fiber category as a continuous variable. Interactions between fiber intake and age, sex, smoking status, alcohol drinking, BMI, and physical activity were tested by introducing an interaction term of the two variables of interest.

Principal component analyses were performed on the original cytokine and chemokine concentrations to identify underlying patterns. Factors were retained if the eigenvalue, i.e. the variance explained by the factor, was greater than one. We applied a varimax rotation to the factor loading matrix to achieve a simpler loading structure. Factor scores for each subject, i.e. a subject's relative standing on each of the factors, were calculated and standardized such that the distribution of the scores had a mean of zero and a variance of one, and were used as inflammatory profiles to estimate the associations with fiber intake.

Results

Table 1 shows the general characteristics of the participants, according to tertiles of fiber intake. Table 2 shows the adjusted mean levels of cytokines and chemokines by tertiles of total fiber intake. No decreasing or increasing trend was observed in marker levels among tertiles of total fiber. In contrast, cereal fibers (Table 3) show several statistically significant associations, in particular with IL-4, IL-5, IL-8, IL-12, IL-13, IFN-γ, TNF-α, and eotaxin. None of the other types of fibers showed statistically significant associations with any of the cytokines or chemokines (data not shown).

We performed factor analysis in search for pro- or anti-inflammatory patterns in relation to cereal fiber intake (Table 3). Four factors were retained with an eigenvalue larger than one. The four factors explained about 73% of the total variance in the original dataset (Appendix 1). Cereal fiber intake was inversely associated with factor 1, which is mainly formed by IL-1β, IL-4, IL-5, IL-6, IL-13, and TNF-α. All of these are pro-inflammatory except IL-4 and IL-13, which are anti-inflammatory.

Table 1. Characteristics of the study population according to tertiles of fibre intake.

	≤17.52		17.52–24.03		>24.03	
Fibre intake (g/day)	Mean	SD	Mean	SD	Mean	SD
Age at recruitment	54.4	8.7	53.7	6.8	53.8	8.2
Alcohol intake at baseline	14.8	18.7	15.0	21.7	21.2	18.8
BMI	26.2	4.1	25.7	2.9	25.1	2.5
% of cereal fibre	38.7	16.0	37.0	13.2	37.1	13.2
% of fruit fibre	24.9	13.6	32.3	12.3	30.0	9.4
% of vegetable fibre	19.5	10.6	17.9	7.5	19.0	10.0
% of legume fibre	3.7	5.8	3.7	3.0	4.1	3.8
% of other fibre	14.1	8.1	9.3	5.6	10.2	4.6
	N	%	*N*	%	*N*	%
Sex						
Men	7	24	12	41	15	52
Women	22	76	17	59	14	48
Centers						
Florence	5	17	10	34	5	17
Varese	15	52	11	38	13	45
Ragusa	1	3	1	3	3	10
Turin	8	28	5	17	4	14
Naples	0	0	2	7	4	14
Education						
None or primary school completed	20	69	15	52	14	48
Above	9	31	14	48	15	52
Smoking Status						
Never smokers	16	55	14	48	11	38
Former smokers	5	17	11	38	8	28
Current smokers	8	28	4	14	8	28
Unknown	0	0.0	0	0.0	2	7
Physical Activity						
Low or medium	8	27	13	45	11	38
High or very high	21	73	16	55	18	62

Discussion

In this cross-sectional study, examining fiber intake and 17 cytokines and chemokines in 88 healthy EPIC-Italy participants, we observed an association between cereal fiber and decreasing levels of predominantly pro-inflammatory cytokines, but associations were not evident for fruit and vegetable fibers.

An analysis in the Iowa Women's Health Study reported an association between whole grain intake and non-cancer, non-CVD inflammatory disease mortality (Jacobs, Jr. et al., 2007). In another study using a similar case definition (Buyken et al., 2010), only fruit fiber was associated with inflammatory disease mortality and only in men. In the NIH-AARP cohort (Park et al., 2011), the investigators observed inverse associations between fiber intake and several causes of death, including a 46% lower risk for respiratory disease death, and a 59% lower risk for infectious disease death, when comparing the highest to the lowest quintile of total fiber intake. The associations were especially strong for grain fiber intake. The authors suggested that the observation could be due to the anti-inflammatory properties of dietary fiber.

It has been recognized that chronic inflammation is acting as an essential component in pathogenesis of a wide variety of chronic diseases, such as diabetes, cardiovascular diseases (CVD), chronic liver diseases, asthma, as well as conditions like obesity and aging (Scrivo et al., 2010). Some inflammatory markers are acting as disease markers (King, 2005). In obese people, increased pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, were associated with diabetes (Badawi et al., 2010; Goldberg, 2009). On the other hand, the TH2-like anti-inflammatory cytokines, such as IL-4, and IL-13 were positively associated with atopic asthma (Lloyd and Hessel, 2010). Adiponectin is a marker inversely related to the degree of adiposity (Nedvidkova et al., 2005). In animal studies, it was shown that adiponectin can

Table 2. Mean levels (pg/ml)* (and their 95% confidence intervals) for cytokines and chemokines, according to tertiles of total fibre intake.

	Total fibre intake: ≤17.52 g/day			Total fibre intake: 17.52–24.03 g/day			Total fibre intake: >24.03 g/day			
	Mean	LL	UL	Mean	LL	UL	Mean	LL	UL	*P trend*
IL-1α	82.27	29.08	232.76	83.10	33.45	208.51	39.65	13.33	117.92	0.40
IL-1β	2.29	1.34	3.94	1.22	0.76	1.95	1.09	0.63	1.93	0.11
IL-2	96.54	36.97	254.68	76.71	32.79	177.68	39.65	14.44	108.85	0.27
IL-4	1.03	0.53	2.01	0.61	0.34	1.12	0.41	0.20	0.84	0.10
IL-5	35.52	14.01	89.12	25.53	11.36	57.40	20.09	7.61	52.46	0.46
IL-6	91.84	45.60	184.93	45.15	24.29	83.10	34.12	16.44	71.52	0.09
IL-8	9.87	4.14	23.81	6.42	2.97	13.87	4.31	1.73	10.80	0.26
IL-10	55.15	22.20	138.38	54.05	24.29	120.30	29.67	11.36	76.71	0.41
IL-12	20.91	8.25	52.98	15.33	6.75	34.47	9.12	3.46	24.05	0.28
IL-13	2.23	1.07	4.62	1.62	0.84	3.06	1.43	0.66	3.06	0.47
IFN-γ	12.06	4.57	32.14	4.31	1.84	10.18	4.48	1.62	12.43	0.23
TNF-α	1.34	0.69	2.61	0.73	0.40	1.30	0.64	0.32	1.27	0.18
RANTES	7259.02	5271.13	9897.13	8022.46	6063.24	10614.75	9604.62	6836.29	13359.73	0.29
ICAM	114480.00	100984.00	127976.00	129384.00	117514.00	141254.00	125206.00	111095.00	139317.00	0.34
VCAM	72707.00	66172.00	79243.00	71209.00	65415.00	77002.00	71124.00	64077.00	78170.00	0.77
Eotaxin	85.63	59.74	123.97	77.48	56.26	106.70	56.26	38.47	83.10	0.17
IP10	37.34	29.37	46.99	41.68	33.78	50.91	42.10	32.79	54.05	0.53

*The means were calculated by exponentiating the natural-log transformed means, which were estimated from multiple linear regression and adjusted for age at recruitment (years), sex, centres (categorical), education (none or primary school vs. above), smoking status (never, former, current, and unknown), alcohol drinking at baseline (g/day), body mass index (BMI, kg/m^2), physical activity (low or medium vs. high or very high), and total energy intake (kcal/day).

Table 3. Mean levels (pg/ml)* (and their 95% confidence intervals) for cytokines and chemokines, according to tertiles of cereal fibre intake.

	Cereal fibre intake: ≤5.94 g/day			Cereal fibre intake: 5.94–8.81 g/day			Cereal fibre intake: >8.81 g/day			
	Mean	LL	UL	Mean	LL	UL	Mean	LL	UL	*P trend*
IL-1α	83.10	19.69	354.25	35.87	9.21	139.77	19.11	4.95	72.97	0.09
IL-1β	2.77	1.30	5.93	3.06	1.51	6.30	1.52	0.76	3.06	0.18
IL-2	48.42	12.81	181.27	27.94	8.00	97.51	18.73	5.47	64.72	0.24
IL-4	1.27	0.50	3.22	0.76	0.86	1.84	0.37	0.16	0.88	0.03
IL-5	90.92	25.79	323.76	31.82	9.68	104.58	19.89	6.11	64.07	0.05
IL-6	51.94	19.30	139.77	60.95	23.81	154.47	27.39	10.91	68.72	0.27
IL-8	11.47	3.49	37.71	4.35	1.42	13.33	2.25	0.74	6.82	0.03
IL-10	55.15	15.64	196.37	32.79	9.97	107.77	16.61	5.10	53.52	0.11
IL-12	27.94	7.92	98.49	9.87	3.03	32.46	4.48	1.39	14.44	0.02
IL-13	3.32	1.23	8.94	2.46	0.96	6.23	1.03	0.41	2.59	0.05
IFN-γ	12.18	3.25	46.06	6.82	1.95	23.57	1.86	0.54	6.36	0.02
TNF-α	2.25	0.91	5.53	1.46	0.63	3.42	0.55	0.24	1.27	0.01
RANTES	4722.06	3071.74	7331.97	6904.99	4582.50	10509.13	7259.02	4865.87	10938.02	0.11
ICAM	111192.00	92689.00	129696.00	126546.00	109127.00	143965.00	126691.00	109495.00	143887.00	0.18
VCAM	75295.00	66333.00	84257.00	71427.00	62942.00	79912.00	68158.00	59822.00	76495.00	0.19
Eotaxin	101.49	61.56	167.34	75.94	47.47	122.73	54.05	33.78	86.49	0.04
IP10	46.53	33.78	64.72	43.82	32.14	59.74	35.52	26.05	47.94	0.15
Factor 1	0.83	0.24	1.42	0.31	-0.25	0.87	-0.07	-0.62	0.49	0.01
Factor 2	0.17	-0.51	0.86	-0.22	-0.87	0.43	-0.34	-0.98	0.30	0.22
Factor 3	0.10	-0.54	0.75	-0.06	-0.67	0.55	-0.40	-1.01	0.20	0.19
Factor 4	0.02	-0.60	0.65	-0.05	-0.65	0.54	-0.11	-0.70	0.47	0.72

*The means were calculated by exponentiating the natural-log transformed means, which were estimated from multiple linear regression and adjusted for age at recruitment (years), sex, centres (categorical), education (none or primary school vs. above), smoking status (never, former, current, and unknown), alcohol drinking at baseline (g/day), body mass index (BMI, kg/m^2), physical activity (low or medium vs. high or very high), total energy intake (kcal/day) and other sources of fibre intake (g/day).

attenuate allergen-induced airway inflammation and hyper-responsiveness associated with IL-13 (Shore et al., 2006; Shore, 2010). High consumption of cereal fiber was shown to be associated with higher plasma adiponectin levels (Qi et al., 2005).

Therefore, we hypothesized that the beneficial effects of fiber can be related to inflammatory properties. However, it is still not clear how fiber modulates the inflammatory/immune status. Dietary fiber has been inversely associated with several inflammatory

markers, such as C-reactive protein (CRP), IL-6, IL-18, and TNF-α, in cross-sectional studies (Ma et al., 2006; Ma et al., 2008; Wannamethee et al., 2009). One of the proposed mechanisms is through the short chain fatty acids (SCFA), which are produced when fiber is fermented in the colon (Meijer et al., 2010), and are associated with a range of processes related to the maintenance of normal health, as well as disease processes in the gut (Andoh et al., 1999). It is hypothesized that fiber could alter the luminal environment and the colonic microflora (Young et al., 2005). Probiotics are living microbes, which are beneficial for the host health. Probiotics were shown to influence the gut microflora and modulated immune response *in vitro* and *in vivo,* and were used to treat or prevent allergic diseases in clinical trials (Borchers et al., 2009; Huffnagle, 2010; Kukkonen et al., 2007; Noverr and Huffnagle, 2004; van de Pol et al., 2011). *In vitro* experiments have shown that butyrate could induce a shift from a more pro-inflammatory TH1-like profile to an anti-inflammatory profile (Maa et al., 2010; Saemann et al., 2000). A combination of butyrate, acetate, and propionate showed a slightly reduced pro-inflammatory profile compared to cultures without SCFAs (Cavaglieri et al., 2003). However, the mechanism by which the SCFAs modulate inflammation is still unknown, probably *via* different mechanisms for different SCFAs (Meijer et al., 2010). Animal studies also suggested that different types of fiber can influence the distribution of SCFAs; this could be because of the physicochemical properties of the fibers, such as water solubility, the location where they get fermented, the rate of fermentation, etc. For example, soluble fiber (mainly found in fruit or oat) is quickly fermented in the proximal colon, while insoluble fiber (that can be found in wheat) is slowly fermented in distal colon (Henningsson et al., 2002; Pylkas et al., 2005; Young et al., 2005). However, such investigations have not yet been performed in human studies.

On the other hand, it is not clear whether cereal fiber itself contributes to the levels of the inflammatory markers or it acts as a marker of other exposures related to inflammation. For instance, intake of cereal fiber could be a marker of whole-grain cereal intake. Whole grain cereals are also rich sources for other bioactive compounds that have anti-inflammatory properties, such as Mg^{2+} or Zn^{2+}, etc (Fardet, 2010). It is possible that these compounds are the major players in inflammation modulation instead of fiber. It is also possible that the associations are from residual confounding from other lifestyle factors. National surveys have suggested that fiber intake, or whole-grain intake, is associated with socio-economic status, smoking, and physical activity (Hulshof et al., 2003; Kyro et al., 2011; Lang and Jebb, 2003; Thane et al., 2007). The inverse association between fiber intake and inflammatory markers could be due to the different levels of these behaviors. However, we have adjusted our observations by education, smoking status, alcohol drinking, BMI, and physical activity, to reduce the impact of confounding.

In the current analyses, we did not observe an association between fruit and vegetable fibers and inflammatory markers. This appears to contradict our observation that all cereal, fruit, and vegetable fibers are inversely associated with inflammatory disease mortality in EPIC (per 5g increase: RR = 0.86, 95% CI = 0.81–0.92 for cereal fiber; RR = 0.92, 95% CI = 0.85–1.00 for fruit fiber; and RR = 0.90, 95% CI = 0.82–0.99 for vegetable fiber; Chuang et al., manuscript in preparation). However, this may result from an inadequate sample size, and an inability to detect the relatively weaker association and narrower distribution of fruit and vegetable fiber. Fruit and vegetables were inversely associated with CRP (Holt et al., 2009; Oliveira et al., 2009; Wannamethee et al., 2006), IL-6 (Holt et al., 2009), and TNF-α (Holt et al., 2009) in previous studies.

A limitation of the study is that we were not able to study how fiber modulates gut microflora populations, and how microflora in turn influences the inflammatory markers. The investigation of microflora would require a different design.

In summary, our analyses suggest that a high cereal fiber intake may be inversely associated with the presence of a cytokine pro-inflammatory profile. Given the existing evidence for the influence of dietary patterns on gut microfloral populations, we speculate that this might be related to an action of fibers on the gut microflora, which in turn may modulate the release of cytokines and chemokines. Further investigations, including randomized trials, would help to isolate the effects of fiber intake.

Appendix

Table A1. Factor loadings and explained variance (VAR) for the major cytokine/chemokine patterns.

	Factor1	Factor2	Factor3	Factor4
IL-1α	0.03642	**0.93063**	0.08166	0.01184
IL-1β	**0.89522**	0.02021	0.07487	−0.06689
IL-2	−0.08451	**0.60698**	**0.60869**	−0.08963
IL-4	**0.63192**	0.27184	0.57748	0.05666
IL-5	**0.65604**	0.45173	0.06972	0.14628
IL-6	**0.96618**	0.06852	0.0261	0.02376
IL-8	0.13851	**0.83417**	0.2609	0.17297
IL-10	−0.02571	**0.90829**	0.01937	−0.03798
IL-12	0.15851	**0.95259**	0.10438	0.08046
IL-13	**0.97433**	0.07775	0.0176	0.03517
IFN-γ	0.04513	0.14004	**0.91666**	0.05659
TNF-α	**0.96524**	−0.00521	0.00569	0.04114
RANTES	−0.03688	−0.04751	0.00095	−0.33095
ICAM	−0.11637	−0.17797	0.35026	0.49326
VCAM	−0.07332	0.07927	−0.11333	**0.82441**
Eotaxin	0.21913	0.55365	0.57271	−0.13376
IP10	0.41101	−0.01728	0.00343	−0.18717
Proportion of VAR explained	35.5	21.64	8.58	6.81
Cumulative VAR explained	35.5	57.14	65.72	72.53

Loadings ≥0.60 were bolded.

Declaration of interest

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