

FAECAL MICROBIAL FLORA AND DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS DURING A VEGAN DIET

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SUMMARY

To clarify the role of the faecal flora in the diet-induced decrease of rheumatoid arthritis (RA) activity, 43 RA patients were randomized into two groups: the test group to receive living food, a form of uncooked vegan diet rich in lactobacilli, and the control group to continue their ordinary omnivorous diets. Based on clinical assessments before, during and after the intervention period, a disease improvement index was constructed for each patient. According to the index, patients were assigned either to a group with a high improvement index (HI) or to a group with a low improvement index (LO). Stool samples collected from each patient before the intervention and at 1 month were analysed by direct stool sample gas–liquid chromatography of bacterial cellular fatty acids. This method has proved to be a simple and sensitive way to detect changes and differences in the faecal microbial flora between individual stool samples or groups of them. A significant, diet-induced change in the faecal flora ($P = 0.001$) was observed in the test group, but not in the control group. Further, in the test group, a significant ($P = 0.001$) difference was detected between the HI and LO categories at 1 month, but not in the pre-test samples. We conclude that a vegan diet changes the faecal microbial flora in RA patients, and changes in the faecal flora are associated with improvement in RA activity.

KEY WORDS: Bacteria, Rheumatoid arthritis, Vegan diet, Faecal flora.

PATIENTS with rheumatoid arthritis (RA) often claim that special diets alleviate their symptoms. Some reports seem to confirm their observations [1, 2]. The alteration of dietary lipids and the change in lipid mediators of inflammation may explain some of the improvement [3]. However, diet can also change the intestinal microflora [4, 5] and this may play a role in the decrease in RA activity. The idea is largely based on the reports connecting RA and elevated faecal counts of bacterial species, e.g. *Clostridium perfringens* [6, 7], but an overall difference in the faecal flora between RA patients and controls has also been observed [8]. The changes in the faecal flora may lead to increased absorption of arthritogenic antigens and to inflammatory joint disease in susceptible individuals [9, 10]. On the other hand, changes in the intestinal microflora induced by a vegetarian diet may be beneficial in RA, e.g. by changing the flora to a less harmful direction. This is implicated by the results in a trial to treat RA with a fast, followed by 1 yr of a vegetarian diet, which was individually adjusted to eliminate all items provoking RA symptoms [5]. In the study, the faecal flora of the RA patients experiencing the highest improvement during the dietary intervention period differed significantly from that of the rest of the patients on the same vegetarian diet. The aim of the present study was to observe the effects of living food, an uncooked vegan diet rich in lactobacilli, on the faecal flora of RA patients, and to test whether the

results of the previous study could be confirmed with a type of vegan diet, which is not individually adjusted but designed and known to change the faecal flora [4].

For studies on the faecal flora, traditional methods are culture based. The human faecal flora is, however, an extremely complex ecosystem with over 400 different species [11]. For studies on the faecal flora, culture-based methods are laborious, insensitive, difficult to interpret and regarded as being outright unsuitable [12]. To overcome these problems, another approach was used. This is possible by computerized comparison of bacterial cellular fatty acid (CFA) profiles produced by gas–liquid chromatography (GLC) directly from stool samples [4, 5, 13]. The CFAs are structural components of bacterial cell membranes. They are long-chain fatty acids (10–20 carbon atoms). Each bacterial species has a special CFA composition [14]. In a stool sample, the CFA profile represents all the bacteria present in the sample. The method has proved to be sensitive, reliable and practical in detecting overall changes in the faecal microflora of stool samples [4].

MATERIALS AND METHODS

Subjects

Forty-three consecutive adult patients with diagnosed (ARA criteria) chronic and active RA (Steinbrocker's functional class II–III) visiting the rheumatic out-patient clinic at Kivelä Hospital, Helsinki, were enrolled in the study. The selected patients had active joint symptoms (more than three swollen or five tender joints) and elevated inflammatory parameters [erythrocyte sedimentation rate (ESR) >20, mean 32.7 in the intervention group and 40.2 in

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the control group, s.d. 16.3 and 26.5, respectively]. The approval of the regional ethics committee was obtained. After informed consent, the patients were randomized into two groups: the test group to receive vegan diet and the control group to continue their previous omnivorous diets. There was no significant difference between the groups in height, weight, body mass index, duration of rheumatic disease, seropositivity, medication or the activity of RA, yet the intervention group was younger (49 vs 56 yr, $P = 0.02$). Caffeine-containing drinks, chocolate, alcohol and tobacco smoking were prohibited in both groups. No antibiotics were used during the study. All patients remained as ambulatory patients, and continued their previous medication for the first month of the study. The medication could later be modified when necessary on clinical grounds by one of the authors (TH, a rheumatologist). The medication of six patients in each group was thus changed. In the test group, the dose of non-steroidal anti-inflammatory drugs (NSAIDs) was reduced in the treatment of five patients, and for one patient corticosteroids were first introduced and then discontinued. In the control group, NSAIDs were discontinued in three cases and increased in one. Corticosteroids were discontinued in two patients and gold in one. The basic data of the subjects are presented in Table I.

TABLE I
Patient characteristics*

	Test group	Control group
Patients		
Randomized for the study	22	21
Started the study	22	20
Completed the study	19	18
Stool samples collected from	18	18
Pre-test sample	yes	yes
1 month sample	yes	yes
Post-test sample	yes	no
Anthropometric data		
Sex: male/female	1/17	1/17
Age (yr)	49.1	55.6
Height (cm)	163.4	164.3
Weight (kg)	68.0	63.6
Body mass index (kg/m ²)	25.5	23.5
Rheumatoid arthritis		
Duration (yr)	12.6	16.1
Seropositive RA	15	14
Medication		
Gold (i.m. or p.o.)	4	6
Methotrexate	10	3
Salazopyrine	1	3
Corticosteroids	11	6
NSAIDs	15	17
Improvement categories†		
High improvement (HI)	5	0
Low improvement (LO)	13	18

*Number of patients or mean values are given.

†Based on clinical assessments before and during the intervention. HI category: decrease ($\geq 20\%$) in five or six of the six clinical parameters of disease activity. LI category: all the other patients.

Diet

Dietary records were collected for 1 week before the intervention and at 1 month. Living food [15] is an uncooked vegan diet, rich in lactobacilli. It does not contain any animal products, raffinated substances or added salt. The items may be soaked, sprouted (seeds, grains), fermented, blended or dehydrated. The test group received all the components of the diet daily from a specialized kitchen in a pre-packed form. The kitchen weighed the components, and the subjects recorded items they did not consume and also the amount of extra food whenever used. All the subjects in the test group were supervised daily in the use of the diet. Compliance was controlled by interviews and analysing urinary sodium excretion, which is known to decrease by two-thirds if dietary compliance is good.

The nutrient contents were calculated with UNIDAP (Unilever Dietary Analysis Program, Paasivaara Ltd, Helsinki, Finland), and the results will be published separately. The corresponding data for another group of volunteers using the same diet have already been published [4].

Clinical follow-up

One of the authors (TH) carried out the clinical evaluation blindly before the intervention period and at 1 month during the trial. A disease improvement index (0–6) was calculated for each patient. The index was based on changes in six disease activity parameters: pain (scale 0–100), Health Assessment Questionnaire (Finnish version), the number of tender joints, the number of swollen joints, subjective evaluation of improvement (scale 0–5) and ESR. The patients with improvement of $\geq 20\%$ in five or six of these parameters were assigned to a high improvement index category (HI) and the rest to a low improvement index category (LO).

Laboratory samples

Blood and stool samples were collected before the intervention period and at 1 month (stool samples from the test group also after the intervention period). The stool samples were initially placed at -20°C to be stored in the microbiological laboratory at -40°C for a few days until analysis.

Direct gas-liquid chromatography of stool samples

GLC was used to produce bacterial CFA profiles of the stool samples. For that purpose, the bacterial material was first separated from faecal vegetable fibres and free fatty acids of the faecal material as follows: 100 mg of the faecal sample were weighed, suspended in 5 ml physiological saline, gently mixed and allowed to sediment for 2 h at $+4^{\circ}\text{C}$. After this, the sample was remixed, allowed to sediment for 15 min, and the bacterial component in the supernatant removed and centrifuged at 1000 g for 15 min at room temperature to produce a pellet.

GLC of the bacterial cellular fatty acids was performed as described previously [14]. The collected bacterial mass was saponified, methylated and analysed

as described previously [14]. In brief, the collected bacterial mass was incubated for 30 min at 100°C in 15% (w/v) NaOH in 50% aqueous methanol and then acidified to pH 2 with 6 N aqueous HCl in CH₃OH. The methylated fatty acids were then extracted with ethyl ether and hexane. The GLC analysis was performed with an HP5890A gas chromatograph (Hewlett-Packard) and an Ultra 2, 004-11-09B fused silica capillary column (0.2 mm × 25 m; cross-linked 5% phenylmethyl silicone; Hewlett-Packard). Ultra-high-purity helium was used as a carrier gas. The GLC settings were as follows: injection port temperature, 250°C; detector temperature, 300°C; initial column temperature, 170°C, increasing at 5°C/min up to 270°C at 20 min; total analysis time, 25 min; sample volume 1 µl. The peak retention time and peak area volumes were recorded by an HP3392A integrator (Hewlett-Packard).

GLC data analysis

The bacterial CFA profiles of stool samples were compared by computer as described previously [14]. The analysis is based on the assumption that the CFA profile represents all bacteria present in the stool sample. To eliminate the effect of different sample quantities, the calculations were made with relative peak areas. The chromatogram of each individual sample was compared to the chromatograms of the other samples in the same study, and similarity indices were calculated for each sample pair. The similarity indices were presented as correlation matrices and further analysed by the method of weighted pair-group cluster analysis of arithmetic averages [14]. The samples were grouped according to the diet (test and control), the sample time (pre-test and 1 month, and for the test group also post-test) and the disease improvement category (HI and LO).

Statistical analysis

The statistical significances for the differences between the groups at the time of randomization in age, height, weight, body mass index, duration of RA, seropositivity, medication and disease activity were analysed by Fisher's exact test. Student's *t*-test was used to calculate significances for the differences between the groups in the improvement indices. The differences between stool sample CFA profiles of different groups were calculated as described previously [5]. The procedure is based on the paired comparisons between individual samples and the calculations of similarity indices between them. The variation of the fatty acid profiles within each group was determined by calculating the mean ± S.D. for all the paired comparisons within the group. The variation between two groups was calculated by comparing each fatty acid profile in one group with all the samples in the other group. The mean ± S.D. was calculated for all these comparisons. Finally, the variation between the groups (mean, \bar{x} ; S.D., s ; number of comparisons, n) was compared to the variation within the groups (x_1 , s_1 and n_1 for the first

group; x_2 , s_2 and n_2 for the second group) by calculating a z value as follows:

$$z = \frac{\bar{x} - \frac{n_1x_1 + n_2x_2}{n_1 + n_2}}{\sqrt{\frac{s^2}{n} + \frac{n_1s_1^2 + n_2s_2^2}{(n_1 + n_2)^2}}}$$

The resulting z values were used to determine the P values by using the z table [16].

RESULTS

The patients in the test group were slightly younger than the controls (mean age 49 *vs* 56 yr, $P = 0.02$). There was no significant difference in height, weight, body mass index, duration of RA, seropositivity or medication between the groups. Three patients in the test group refused to continue the diet after a few weeks. In the control group, one stopped the study for personal reasons and one because of hepatic toxicity due to medication. In the test group, one was excluded because of poor diet compliance (high urinary sodium excretion) and another for not producing a stool sample at 1 month. This left 18 subjects in both groups. The distribution of the patients into the disease improvement categories is shown in Table I.

Five patients in the test group (27.8%) and none in the control group belonged to the HI category. The mean values of improvement indices were 3.1 and 2.0, respectively. The difference is significant ($P = 0.027$, Student's *t*-test).

The effect of living food on the faecal microbial flora

When the stool sample CFA profiles were compared, the test-period samples differed significantly ($P < 0.001$) from the pre-test as well as from the post-test samples in the test group, while no significant difference was seen in the control group (Fig. 1).

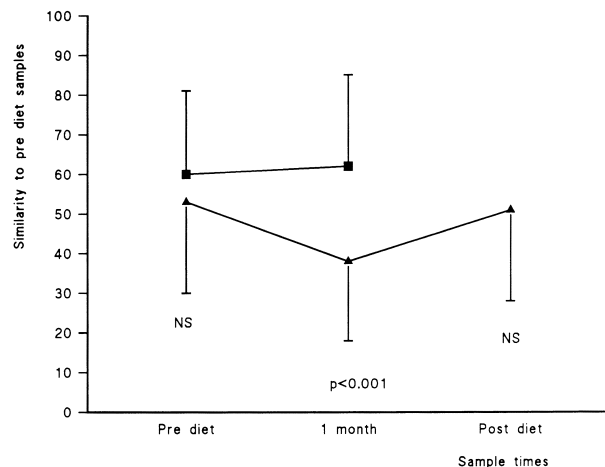


FIG. 1.—The effect of living food on the faecal flora of RA patients. Horizontal axis: sample times. Vertical axis: the mean value of similarity indices to the pre-test samples of each group; 100 indicates complete similarity and 0 complete dissimilarity. Test group, ▲; control group, ■. NS, non-significant.

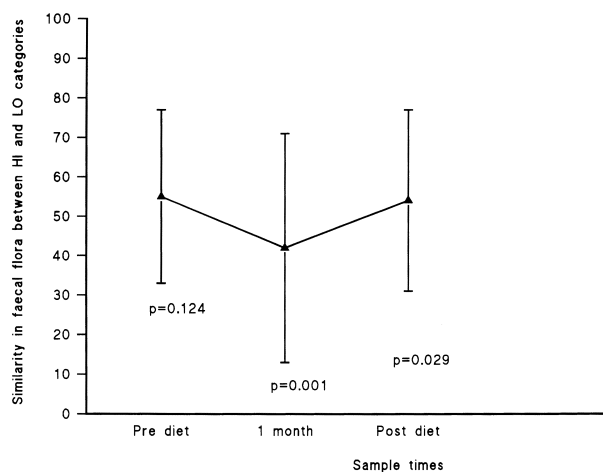


FIG. 2.—Difference in the faecal flora between disease improvement categories in the test group; 100 indicates complete similarity and 0 complete dissimilarity. HI, high improvement category; LO, low improvement category. Horizontal axis: sample times (before, during and after the dietary intervention). Vertical axis: the mean value of similarity indices between the categories. *P* values are presented for the differences in the faecal microbial flora between the two improvement categories.

Correspondingly, similar results ($P < 0.001$ for the difference) were obtained when the test group was compared to the control group at 1 month. These results indicate that the living food diet changes the faecal flora in RA patients significantly, while there is no such change in the control group during the same period.

The role of the faecal flora in the changes in disease activity

When the CFA profiles of the stool samples were compared between the HI and LO categories, a significant difference ($P = 0.001$) was observed at 1 month and even after the intervention period ($P = 0.029$), but not in the pre-test samples (Fig. 2).

DISCUSSION

Dietary manipulations have been reported to improve both subjective and objective RA activity parameters [1, 2]. How the diets induce this improvement is not fully understood. Our results in this and in a previous study [5] support the idea that changes in the faecal flora may play a role. The findings in the present study not only confirm our earlier observations that living food changes faecal flora [4], but also connect the alteration of the faecal flora to the change in RA activity.

The GLC method used to study overall changes in the faecal flora has proved to be considerably more sensitive to detect differences in the faecal floras than the classical quantitative culture of stool samples [4]. It is also technically simple to perform, while the classical bacteriological techniques are enormously laborious and outright unsuitable for the study of faecal microecology in a large number of individuals [12]. The

clinically important change in the faecal flora does not necessarily have to be due to changes in one or even a few species, but due to a combination of changes in a large number of different bacterial species at the same time as a response to the same environmental factor, such as diet. In a situation like that, the quantitative culture of stool samples is too insensitive, and the results too complicated for reliable interpretation.

The automated analysis of stool sample GLC fatty acid profiles readily detects changes and differences between stool samples. The differences between the dietary groups and between the improvement categories were highly significant. In this study, the vegan diet both changed the faecal microflora and also induced a decrease in disease activity in some of the RA patients. Because the study was conducted in parallel for all patients, and in the test group the diet was strictly monitored and remained uniform for the whole intervention period, we were given a valuable opportunity to observe in most controlled circumstances whether disease improvement is associated with changes in the faecal flora. The methods and the experimental design do not allow us to determine what exactly in the faecal flora in the HI category is different from the LO category and whether the difference is linked to the disease improvement causally. Even if the association were causal, it remains open which is primary—disease improvement or change in the faecal flora—although the early appearance of the changes (at 1 month) favours the primacy of the change in the faecal flora.

We conclude that living food induces a significant change in the faecal microbial flora of RA patients. There is also a connection between the changes in clinical activity of RA and in intestinal microbial flora, suggesting that faecal bacteria may have a role in diet-induced changes in disease activity in RA patients.

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