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Fecal Microbiota Composition and Frailty

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The relationship between fecal microbiota composition and frailty in the elderly was studied. Fecal samples from volunteers with high frailty scores showed a significant reduction in the number of lactobacilli (26-fold). At much higher population levels, both the *Bacteroides*/*Prevotella* (threefold) and the *Faecalibacterium prausnitzii* (fourfold) groups showed a significant reduction in percentage of total number of hybridizable bacteria in the elderly with high frailty scores. In contrast to this, the number of *Enterobacteriaceae* was significantly higher (sevenfold) in samples from very frail volunteers.

The gut microbiota forms an essential part of a complex ecosystem that plays an important role in human health and nutrition (5, 8, 34). Bacterial colonization of the gastrointestinal (GI) tract is affected by various factors such as host, microbiological, physiological, dietary, and environmental factors. Studies of babies (10) and adults (6, 12, 37) have shown that after birth the gut microbiota composition keeps evolving, even in old age (23).

Knowledge about elderly gut microbiota composition is far from complete (3, 13, 16, 17, 27). Changes in gut microbiota composition related to ageing may have implications for the health of elderly persons and may be caused by factors which include psychosocial stress, mobility, and nutrition. Psychosocial stress factors may contribute to the development of anorexia or changes in the immune system, which may in turn affect the gut microbiota composition (15, 19). Small bowel motor pattern changes have been reported with ageing, and in addition, the degree of mobility of the elderly person may influence bowel motility. Reduced bowel movement has a negative effect on digestion but also causes constipation and may therefore be associated with changes in the gut microbiota.

Frailty comprises several of the above-mentioned factors (28, 30). Frailty can generally be defined as a state of decreasing reserves with respect to those functions and resources that are essential for a person to maintain an acceptable level of functioning. The Groningen Frailty Indicator (GFI) (28, 30) is a short 15-item questionnaire aiming to identify patients that have diminished reserves in one or more of the core domains of functioning. These domains are: mobility, physical fitness, comorbidity, weight loss, vision, hearing, cognition, and psychosocial resources. Of all these domains it has empirically been shown that these are associated with a variety of adverse outcomes. The current view on the clinical measurement of frailty encompasses the whole person's functioning and physiology, emphasizing the interaction of physical and psychosocial systems (25). The GFI builds on the assumption that strong interaction effects often exist between different weaknesses of older patients, reinforcing each other into a downward spiral of further decline.

Because it is often difficult to separate different problems in causal order in elderly patients with multiple problems, it is deemed possible to identify the core domains of functioning and summate the affected domains because each of them contributes to the latent variable frailty. Psychometric analyses of the GFI in different development studies have shown that it is a one-dimensional (with principal component analysis) and internally consistent scale (KR-20 = 0.71) (28). With respect to the validity and predictive value of the GFI in hospital patients and in general practitioners' patients, it has been shown that the GFI is able to predict adverse outcome in different groups of patients (cancer patients, emergency room admissions, osteoporotic fractures, surgery). All studies showed that the GFI was a better predictor than calendar age.

In this paper, we describe the fecal microbiota composition in relation to frailty. Advances in the field of molecular phylogeny have made it possible to study bacterial populations by a culture-independent approach (1, 6, 9, 11, 18, 31, 35, 36). The predominant microbiota composition of fecal samples of volunteers was determined with fluorescent in situ hybridization (12), and the level of frailty was determined by the GFI (28).

Fresh fecal samples from 23 elderly volunteers (aged 70 to 100 years, with a median of 86 years) were collected. All volunteers were living in the Heymans Elderly Center, Groningen, The Netherlands. The study was compliant with the Medical Research Involving Human Subjects Act and informed consent was obtained. Samples came from elderly of two different levels of care in this center, varying from relatively little to complete nursing care. All volunteers were supplied with the same food menu. At least 4 weeks prior to collection of the samples, the volunteers did not receive antibiotics. Samples were stored at 4°C immediately after collection and processed within 24 h.

The total amount of cells and the total number of bacteria and specific bacterial groups of each fecal sample were enumerated as described previously (12), using the set of probes listed in Table 1. The level of frailty of the volunteers was determined using the GFI questionnaire (28, 30). One point

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TABLE 1. Fecal microbiota composition of elderly volunteers with low and high frailty scores as determined by DAPI staining (total cells) and hybridization with bacterial probes

Bacterial group or parameter	Stain or probe	Reference	Low-frailty group (n = 13)				High-frailty group (n = 10)			
			Median no. of cells/g of feces[a]		Median % microbiota		Median no. of cells/g of feces[a]		Median % microbiota	
			Dry wt	Wet wt	DAPI	Eub338	Dry wt	Wet wt	DAPI	Eub338
Total cells	DAPI		2.0×10^{11}	5.3×10^{10}	100		2.0×10^{11}	6.6×10^{10}	100	
Total bacteria	Eub338	2	1.3×10^{11}	3.5×10^{10}	53.9	100	1.1×10^{11}	3.4×10^{10}	54.2	100
Bacteroides/Prevotella group	Bac303	22	2.7×10^{10}	7.7×10^{9}	11.0	24.2*	8.7×10^{9}	2.9×10^{9}	4.5	9.4*
E. rectale/C. coccoides group	Erec482	6	1.6×10^{10}	5.1×10^{9}	10.6	19.7	1.3×10^{10}	4.1×10^{9}	6.7	13.2
Ruminococcus group	Rfla729/Rbro730	12	1.9×10^{10}	4.5×10^{9}	6.3	15.2	2.9×10^{10}	7.9×10^{9}	12.7	23.8
Atopobium group	Ato291	11	5.3×10^{9}	1.3×10^{9}	2.1	3.4	9.0×10^{9}	2.4×10^{9}	4.3	10.6
Faecalibacterium prausnitzii group	Fprau645	32	2.2×10^{9}	6.1×10^{8}	1.2	3.1*	4.6×10^{8}	1.6×10^{8}	0.3	0.7*
Eubacterium cylindroides group	Ecyl387	12	1.5×10^{9}	4.9×10^{8}	0.9	1.4	1.6×10^{9}	4.2×10^{8}	0.8	0.9
Bifidobacterium	Bif164	21	2.0×10^{9}	4.6×10^{8}	0.9	1.3	4.6×10^{8}	1.4×10^{8}	0.2	0.5
Streptococcus group	Strc493	6	1.1×10^{9}	3.8×10^{8}	0.7	1.1	8.7×10^{8}	2.8×10^{8}	0.4	0.8
Lactobacillus/Enterococcus group	Lab158	9	3.1×10^{8}*	9.8×10^{7}**	0.1*	0.3**	2.5×10^{7}*	7.1×10^{6}**	0.03*	0.04**
Clostridium group	Chis150/Clit135	6	2.4×10^{8}	5.4×10^{7}	0.1	0.2	1.8×10^{7}	5.8×10^{6}	0.01	0.02
Enterobacteriaceae	Ecoli1531^{23S}	24	6.6×10^{7}*	2.1×10^{7}*	0.05*	0.1	4.7×10^{8}*	1.4×10^{8}*	0.3*	0.6
Veillonella	Veil223	12	9.2×10^{6}	2.7×10^{6}	0.005	0.01	1.6×10^{7}	4.9×10^{6}	0.01	0.01
Phascolarctobacterium group	Phasco741	12	4.3×10^{5}	1.0×10^{5}	0.0002	0.001	4.1×10^{6}	1.3×10^{6}	0.002	0.004
Sum of specific probes					34.0	69.9			30.3	60.6
Lachnospira group	Lach571	12	2.0×10^{8}	4.5×10^{7}	0.1	0.2	1.5×10^{7}	4.0×10^{6}	0.01	0.01
Eubacterium hallii group	Ehal1469	12	1.3×10^{8}	3.3×10^{7}	0.1	0.1	9.9×10^{7}	2.7×10^{7}	0.1	0.1
Enterococcus faecium/Enterococcus faecalis	Enfm2/Enfl3	—[b]	3.6×10^{5}	1.0×10^{5}	0.0002	0.001	1.8×10^{6}	5.0×10^{5}	0.001	0.002

[a] The data were analyzed assuming that when no bacteria of the target group were detected, the bacterial number was 1.0×10^{5} per gram, wet weight. *, statistically significant ($P \leq 0.05$, two-sided); **, statistically significant ($P \leq 0.01$, two-sided).

[b] K. Waar, personal communication.

could be scored on each question. Mobility (four questions) and psychosocial factors (five questions) are the major components in the total frailty score. In some cases the questions about mobility and psychosocial factors were answered by the medical staff or the relatives of the elderly. The volunteers were divided into two groups, one ($n = 13$) with a low frailty score (1 to 4) and one ($n = 10$) with a high frailty score (5 or more) (30). Both groups consisted mainly of females, with two males in each group.

The results were statistically analyzed by SPSS for Windows, release 10.0.5, standard version. Analysis with Spearman's rank-order correlation coefficient showed that the age and frailty scores were not significantly related (correlation coefficient = 0.058). To compare the enumeration results of fecal samples of the groups with low and high frailty scores, the Mann-Whitney U test was applied. The enumeration results are listed in Table 1. The total number of cells was the same in both frailty groups, i.e., 2.0×10^{11} cells/g (dry weight). Of the total cells, the percentage of total hybridizable bacterial cells was approximately 54%. With group-specific probes, 34.0% and 30.3% of the total number of cells and 69.9% and 60.6% of the total number of hybridizable bacteria in the low- and high-frailty volunteer groups could be accounted for, respectively.

The percentages of hybridizable bacteria of the three largest groups of the fecal microbiota of healthy (low frailty scores) elderly, i.e., *Bacteroides*/*Prevotella*, *Eubacterium rectale*/*Clostridium coccoides*, and *Ruminococcus* (type species *R. flavefaciens*) were 24.2, 19.7, and 15.2%, respectively. The same groups in healthy adults aged 20 to 55 yrs were rather similar, 27.7, 22.7, and 10.3%, respectively (12). Larger differences were observed when these three percentages were compared to those of the fecal microbiota of elderly with high frailty scores, 9.4, 13.2, and 23.8%, respectively.

More differences in fecal microbiota composition can be observed between elderly persons with low and high frailty scores. The most predominant were the *Bacteroides*/*Prevotella*, *E. rectale*/*C. coccoides*, *Ruminococcus*, *Atopobium*, and *F. prausnitzii* bacterial groups. Of the total number of cells, the percentages of three of these groups were decreased in the high-frailty volunteers, i.e., *Bacteroides*/*Prevotella* (from 11.0 to 4.5%), *E. rectale*/*C. coccoides* (from 10.6 to 6.7%), and *F. prausnitzii* (from 1.2 to 0.3%). In contrast to this, the percentages of the *Ruminococcus* (from 6.3 to 12.7%) and *Atopobium* (from 2.1 to 4.3%) groups were increased in the high-frailty volunteers.

Four groups of bacteria were significantly different between fecal samples from the two volunteer groups, i.e., those that hybridized with the Lab158, Ecoli1531 (*Enterobacteriaceae*), Bac303 (*Bacteroides*/*Prevotella*), and Fprau645 (*F. prausnitzii*) probes. *Lactobacillus* numbers were obtained by subtracting those obtained with the Enfm2/Enfl3 probes from those determined with the Lab158 probe. The number of lactobacilli was 3.0×10^8 cells/g (dry weight) in low-frailty volunteers and 1.1×10^7 cells/g (dry weight) in high-frailty volunteers ($P = 0.005$). Of the total number of cells the percentage of lactobacilli was 0.1% in low-frailty volunteers and 0.01% in high-frailty volunteers ($P = 0.005$). Additionally, of the total number of hybridizable bacteria the percentage of lactobacilli was 0.3% in low-frailty volunteers and 0.02% in high-frailty volunteers ($P = 0.004$). As analyzed by Spearman's rho, the number of lactobacilli showed a significant negative correlation with the GFI (correlation coefficient = -0.694, $P < 0.0001$).

At much higher population levels, both the *Bacteroides*/*Prevotella* and *F. prausnitzii* group showed a significant reduction in percentage of the total number of hybridizable bacteria for the high-frailty volunteers. The *F. prausnitzii* group showed a significant reduction in percentage from 3.1% for the low-frailty volunteers to 0.7% for the high-frailty volunteers ($P = 0.03$). Analysis with Spearman's rho showed a strong correlation between the *F. prausnitzii* group and lactobacilli (correlation coefficient = 0.770, $P < 0.0001$). The *Bacteroides*/*Prevotella* group also showed a significant reduction in percentage, from 24.2% for the low-frailty volunteers to 9.4% for the high-frailty volunteers ($P = 0.05$).

For the number of lactobacilli, *F. prausnitzii*, and *Bacteroides*/*Prevotella* groups, it could be observed that the variation was the largest in the high-frailty volunteers. This might be explained by an increasingly unstable gut microbiota composition as frailty increases. The number of *Enterobacteriaceae* cells was 6.6×10^7 cells/g (dry weight) in the low-frailty volunteers and 4.7×10^8 cells/g (dry weight) in the high-frailty volunteers ($P = 0.041$). Of the total number of cells, the percentage of *Enterobacteriaceae* was 0.05% in low-frailty volunteers and 0.3% in high-frailty volunteers ($P = 0.041$).

The differences between fecal samples of elderly with low and high frailty may have several explanations. The GFI consists of components that can be expected to correlate with changes in the gut microbiota. A major component in the GFI is physical functioning. Due to low mobility, constipation may occur in volunteers. With altering fecal transit times, microbial niches may shift to different environmental conditions such as loss of contact with mucosa, depletion of energy substrates (29) or the increase of toxic compounds. Therefore it can be expected that some intestinal bacterial groups may increase in number while others may decrease (14). For some groups of bacteria different environmental conditions may result in less active cells yielding lower rRNA contents for fluorescent in situ hybridization measurements.

Another major GFI component is the psychosocial factor. Psychosocial stress has been suggested to be correlated with the gut microbiota (15). Host and gut microbiota form a complex ecosystem and as such, changes in gut microbiota composition may in its turn have implications for the health of the elderly host. Lactobacilli are involved in the stimulation of immune functions, aid in the digestion and/or absorption of food ingredients and minerals, and inhibit growth of exogenous or harmful bacteria (7). Several strains of *Lactobacillus* are mentioned as being beneficial to elderly health upon oral consumption in several studies (14).

Bacteroides is one of the five numerically predominant genera and plays an important role in the colonic ecosystem (26). The major part of polysaccharide digestion that occurs in the large intestine is accounted for by *Bacteroides* spp., which are known to ferment a wide variety of carbohydrates. Alterations in the numbers of such a nutritionally important bacterial group may affect bacterial metabolism and the complex colonic ecosystem of cross-feeding species (3). Colonic strains of *Bacteroides* have also been reported to produce bacteriocins.

In addition, some colonic *Bacteroides* are opportunistic pathogens (26).

F. prausnitzii is another abundant group which consists mostly of strains that are difficult to culture or unculturable and therefore at present the knowledge about this group is limited (4, 32). *F. prausnitzii* plays a role in carbohydrate breakdown and fermentation in the large intestine and is an important producer of butyrate, its main carbohydrate fermentation product. Butyrate plays a role in providing protection against colorectal cancer and ulcerative colitis. *Enterobacteriaceae* are potential pathogens that may cause autogenous infections when host resistance is reduced.

It is difficult to compare our results with other elderly gut microbiota results since the frailty status of the elderly in those studies was not established. The present study could confirm a not-significant reduction in bifidobacteria that has been reported with ageing (14) and an increase in the percentage of the *Lactobacillus*/*Enterococcus* group (23, 13) compared to younger adults. Bartosch et al. (3) characterized bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by real-time PCR. They found a marked reduction of the mean 16S rRNA gene copy numbers of the *Bacteroides*/*Prevotella* group following hospitalization. Additionally, reductions in the total number of bacterial 16S rRNA genes and mean 16S rRNA gene copy numbers of bifidobacteria and *F. prausnitzii* in the hospitalized patients were reported. In contrast to this, the mean 16S rRNA gene copy numbers of enterobacteria and *Enterococcus faecalis* increased in these patients. If low frailty is regarded as analogous to healthy and high frailty to hospitalized, then these results are consistent with our findings.

Our results may have interesting implications. Probe sets may be used as sensitive tools in the analysis of gut microbiota composition in relation to human health and disease. Some groups of bacteria, like lactobacilli, might serve as indicator organisms for health monitoring. It is difficult to interpret the observed differences in bacterial composition and directly link them to frailty components. Whether modulation of gut microbiota composition (14, 20, 33) will have an effect on the development of frailty or components thereof remains to be investigated.

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