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The effect of ageing with and without non-steroidal anti-inflammatory drugs on gastrointestinal microbiology and immunology

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Elderly individuals are more susceptible to gastrointestinal problems such as constipation than young adults. Furthermore, the common use of non-steroidal anti-inflammatory drugs (NSAID) among the elderly is known to further increase such gastrointestinal ailments. To describe the specific changes in elderly, intestinal microbes, their metabolites and immune markers were measured from faecal samples obtained from fifty-five elderly individuals (aged 68–88 years), using either NSAID or not, and fourteen young adults (aged 21–39 years). The faecal DM content increased with age but was significantly lower among the elderly NSAID users. The microbial metabolism was especially influenced by NSAID use and/or ageing, although fewer changes were observed in the composition of the microbial community, whilst the level of aerobes was increased in the elderly and the level of *Clostridium coccoides–Eubacterium rectale* reduced in the elderly NSAID users as compared with young adults. An increase in the concentrations of some branched SCFA and L-lactate but a decrease in some major SCFA concentrations were observed. Evidently, the decreased defecation frequency in the elderly directed colonic fermentation toward an unfavourable microbial metabolism but this was partially offset by the use of NSAID. Irrespective of the use of NSAID, the elderly subjects had significantly lower concentrations of faecal PGE_2 than the young adults, reflecting possibly a reduced immune response. According to the present study more attention should be paid to the development of dietary products that seek to enhance bowel function, saccharolytic fermentation and immune stimulation in the elderly population.

Intestinal bacteria: Immune responses: Ageing: Non-steroidal anti-inflammatory drugs

Ageing is associated with significant changes in gastrointestinal function. Due to changes in gut physiology, immune system reactivity and diet, elderly individuals are more susceptible to gastrointestinal problems and diseases than young adults. Diseases such as peptic ulcer and gastric cancer, irritable bowel syndrome, diverticulosis and colon cancer are relatively common problems of ageing. Constipation, another common concern in the elderly, often results in increased use of laxatives with ageing[1].

Furthermore, the common use of non-steroidal anti-inflammatory drugs (NSAID) among the elderly may affect their intestinal health. NSAID have analgesic, antipyretic and anti-inflammatory effects. The most common adverse effect caused by NSAID is damage to the mucosa in the gastrointestinal tract[2,3]. According to a recent study by Hartikainen *et al.* [4], 70 % of the over-75-year-olds in the city of Kuopio (Finland) take at least one analgesic, of which NSAID are the most commonly used.

During infancy, the intestinal microbiota changes dramatically[5] after which it remains relatively stable during childhood and throughout adult life[6]. However, during old age, it has been observed that changes in the composition of the microbiota occur again. Using culture-based methods, it is generally shown that ageing correlates with a decrease in *Bifidobacterium* levels[7–9]. Using molecular biological methods, such a decrease in total faecal *Bifidobacterium* counts in the elderly has not been observed. In contrast, levels similar to those normally observed in young adults have been detected[9,10]. Other reported changes in the microbiota of seniors are an increase in the numbers of lactobacilli, clostridia and facultative anaerobes[8,11]. These changes have, however, received less attention in recent molecular analyses of the intestinal microbiota of the elderly, and appear to be less pronounced than previously considered[9,10].

Weakened immunity in the elderly has been observed by many studies addressing different arms of immune responses both in animal models and, to a lesser degree, in human studies[12]. In the present study, faecal IgA, TNF-α and PGE_2 were measured. Intestinal IgA has an important role, not only in neutralising intestinal pathogens but also in the maintenance of gut homeostasis[13]. TNF-α mediates the inflammatory responses necessary for successful defence against intracellular infections. Prostaglandins have an essential role in mucosal functions. They inhibit gastric acid secretion, stimulate bicarbonate and mucus secretion and are involved in the regulation of motility and epithelial barrier functions[14]. PGE_2 has been found to be able to induce proliferative responses in colonocytes as well as in colon cancer

Abbreviation: NSAID, non-steroidal anti-inflammatory drugs.

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cells[15,16]. Prostaglandins also perform an important role in the regulation of inflammatory responses[17].

The aim of the present study was to compare gut function, immunological status and the key faecal microbiota components between the elderly subjects, both users and non-users of NSAID, and young adults. This information can aid in the development of products suitable for the different elderly target groups that suffer from gastrointestinal complications.

Materials and methods

Study subjects

The study was carried out in two stages. The first part consisted of elderly subjects living in a nursing home and young adults living at a boarding school. The second stage consisted of elderly individuals not living in a nursing home. In total, twenty-six elderly users of NSAID, twenty-nine elderly non-users of NSAID and fourteen young adults took part in the study in the cities of Kuopio and Helsinki (both in Finland).

The elderly were defined as NSAID users when they used the medication three or more times per week. The NSAID used included indomethacin, ibuprofen, ketoprofen, diclofenac, piroxicam, celecoxib, nimesulid and aspirin. The exclusion criteria were critical illnesses, inflammatory bowel disease, coeliac diseases and major malignancies in the gastrointestinal tract. Subjects using antibiotics during the study or within 2 months before the screening were excluded. The use of probiotic and prebiotic products was not allowed during the study. The inclusion and exclusion criteria were monitored during the run-in period before randomisation. Throughout the sampling both the elderly and the young study subjects followed their habitual diet and most of them used the uniform meal service provided at least once per d.

The study protocols were approved by the Research Ethics Committees of the Hospital Districts of Northern Savo and Helsinki-Uusimaa. The purpose of the study was explained to the participants, and written consent to the study was given by all of the subjects. Information on the demographic data, diseases, current medication and use of probiotics and prebiotics were obtained at the beginning of the study.

Faecal samples

Spot samples of faeces were obtained three times from each subject over 3 consecutive weeks. Subjects were personally instructed to take the faecal samples according to the written protocol. All the equipment and documentation papers were provided to the subjects before sampling. All faecal samples were initially stored at −20°C, transferred to the laboratory within 12 h of defecation and stored at −70°C until analysed.

Physico-chemical analyses

The concentration of ammonia in the faecal samples was analysed with an enzymic test kit (Boehringer Mannheim/R-Biopharm catalogue no. 11 112 732 035; R-Biopharm AG, Darmstadt, Germany). The pH of the faecal samples was measured with a Mettler Toledo InLab 427 (Mettler Toledo Inc., Columbus, OH, USA) electrode according to protocol 104A:1984 of the International Dairy Federation. For DM determination, about 1 g of faecal sample was weighed and dried (105°C for 16 to 18 h), cooled down in a desiccator to room temperature and reweighed.

The concentrations of acetic, propionic, butyric, valeric, isovaleric, capronic and isocapronic acid were determined by capillary GC (HP-6890; Hewlett Packard, Palo Alto, CA, USA) by adapting the method described by Hoverstad *et al.*[18] whereby faecal samples were diluted 1:10 with 0·1 M-potassium phosphate buffer (pH 7·0), homogenised for 1 min with a Stomacher blender (Seward, Thetford, Norfolk, UK) and filtered. The filtrate was sonicated for 1 min at 4°C and centrifuged (450 ***g***; 15 min; 4°C). The samples were stored at −20°C. For analysis, a 4 ml sample of faecal suspension was mixed with an internal standard (2-ethyl butyric acid; 5 mg/100 g), 250 μl sulfuric acid *pro analysi*, 10 ml diethyl ether and approximately 3·5 g $NaSO_4$ and sonicated for 5 min. The 4 ml sample was then transferred into a heptane-regenerated solid-phase extraction Varian NH2 cartridge (Varian Inc., Palo Alto, CA, USA). The cartridge was then washed with 5 ml isopropanol–heptane mixture (1:3, v/v). The sample was ready for GC after elution with 5 ml of 3 % HCOOH in diethyl ether.

To analyse the D- and L-lactic acid concentrations, 1 g of the sample was mixed with 6 ml water. The sample was shaken thoroughly and centrifuged at 7000 rpm for 10 min and 0·4 ml of the supernatant fraction was transferred into a 1·5 ml microfuge tube. To precipitate protein, 0·4 ml of 0·4 M-$HClO_4$ was added. The sample was kept on ice for 5 min and centrifuged (16 000 ***g***; 5 min). To neutralise the sample, 0·6 ml of the supernatant fraction was transferred into a new 1·5 ml microfuge tube and 70 μl 2 M-KOH was added. The sample was kept on ice for 5 min and centrifuged (16 000 ***g***; 5 min). D- and L-Lactic acid were then analysed enzymically (R-Biopharm E1112821; Darmstadt, Germany) from the supernatant fraction.

Microbial analyses

The presence and quantity of yeast, anaerobic and aerobic bacteria were analysed by cultivation methods. Faecal samples were thawed in an anaerobic chamber and diluted to a ratio of 1:10 with Wilkins–Chalgren medium (Oxoid Ltd, Basingstoke, Hants, UK) in plastic bags. The mixture was homogenised, a tenfold dilution series was made and the appropriate dilutions were plated on agar. Aerobic bacteria were cultivated for 3 d and the anaerobic bacteria anaerobically for 5 d on brain heart infusion agar (Oxoid) at 37°C. The yeasts were grown on yeast extract–glucose–chloramphenicol agar (YGC agar; International Dairy Federation standard 94B:1990) at 25°C for 5 d.

The total bacteria cell counts in faecal samples were determined by flow cytometry (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA) as previously described[19]. The bacterial fractions were recovered by suspending faecal samples in a buffer, followed by centrifugation and washing. The cell samples were diluted, fixed and stained with a fluorescent nucleic acid-binding dye (SYTO 24; Molecular Probes, Leiden, The Netherlands). The results are expressed as the quantity of bacteria/g wet faeces.

DNA was extracted from the recovered bacteria using the method described by Apajalahti *et al.* [20] whereby bacteria were subjected to five freeze–thaw cycles and subsequently treated with lysozyme and proteinase K. The recovered bacterial DNA was used to quantify the total bifidobacteria, as described by Gueimonde *et al.* [21]. The primers and probe for the detection of sulfate-reducing bacteria were designed using the adenosine-5′-phosphosulfate reductase α gene of *Desulfovibrio intestinalis*, and for the detection of *Clostridium perfringens* by using the α-toxin gene with PrimerExpress software (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR was performed using 1 μg of isolated bacterial DNA with primers and probes specific for *C. perfringens* and sulfate-reducing bacteria (Table 1). A 25 μl amplification reaction consisted of 1 × TaqMan universal master mix (Applied Biosystems) with 300 nM of both reverse and forward primers, and 200 nM TaqMan probe (Applied Biosystems). All assays were run on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using the instrument's default settings for thermal cycling and fluorescence measurements. For standard curves bacterial genomic DNA from *C. perfringens* (ATCC 13124) and in-house isolated genomic DNA from *D. intestinalis* (DSM 11275) were applied. Based on the genomic sizes, the weight of one copy of each the *C. perfringens* and *D. intestinalis* genome was calculated, and the amount of chromosomes in 1 pg was estimated. Standard amplification curves were constructed by using 1, 10, 100 and 1000 pg of bacterial genomic DNA as a template. The results are expressed as the quantity of bacteria/g faeces (fresh weight).

For fluorescence *in situ* hybridisation (FISH) analysis faecal samples were diluted to the ratio of 1:10 with PBS and homogenised in filter bags for 2 min with a Stomacher blender. A quantity of 5 ml of the suspension was transferred into a tube containing glass beads (diameter 2 mm). The sample was mixed for 30 s with a test-tube mixer and centrifuged for 2 min (250 ***g***) to remove the coarse material. Of the supernatant fraction, 375 μl were mixed to a ratio of 1:4 with fresh 4 % paraformaldehyde (pH 7·2) and fixed overnight at 4°C. The fixed bacterial cells were collected (13 000 ***g***; 5 min), washed twice with PBS and re-suspended, first in 150 μl PBS, after which an equal volume of 94 % ethanol was added. The suspension was then mixed and stored at −20°C until used. The cell suspension was diluted to a ratio of 1:20 with 0·9 M-NaCl-20 mM-tri(hydroxymethyl)-aminomethane–0·1 % SDS (pH 7·2) hybridisation buffer at 50°C and a fluorescent probe was added to the final concentration of 5 ng/ml. Hybridisation was carried out overnight at 50°C, then the washing and filtering were performed according to Franks *et al.* [22]. The filters were mounted on a slide with AntiFade reagent (Molecular Probes) and covered with glass. The cells were counted visually with an epifluorescence microscope, examining at least fifteen fields in every sample. The oligonucleotide probes used in the FISH analysis were Cy3-labelled Bfra602 described for the *Bacillus fragilis* group, and Bdis656 for the *B. distasonis* group[22], Bif164 for the bifidobacteria[23], Erec482 for the *C. coccoides–Eubacterium rectale* group, Chis150 for the *C. histolyticum* group[22], Lab158 for the lactobacilli and enterococci[24] and Fprau0645 for the *Faecalibacterium prausnitzii* [25].

Table 1. Primers and probes used for the quantitative determination of *Clostridium perfringens* and *Desulfovibrio intestinalis*

Primer name	Sequence 5′ → 3′	Label
D. intestinalis		
apsA1F	GGC GCT GAA ATG ACC ATG AT	
apsA1R	GGC CGT AAC CGT CCT TGA A	
apsA1Probe	TTC GTG CCC GCC CG	5′FAM
C. perfringens		
CPTAFW	TTT GGA GAT ATA GAT ACT CCA TAT CAT CCT	
CPTARV	GTG CAA AAG TCT CAA ACT TAA CAT GTC	
CPTAPRO	TAA TGT TAC TGC CGT TGA T	5′FAM

Immunological analyses

The concentrations of IgA, TNF-α and PGE_2 were measured from the soluble fraction of the faeces. The frozen samples were thawed and extracted with bovine serum albumin as described previously[26] and stored at −20°C before analysis. Concentrations of IgA, TNF-α and PGE_2 were determined using ELISA according to the respective manufacturer's instructions (E80-102; Bethyl Laboratories, Inc., Montgomery, TX, USA; Biosource Europe S.A., Nivelles, Belgium; Cayman Chemical Company, Ltd, Ann Arbor, MI, USA) and the results were expressed as μg or pg/g faeces (fresh weight).

Statistical analysis

The summary statistics (mean, minimum, maximum) were separately calculated for each group. After performing the initial analysis, the effects of NSAID and age were studied separately by comparing the mean levels of parameters in the various groups using the *t* test.

The relationships of the correlated variables were studied using principal component analysis. The eigenvalues were calculated, and the percentages of total variation explained by them and the first ten principal components. The statistical analysis was performed using SAS statistical analysis software (version 9; SAS Institute, Cary, NC, USA).

Results

The baseline characteristics, defecation frequency, quality of faeces and use of laxatives of the participants are presented in Table 2. The mean age of the elderly subjects was 75·6 years (without NSAID) and 77·8 years (with NSAID), compared with the mean age of 28·1 years for the young adults. The self-reported defecation frequency was highest in young adults (1·6 times/d) decreasing in order in the elderly NSAID users (1·1) and non-NSAID users (0·6). Hard faeces were reported by 38 % and laxatives were used by 35 % of the elderly but by none of the young adults. The results of the physico-chemical, microbiological and immunological measurements are presented in Table 3. In general, the most important variables, explaining 77 % of the variation in the present study, were, in descending order, the use of NSAID, age, IgA, PGE_2, TNF-α, DM, pH, ammonia and acetic acid (data not shown). Since NSAID use and age were the variables

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Table 2. Baseline characteristics of the sixty-nine participants of the three groups

	Elderly, no NSAID (*n* 26)	Elderly, with NSAID (*n* 29)	Young adults (*n* 14)
Men (*n*)	10	7	9
Women (*n*)	16	22	5
Age (years)			
Mean	75·6	77·8	28·1
SD	4·9	5·0	6·5
Range	68–84	70–88	21–39
Defecation frequency (times/d)			
Mean	0·6	1·1	1·6
SD	0·3	1·0	0·7
Quality of faeces			
Very loose	1	–	–
Loose	1	3	1
Normal	10	16	13
Hard	11	10	–
Very hard	–	–	–
Laxative users (*n*)	11	8	–

NSAID, non-steroidal anti-inflammatory drugs.

that explained most of the variation in the study, the effects of NSAID use and age are considered in more detail below.

Effect of use of non-steroidal anti-inflammatory drugs

The effect of NSAID use was first studied by comparing the elderly NSAID users with the elderly without NSAID, and second with the young subjects, none of whom used NSAID (Table 3; groups 1 *v.* 2, and groups 2 *v.* 3).

The mean proportion of faecal DM and the concentrations of isobutyrate, isovaleriate and L-lactate were significantly lower in the elderly with NSAID than in the elderly non-NSAID users ($P<0·001$, $P<0·01$, $P<0·01$; $P<0·05$, respectively). The use of NSAID did not have an effect on the measured faecal immune parameters or cause any significant changes in microbiota when compared with the elderly non-NSAID users (Table 3; groups 1 *v.* 2).

The elderly NSAID users had significantly lower levels of total SCFA ($P<0·05$), butyric acid ($P<0·05$), propionic acid ($P<0·01$), isocapronic acid ($P<0·0001$), *C. coccoides–E. rectale* bacteria ($P<0·05$) and PGE_2 ($P<0·01$) when compared with the young adults (Table 3; groups 2 *v.* 3). In addition, a trend for lower acetic acid ($P=0·0542$) and valeric acid ($P=0·0675$) concentrations and higher numbers of bifidobacteria ($P=0·0657$) were detected in the elderly NSAID users when compared with the young adults.

The effect of age

The effect of age was assessed by comparing the elderly non-NSAID users with the young adults (Table 3; groups 1 *v.* 3). The elderly non-NSAID users exhibited higher proportions of faecal DM ($P<0·01$) and higher numbers of aerobic bacteria than the young adults ($P<0·05$). Moreover, they had higher concentrations of isobutyric acid ($P<0·05$), isovaleric acid ($P<0·05$), isocapronic acid ($P<0·0001$) and L-lactate ($P<0·05$) than the young adults. Furthermore the elderly non-NSAID users had lower concentrations of propionic acid ($P<0·05$) and PGE_2 ($P<0·05$) than the young adults.

The combined effects of non-steroidal anti-inflammatory drugs and age

A comparison of the elderly subjects, irrespective of the use of NSAID, with the young adults revealed significantly higher quantities of aerobic bacteria ($P<0·05$) and higher concentrations of isocapronic acid ($P<0·0001$), but lower concentrations of PGE_2 ($P<0·01$) and propionic acid ($P<0·001$) in the elderly (Table 3; groups 1 and 2 *v.* 3). Moreover, there were trends for higher quantities of *Lactobacillus/Enterococcus* ($P=0·0585$) and lower amounts of *C. coccoides–E. rectale* ($P=0·0676$) and total bacteria ($P=0·0686$), lower concentrations of acetic acid ($P=0·065$), butyric acid ($P=0·075$) and the total SCFA ($P=0·0599$) in the elderly, when compared with the young adults.

Discussion

Constipation is known to be a common problem among the elderly, as evidenced by the high rate of laxative use[1]. In this study population, 35 % of the elderly used laxatives but none of the young adults. Generally the most common defecation frequency is once daily[27]. According to this survey the defecation frequency was highest in young adults then decreasing in order in the elderly with NSAID and then without NSAID use. The impaired bowel habit in non-NSAID users supports our finding that non-NSAID users had drier faeces compared with NSAID users. The use of NSAID is known to be associated with gastric damage but not with constipation, which is a typical problem with opioids[28].

The self-reported stool type changed from normal to hard with advancing age: young adults reported a normal stool type but over one-third of elderly reported hard faeces. This is also consistent with the occurrence of increasing DM content of faeces associated with ageing. The faecal DM also correlated negatively to the main SCFA (acetic, propionic and butyric acid; R −0·391, $P=0·001$; R −0·335, $P=0·006$; R −0·304, $P=0·013$, respectively) and the total SCFA (R −0·313, $P=0·01$) concentrations, giving support to the hypothesis that accelerated intestinal transit is associated with increased colonic SCFA concentration, as indicated by Lewis & Heaton[29]. The non-NSAID users in particular had altered physical bowel functioning, i.e. significantly higher faecal DM and decreased defecation frequency, which could lead some bacterial groups to decrease or become less active. Such consequences could be caused by a loss of contact by microbes with intestinal mucosa, a lack of energy, or by increased concentrations of toxic substances[30]. The NSAID users had more moist faeces than the non-NSAID users, but compared with the young subjects, still lower *C. coccoides–E. rectale* counts and reduced SCFA production.

Clear differences in colonic fermentation were demonstrated: NSAID use and/or age decreased concentrations of SCFA (acetate, propionate, butyrate) but increased concentrations of branched-chain fatty acids and L-lactic acid. Decreased concentrations of the major SCFA may indicate decreased availability of butyrate to the mucosa. Butyrate is the main source of energy in the colonic mucosa[31] and thus any dietary change that can increase its availability in the elderly could have important health implications. Branched fatty acids are themselves not

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Table 3. The comparison between elderly non-steroidal anti-inflammatory drug (NSAID) users and non-users and young adults (per g or kg faecal fresh weight (fw))
(Mean, minimum (Min) and maximum (Max) values)

	Elderly non-NSAID users (group 1)				Elderly NSAID users (group 2)				Young adults (group 3)				Significance of difference between groups: *P*			
Variable	*n*	Mean	Min	Max	*n*	Mean	Min	Max	*n*	Mean	Min	Max	1 *v.* 2	1 *v.* 3	2 *v.* 3	1 and 2 *v.* 3
Physico-chemical																
DM (%)	25	29·42	17·94	42·28	29	22·50	9·34	33·10	13	24·01	18·20	30·80	***	**		
pH	26	7·09	4·69	7·90	28	7·04	6·17	7·69	14	7·13	6·20	8·40				
Ammonia (g/kg fw)	26	0·69	0·21	1·78	28	0·79	0·24	1·68	14	0·61	0·32	1·22				
Microbial metabolites																
SCFA (mg/100 g fw)																
Total SCFA	26	488·76	116·66	1079·00	28	418·77	86·24	829·70	13	599·17	365·18	1279·15			*	
Acetic acid	26	229·74	68·97	544·00	28	213·68	41·55	414·63	13	290·99	172·71	672·62				
Butyric acid	26	100·03	9·98	295·00	28	85·17	0·00	265·00	13	133·35	56·22	292·09			*	
Propionic acid	26	76·06	13·73	191·00	28	66·57	13·87	138·09	13	114·38	61·05	251·89		*	**	***
Isovaleric acid†	26	34·80	5·65	112·76	28	19·95	0·50	36·00	13	22·75	9·34	34·26	**	*		
Isobutyric acid†	26	20·57	<1	64·86	28	11·09	<1	22·00	13	20·07	<1	43·65	**	*		
Valeric acid†	26	20·44	<1	61·37	28	13·92	<1	38·70	13	13·54	<1	22·61				
Capronic acid†	25	7·65	<1	42·00	28	8·61	<1	38·00	13	4·06	<1	16·91				
Isocapronic acid†	26	0·29	<1	0·50	28	0·34	<1	0·50	13	<1	<1	<1		****	****	****
L-Lactic acid (μmol/kg fw)‡	24	1084·00	<10	9992·00	29	161·82	<10	1552·66	14	231·35	<10	642·84	*	*		
D-Lactic acid (μmol/kg fw)‡	24	654·76	<10	9180·00	29	376·55	<10	1263·30	14	341·65	40·11	797·86				
Microbes (log_{10} cells/g fw)																
Total counts	26	11·18	10·61	11·58	27	11·18	10·50	11·77	14	11·32	10·78	11·62				
Anaerobes (log_{10} cfu/g fw)	26	9·29	8·15	10·11	29	9·22	8·04	10·23	14	9·11	8·20	9·83				
Aerobes (log_{10} cfu/g fw)	26	7·26	5·00	8·91	29	7·19	4·67	9·15	14	6·61	5·30	7·92		*		*
Yeasts (log_{10} cfu/g fw)§	26	2·12	<2	4·48	29	2·57	<2	6·63	14	2·46	<2	4·72				
Lactobacillus/*Enterococcus*	26	8·28	7·41	9·33	29	8·25	7·36	9·32	14	7·94	7·06	9·40				
Clostridium coccoides–*Eubacterium rectale*	26	10·19	8·88	11·02	28	10·13	9·29	10·70	14	10·31	10·01	10·71			*	
Faecalibacterium prausnitzii	26	8·92	5·73	10·07	29	9·13	2·85	10·05	14	8·72	5·40	9·99				
C. histolyticum	26	6·81	3·00	8·08	29	7·10	5·40	9·87	14	7·09	6·30	7·60				
Bacteroides	26	9·59	8·59	10·37	29	9·72	8·23	10·45	14	9·42	8·54	10·33				
Bifidobacterium	26	9·59	5·88	10·31	27	9·83	7·67	10·44	14	9·54	6·10	10·05				
C. perfringens§	25	6·44	<2	7·83	27	6·70	<2	8·05	14	4·97	3·80	5·53				
Sulfate reducers§	25	8·06	2·47	9·09	26	7·82	<2	9·06	10	7·31	3·63	8·25				
Immunological																
IgA (μg/g fw)	26	141·53	7·58	657·78	28	123·47	8·00	515·00	14	153·47	21·51	585·13				
PGE_2 (pg/g fw)	26	538·88	142·00	1111·00	28	530·46	233·00	1203·00	14	1084·50	106·00	2674·00		*	**	**
TNF-α (pg/g fw)	26	1·27	0·02	5·09	28	2·56	0·02	25·60	14	3·00	0·02	13·28				

cfu, Colony-forming units.
*$P<0·05$, **$P<0·01$, ***$P<0·001$, ****$P<0·0001$ (pairwise *t* test).
† Detection limit for isovaleric acid, isobutyric acid, valeric acid, capronic acid and isocapronic acid was 1 mg/100 g fw.
‡ Detection limit for L- and D-lactic acid was 10 μmol/kg fw.
§ Detection limit for yeasts was 2 log_{10} cfu/g and for *C. perfringens* and sulfate reducers was 2 log_{10} cells/g.

toxic, but are metabolites produced during protein fermentation (for a review, see Blachier *et al.* [32]), which are indicating the production of other potentially toxic metabolites, such as indolic and phenolic compounds, which have been linked with long-term adverse health effects such as the development of colon cancer[33].

In the present study, relatively few significant differences in the microbiota of elderly subjects were found when compared with the young. The levels of aerobic bacteria were higher in the elderly non-NSAID users, which is consistent with observations by Woodmansey *et al.* [34]. Although the levels of *Lactobacillus/Enterococcus* have previously been reported to have increased in the elderly[11,35,36], conflicting results have also been published[9,34]. Furthermore, a recent study indicated that age-related differences in *Lactobacillus/Enterococcus* levels could be country specific[36]. There does not seem to be a consistent trend regarding the changes in the levels of these taxonomic groups in the elderly. The present study indicated a trend for increased *Lactobacillus/Enterococcus* levels in the elderly compared with the young. Although animal studies have indicated increased small-intestinal colonisation with enterococci when treated with the NSAID indomethacin[37], we did not observe changes in the levels of *Lactobacillus/Enterococcus* in the NSAID users group. This may relate to methodology which did not distinguish between enterococci and lactobacilli, or the fact that in the present study we analysed faecal rather then small-intestinal microbiota. Consistent with the findings of others[30,35,36] in the combined group of elderly NSAID users and non-users the present study found that the *C. coccoides–E. rectale* group, which is among the most numerous groups in adults, had decreased significantly. The commonly reported often-mentioned decrease in faecal bifidobacteria in the elderly[11,38] could not be demonstrated in the present study. A similar difference in results had also been reported earlier by Bartosch *et al.* [10] and may partly be explained by the differing use of culture-based techniques[11] or molecular-based techniques by Bartosch *et al.* [10] and the present study.

In the elderly faecal PGE_2 levels were significantly decreased to approximately half the levels of those in the young adults irrespective of the use of NSAID. The functions of PGE_2 in the large intestine are not fully understood but it has been proposed that ageing in general is associated with the development of a cancer-promoting microenvironment characterised by chronically elevated levels of PGE_2[39]. Increased concentrations of PGE_2 have been demonstrated previously *in vitro* in macrophages and in bronchoalveolar lavages obtained from elderly subjects[40,41]. In the present study, however, faecal concentrations of PGE_2 were clearly decreased in the elderly. This supports previous findings suggesting that ageing decreases prostaglandin concentrations, and thus mucosal protection, in the stomach and duodenum[42].

The volunteers did not exhibit acute-type inflammation in the large intestine, as judged by the low faecal TNF-α concentrations in all test groups, and therefore no indication of increased background inflammation was found[43].

Interestingly, the faecal concentrations of IgA were similar in all test groups. Although it has previously been suggested that cellular-type immune responses are especially compromised by advanced age, and furthermore that responses are deviated towards humoral immunity, characterised by an increase in serum, bronchoalveolar lavage, and saliva IgA and IgG concentrations[41,44,45], ageing does not appear to influence the intestinal IgA concentrations[44]. The difference between the intestine and other mucosal sites and serum may be explained by the differing types of regulation of immune responses at the different sites, or by the fact that different immunoglobulin subclasses may change differentially with age[46,47]. Furthermore, measurement of specific IgA concentrations after oral vaccination would give a more direct measure of the mucosal responsiveness[48].

In summary, the most significant change in gut content with ageing was the increase in faecal DM and, especially in the elderly NSAID users, a decrease in the activity of microbial metabolism, such as butyrate concentrations. In contrast, an increase in the concentrations of branched fatty acids was detected in both the elderly NSAID users and non-users. Evidently, the decreasing bowel function in the elderly contributes to the changes in colonic fermentation towards an unfavourable putrefactive microbial metabolism. Dietary products that can enhance bowel function and saccharolytic fermentation would be especially beneficial for the elderly. In contrast to systemic and respiratory immune responses, ageing results in a different type of immune profile in the intestine, which is characterised by no change in IgA concentrations and a clear decrease in PGE_2. Therefore, dietary intervention that can normalise the faecal PGE_2 concentrations in the elderly will probably reflect an improvement in the protection of the mucosa.

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