Fasting hypochlorhydria with Gram positive gastric flora is highly prevalent in healthy old people

E Husebye, V Skar, T Høverstad, K Melby

Abstract
Fifteen healthy old people mean age 84 years (range 80–91 years), were examined to assess the effect of advanced age on the microecology of the upper gastrointestinal tract. Twelve of 15 (80%) were hypochlorhydric with pH 6-6 (0-3) (mean (SEM)) and a mean bacterial count of 10³ colony forming units (CFU) per ml (range 10⁰–10⁶) in fasting gastric aspirate. Normochlorhydric subjects had low counts (<10¹ CFU/ml). The microbial flora was dominated by viridans streptococci, coagulase negative staphylococci, and Haemophilus sp. Only one subject harboured significant concentrations of Gram negative bacilli with Escherichia coli (10⁴ CFU/ml) and Klebsiella (10⁶). Strict anaerobes were not found. The total concentration of short chain fatty acids in gastric aspirate was 10-6 (2-9) mmol/l (mean (SEM)). Absence of significant, intraluminal fermentation of xylose to CO₂, was shown by the "C-ḍ Xylose breath test, and ambulatory manometry showed preserved fasting motility pattern of the small intestine. Serum immunoglobulins were normal. Advanced age is accompanied by fasting hypochlorhydria and colonisation with mainly Gram positive flora in the upper gut. Other factors than old age and fasting hypochlorhydria are required for colonisation with Gram negative bacilli.

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Gastric juice from healthy fasted subjects harbours ≤10¹ colony forming units (CFU) per ml, and aspirate from the proximal small intestine contains ≤10⁶, provided that gastric acidity is preserved. Gram positive organisms dominate, with lactobacilli, staphylococci, and fungi as the most common species. Entero-bacteria are absent or present in low counts, but there may be anaerobes of oral origin.

Host defence mechanisms are partly responsible for this selection and control of microbial growth. Gastric juice represents a barrier to microorganisms in saliva and ingested foods, mainly by the bactericidal activity of hydrochloric acid. Accordingly, fasting gastric juice pH>3-4 is accompanied by increasing concentrations of microbes in the stomach, and proximal small intestine. In the absence of gastric acid, individuals usually harbour the same bacterial species, in similar concentrations, in aspirates from the stomach and proximal small intestine. A typical qualitative pattern of microbes accompanying hypochlorhydria has not been found. Colonise flora, defined as microbial species usually confined to the lower gastrointestinal tract in healthy individuals, is quite common after Biroth II partial gastrectomy, present in about every other patient with pernicious anaemia and hypogammaglobulinaemia with achlorhydria. These clinical conditions exhibit rather complex and heterogeneous pathophysiology that may affect intestinal flora through changes in defence mechanisms other than gastric acidity. The importance of changed motility for microbial colonisation has been shown after gastric surgery and drainage is probably just as important in other clinical conditions with hypochlorhydria.

Ageing is accompanied by an increasing prevalence of gastritis and declining output of gastric acid, whereas contradictory findings have been reported for gastric emptying. It is currently believed that absence of gastric acid can lead to microbial growth, including that of Gram negative bacilli, in the upper gut. As the clinical consequences of bacterial overgrowth are associated with presence of these species, and anaerobes in particular, age related hypochlorhydria may represent a pathogenetic factor in the elderly. Reports of bacterial overgrowth without a blind loop in the elderly have brought this issue to current interest, and the need for studies on intestinal flora and factors regulating microbial growth in ageing has recently been emphasised.

This prospective study examined gastric pH, microbial flora, and metabolic markers in the upper gastrointestinal tract of healthy old (>80 years) people. Concomitant analyses of small intestinal motility and serum immunoglobulins are presented to illustrate the relationship between changes in proximal intestinal flora and major host defence mechanisms.

Methods

SUBJECTS
Fifteen healthy volunteers with a mean age of 84 years (range 80–91) years took part in the study. There were seven men (mean 84 years) and eight women (83 years). The mean weight was 72 kg (range 62–80 kg) for men and 56 kg (range 43–63 kg) for women. These subjects were selected from a random sample of individuals above 75 years carefully examined to determine the prevalence of dementia in the general population. People without signs of dementia, who looked lively and in good clinical condition and who had no diseases or symptoms that interfered significantly with daily activities or normal functions were invited to participate.

The entrance criteria included absence of gastrointestinal disorders; previous gastric, biliary, or small intestinal surgery; and endocrine, systemic, neuromuscular, liver, or kidney
months as means and 95% confidence intervals (CI) with the number of individuals with values below (L) and above (H) the reference limits in brackets: haemoglobin 14·1 g/100 ml (13·6–14·7 g/100 ml); whole blood folic acid 437 nmol/l (334–540 nmol/l) (1H); serum vitamin B12 238 pmol/l (159–318 pmol/l) (3L). Intrinsic factor antibodies were not detected, while low, unspecific titres of antibodies against parietal cells were present in four subjects. Further details regarding clinical data and laboratory tests have been given elsewhere. All subjects gave their written informed consent, and the study was approved by the ethics committee for Health Region 1 in Norway.

Sixteen asymptomatic healthy laboratory workers with a mean age of 43 years (range 32–68 years) served as controls for the 14C-d Xylose breath test. Their mean weights were 70 kg (range 55–83 kg), their mean height was 173 cm (range 160–186 cm) and their mean body mass index was 23 kg/m² (range 18–30 kg/m²). The presence of bile colour or vomiting did not correlate with the pH level or difference. The average of the two measurements in individuals with pH less than four (24 outpatients), was pH 1·87 (0·54) (mean (SD)). Accordingly, the cut off level was set at pH 3 (mean ±2SD). The terms low gastric pH and fasting normochlorhydria are used synonymously for pH ≤3 in fasting gastric aspirate, and fasting hypochlorhydria for pH >3.

**GASTRIC ACIDITY**

The pH in aspirates was measured by glass electrode (pHm 62 Radiometer, Copenhagen) within two hours, after adjustment with standard buffers.

Gastric intubation and aspiration induce duodenogastric reflux. A methodological study was undertaken to estimate approximately the magnitude of this bias and to establish the reliability of pH measurement in gastric aspirates. Twenty nine consecutive outpatients referred for gastroscopy, who had had no previous gastric surgery or taken medication that inhibits the secretion of gastric acid, were examined (14 men and 15 women, aged mean 53 years (range 18 to 84 years)). Five ml of gastric juice were aspirated through an internal sterile polyethylene catheter after the endoscope entered the stomach. After appropriate procedures, aspiration was repeated in the same way but with a new catheter before removing the endoscope. In the meantime vomiting was noted, as was the colour of the gastric juice. Good reproducibility was found between the two samples (Fig. 1). There was a slight increase in pH of 0·22 (0·02; 0·42) (mean 95% CIs) (range -0·99 to 1·39). The presence of bile colour or vomiting did not correlate with the pH level or difference. The average of the two measurements in individuals with pH less than four (24 outpatients), was pH 1·87 (0·54) (mean (SD)). Accordingly, the cut off level was set at pH 3 (mean ±2SD). The terms low gastric pH and fasting normochlorhydria are used synonymously for pH ≤3 in fasting gastric aspirate, and fasting hypochlorhydria for pH >3.

**BACTERIOLOGICAL METHODS**

One ml of the aspirate was added to a sterile tube with 9 ml prereduced thiogluconate broth and 1% glucose. A series of 10 fold dilutions was made with the same broth. Sterile Durham tubes (1·8 ml) were placed upside down within the broth tubes to detect the production of gas. Anaerobiosis was secured by the pyrogalol method, and finally, the tubes were closed by sterile airtight caps and incubated at 37°C for 48 hours. From the first tube, 0·001 ml was inoculated onto blood agar, lactose-bromthymol blue agar, and chocolate agar plates. In addition, 0·01 ml was inoculated onto chocolate agar plate. These plates were incubated at 37°C in a moist 5% CO₂ atmosphere. After 48 hours, inoculation was performed from the first and last tubes with visible growth onto blood agar, lactose-bromthymol blue agar, and mannitol salt agar plates for aerobic growth. Two blood agar plates, to one of which had been added gentamicin 10 mg/l, were incubated at 37°C in anaerobic jars (BBL GasPak Plus, Becton Dickinson & Co, Cockeysville, MD, USA) for 48 hours, while aerobic plates were inspected after 24 hours. Identification was performed according to conventional methods.

Visible bubbles of gas in the Durham tubes were noted, and the gas ratio was calculated as log₁₀ to the concentration of microbes corresponding to the last tube with visible gas, divided by log₁₀ to the total concentration of microbes. The term COL flora was used if enterobacteriaceae, strict anaerobes of the bacteroides fragilis group, or Clostridium sp occurred in counts >10⁷ per ml, and COL in brackets if counts were <10⁴. Mainly Gram positive flora without the above mentioned COL species were denoted.
Gastric flora and pH in healthy old people

**GASTRIC MICROBIAL FLORA**

The total bacterial count was median 10^5 CFU/ml; mean 10^4 (range 10^3-10^5) in hypochlorhydric individuals, and ≤10^4 in the normochlorhydric subjects (Table, Fig 2). Fifty five per cent of the variation in the total bacterial count could be explained by gastric pH (r=0.74, p<0.005). A distinct pattern of microbial flora was present in the hypochlorhydric subjects (Table, Fig 3) with microbes belonging to the indigenous upper respiratory tract flora, mainly facultative Gram positives. Viridans streptococci was the dominant species, and coagulase negative staphylococci were present.

**BLOOD SAMPLES**

Gastrin was measured by radioimmunossay (Becton Dickinson & Co, New York). Electrophoresis of plasma proteins with quantification of immunoglobulins and the blood tests for the old people were analysed at Department for Clinical Chemistry, Ullevål Hospital according to standard procedures.

**STATISTICS**

Data are presented as mean and (SEM) unless otherwise stated. Means are compared by Student's t test for unpaired samples. Furthermore, Student’s matched pair test and correlation techniques are applied. The reliability of pH in gastric aspirates is presented by the relation (pH2-pH1)/[pH1+pH2]/2, and judged by testing the difference between the two measurements.

**Results**

**GASTRIC ACIDITY AND SERUM GASTRIN**

Twelve of the 15 (80%) healthy old people were hypochlorhydric, with pH 6.6 (0.3) in fasting gastric juice (Fig 2). The 95% CIs for the prevalence of fasting hypochlorhydria were 60%, 100%. Serum gastrin concentration were 88 (31) pmol/l (Table); 105 pmol/l (range 8 to 400 pmol/l) in hypochlorhydric individuals (n=12) and 21 pmol/l (range 12 to 35 pmol/l) in the low pH group (n=3).

**SMALL INTESTINE MOTILITY**

Digital ambulatory manometry was undertaken as previously described by Husebye et al, and the results have been presented in detail elsewhere. During fasting, abnormally migrating bands of regular phasic contractions (phase III) recur at intervals, intercepted by periods of silence (phase I), and irregular contractions (phase II). The cumulated phase III index is the sum of the duration (in minutes) of all phase III activities recorded after the intake of a standardised meal of 1700 kJ at 6 pm until 7.25 am the next day.

**Microbial flora, pH, and short chain fatty acid (SCFA) concentrations in fasting gastric juice, and IgA and gastrin in serum of healthy old people**

<table>
<thead>
<tr>
<th>Subject no</th>
<th>pH (log [H+])</th>
<th>Serum gastrin (pmol/l)</th>
<th>Bacterial count (log CFU/ml)</th>
<th>Type of flora</th>
<th>Last dilution with gas</th>
<th>Gas ratio*</th>
<th>Total SCFA (mmol/l)</th>
<th>Serum IgA (g/l)</th>
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<tr>
<td>B1</td>
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<td>0</td>
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<td>18</td>
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<td>0</td>
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<td>9</td>
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<td>4</td>
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<tr>
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<td>73</td>
<td>6</td>
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<td>3.4</td>
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<td>URT</td>
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<td>URT</td>
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<td>0.2</td>
<td>10.6</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**URT**: upper respiratory tract flora; COL: colonic type of flora; () indicates <10^5 colony forming units (CFU) per ml. For detailed definitions cf text. * The ratio: last dilution with gas/bacterial count.
cocc and *Haemophilus* sp were commonly present in counts exceeding $10^5$ CFU/ml. In some subjects, non-haemolytic streptococci, *Moraxella* sp, and diphtheroids were recovered, and two subjects had low counts of *Escherichia coli*, outnumbered by URT flora. One individual had COL flora in gastric aspirate with *E. coli* ($10^{10}$ CFU/ml) and *Klebsiella* ($10^{10}$) as dominating species (B13). Strict anaerobes were not detected.

**GASTRIC MICROBIAL METABOLISM**

Gastric juice contained $10^{-6}$ (2-9) mmol/l SCFA (Table). The relative contributions of C2; C3; ic4; and nc4 were 86-6; 12-0; 0-1; and 1-5% of the total concentration, respectively. Subjects with low gastric pH had rather low concentrations, but no statistically significant correlation was found between SCFA and gastric pH or the total bacterial count. Very high concentrations ($>20$ mmol/l) were present in three individuals with URT flora, but no consistent relationship was found between the levels of SCFA and the type of gastric microflora (Table).

Production of gas from glucose under anaerobic growth conditions occurred in samples from six individuals (Table, Fig 4). The individual with COL flora had considerable gas production (B13), while slight to moderate production was seen in the remaining five. Gas production occurred in the presence of abundant URT flora (B3 and B6), but only in the initial steps of the dilutions, as reflected by low gas ratios in individuals with URT flora (Fig 4). Hypochlorhydric individuals with less than $10^4$ CFU/ml of *E. coli* had a gas ratio of 0-14 (0-20) (mean (SD)), while the two individuals with higher concentrations of *E. coli* (B13 and B14) had gas ratios of 0-9 and 0-8, respectively.

**INTESTINAL MICROBIAL METABOLISM**

Intraluminal bacterial metabolism of xylose to CO$_2$ was sparse, as practically similar levels of expired CO$_2$ were found in the healthy old people and controls, apart from one outlying observation (Fig 5). Cumulated expired CO$_2$ within the first 180 minutes were 8-0 and 6-1% of the dose in these groups respectively, a slight difference of borderline statistical significance (p=0.06). The old person with considerable fermentation of xylose (B13), indicating bacterial overgrowth of the small intestine, had COL flora in gastric juice. In individuals with URT flora (n=13), the expired CO$_2$ value was not related to total bacterial count ($r=0.3$, p=0.4).

**SMALL INTESTINAL MOTILITY**

Ambulatory manometry showed preserved fasting motility with a median of 4 (range 2 to 6) migrating motor complexes during one night. The estimate for the cumulated phase III index was 28-2 minutes (23-2; 33-1 minutes) (95% CIs), and minimum values were 17-5 minutes (B6) and 17-6 minutes (B13).
**Gastric flora and pH in healthy old people**

**SERUM IMMUNOGLOBULINS**

Fourteen subjects had serum IgA concentrations above the lower reference limit of 0.5 g/l, while one individual (B13) had IgA deficiency, as shown in the Table. All individuals had serum IgM (mean 0.9 (range 0.6 to 1.5 g/l)) and serum IgG values (mean 12.5 (range 7.1 to 16.5 g/l)) within the normal reference limits.

**Discussion**

Fasting hypochlorhydria was prevalent in the healthy old people, and was accompanied by colonisation of the proximal gastrointestinal tract by microbes belonging to the indigenous flora of the upper respiratory tract and oropharynx. Principally, the same bacterial species were found that have previously been encountered, in low concentrations, in the proximal gut of normochlorhydric healthy adults.

The most significant finding in the present study was the low frequency of Gram negative bacilli in gastric juice, despite advanced age and fasting hypochlorhydria. Correspondingly, the intraluminal bacterial metabolism of xylose was sparse, which strongly indicates that colonisation with URT flora, even at high counts, does not result in a positive 14C-d Xylose breath test. Patients with bacterial overgrowth usually expire significantly higher amounts of 14C, as indicated by the only subject with high counts of Gram negative bacilli in the stomach (B13).

Production of gas in Durham tubes discloses the ability to ferment glucose anaerobically to H2, CO2, and water vapour in vitro. In faecal samples, this phenomenon was closely related to the presence of enterobacteria. Hence, the absence of gas in the samples from most old people in this study agrees with the results obtained by culture and the 14C-d Xylose breath test. Furthermore, it shows the usefulness of this in vitro test for gastric aspirates, too. Small amounts of gas in some individuals without enterobacteria were probably caused by anaerobic fermentation by facultative species of the URT flora. The gas ratio discriminated well between these microbial sources of gas production, and may be a suitable way of identifying enterobacteria in aspirates from the upper gut.

Gianella et al. examined nine hypochlorhydric subjects with a fasting pH > 6 and found a similar prevalence of Gram negative bacilli as in patients with pernicious anaemia. Information regarding the selection and clinical state of the hypochlorhydric individuals, however, is not given, and it is difficult to decide if they are comparable with our study group. In the present study, 10 individuals had a fasting pH > 6, and Gram negative bacilli in significant counts were not found among these (Table).

It is well established that oropharyngeal colonisation with Gram negative bacilli is associated with the severity of illness. In a study of the pH and microflora of the gastric aspires in normal subjects and those with diarrhoea in Brazil, malnutrition was associated with presence of Gram negative bacilli. The increase in pH in this group was comparable with the findings in a control group of breast fed children with normal flora, and the authors concluded that other factors besides pH regulate the growth of Gram negative bacilli. In achlorhydria accompanying pernicious anaemia, Stockbrugger et al. found significant gastric amounts of Gram negative bacilli in 14 of 22 patients. In these patients, the clinical state may account for oropharyngeal colonisation that induces gastric overgrowth in the absence of the acid barrier. The combination of hypop gammaglobulinaemia and pernicious anaemia is well known, and concurrent immune deficiency may also contribute to the problem. Thus, even if fasting hypochlorhydria is a prerequisite for growth of Gram negative bacilli in the stomach, our data show that such colonisation is by no means a necessary consequence of a raised gastric pH, even at advanced age. The low prevalence of oropharyngeal colonisation with Gram negative bacilli in healthy elderly people (<10%) accords well with our findings.

The hypochlorhydric stomach serves as a reservoir for microbes from saliva and ingested materials that continuously seeds the small intestine. With preserved migrating motor complexes, these microbes are transported aborally before significant multiplication takes place. A close relationship has been shown between intestinal stasis and bacterial overgrowth, and impaired fasting motility of the small intestine has been reported in patients with bacterial overgrowth syndrome. In these disorders,
ascending colonisation with Gram negative bacteria may occur, and preserved small intestinal motility during fasting seems to be an important factor in maintaining the microecological balance of the upper gut. Our healthy old people had migrating motor complexes that swept down the intestine at regular intervals, and the absence of intestinal colonisation with Gram negative bacilli was confirmed by the xylose breath test. The only individual with significant amounts of these bacilli (B13) had selective IgA deficiency combined with a modest increase in the gastric pH and borderline fasting small intestine motility, factors that suggest a multifactorial genesis. Even if patients with selective IgA deficiency usually have normal intestinal flora, the combination of this abnormality with impairment of other defence mechanisms aggravates bacterial colonisation.

The influence of gastric acidity on the gastric microbial flora has also been studied in pharmacological models, using antacids, histamine, blockers, and omeprazole. In healthy subjects, total bacterial counts increased because of colonisation with mainly Gram positive flora, in agreement with our findings. However, the gastric pH levels achieved in these pharmacological studies were lower than in the old people examined in the present study.

The total concentration of gastric SCFAs was 10 to 15 times higher in the hypochlorhydric old people than in healthy young adults examined by the same technique. This probably reflects anaerobic metabolism in the stomach in vivo, and some contribution from swallowed saliva. The wide range of SCFA concentrations, unrelated to the type of flora, indicates that these metabolites are not reliable markers of gastric overgrowth with Gram negative bacilli flora in humans. Borello et al did not find any SCFAs in gastric or jejunal aspirates from patients with hypogammaglobulinaemia and pernicious anaemia for reasons that remain unclear.

It must be emphasised that identification and quantification of microbes in samples from the intestinal tract can be problematical because of the complexity and interactions of the gut flora, and the results are significantly influenced by the culturing technique. The present approach, including strict anaerobiosis, was chosen to yield a high recovery rate of enterobacteria and the bacteroides fragilis group, as tracers of lower gastrointestinal tract flora. The outcome of the C13 Xylose breath test renders false negative cultures, in this respect, less likely. Furthermore, plates were selected to favour growth of Haemophilus sp., in particular, and coagulase negative staphylococci, as tracers of upper respiratory tract flora.

By continuous 24 hour recording, Fimmel et al showed that the average gastric pH is 1-98 (range 1-56 to 2-80), in healthy adults taking four meals. Thus, pH mediated control of microbial growth in the stomach will depend mainly on the fasting levels. The pH of fasting gastric juice is quite stable, despite fluctuations in concert with the gastric component of the migrating motor complex, and also robust, showing little change as a result of the aspiration procedure, as found in the present study. Moreover, Stockbrugger et al have shown a close correlation between bacterial counts and basal, but not peak, acid output. Hence, the pH in fasting aspires reflects more directly the influence of gastric acid on microbial growth.

The criteria for hypochlorhydria varies as no consensus has been established. In fasting gastric aspires, pH 3 and pH 4 are commonly used upper limits for healthy controls. Peterson et al performed gastric aspiration every half hour for 24 hours in eight individuals with preserved secretory capacity. During the night time the pH was mean 1-5 and only two readings showed values above pH 3, and none above pH 4. Accordingly, pH>3 applies as well as criterion for fasting hypochlorhydria, even if vomiting occurs during the sampling procedure.

Our study indicates that about 3 of 4 healthy individuals above 80 years have fasting hypochlorhydria. The prevalence of achlorhydria was 17-5% in a previous Scandinavian study on 348 patients between 70 and 89 years old with gastrointestinal disorders. A progressive increase in prevalence during this age is likely, and individuals with preserved ability to secrete acid in response to maximal stimulation may still have a considerably raised fasting pH. This finding of Feldman et al represents an important objective against peak acid output as a criterion for hypochlorhydria in the present context. Bird et al examined 657 patients above 65 years by the azuresin test and found achlorhydria in 68% and hypochlorhydria in further 14%, in accordance with our findings. Thus, the prevalence of hypochlorhydria is conditioned by the definition, and exceeds the current estimate of 20% considerably, if it is based on a pH in fasting gastric aspires. The present definition of fasting hypochlorhydria may be more relevant in studies on microbial growth.

The gastrin concentrations agree with previous findings in 1405 individuals aged 70, 75, and 79 years, but tend to be lower than those reported by Ganguli et al in hypochlorhydric individuals. The variable gastrin values may reflect the reduced capacity of the elderly oxyntic mucosa to secrete gastrin.

Fasting hypochlorhydria associated with gastric colonisation of microbes belonging to the oro- and nasopharyngeal flora is highly prevalent in healthy old people. These microecological changes may be largely attributed to the change in fasting gastric acidity, as small intestinal motility and immunoglobulins were normal. Old age per se does not result in colonisation with Gram negative bacilli, despite coexisting fasting hypochlorhydria.

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