A study was conducted in healthy elderly living independently in senior housing to assess the impact of a probiotic yoghurt supplement on small intestinal bacterial overgrowth. Twenty-three participants with positive and thirteen participants with negative hydrogen breath test were studied before and after a period of 4 weeks of probiotic yoghurt administration. Intestinal permeability, plasma endotoxin levels, phagocytic activity of leucocytes, cytokine production by monocytes and free radical response of neutrophils were determined. Intestinal permeability was similar for the two groups and was unaffected by probiotic treatment. Both plasma endotoxin levels and the basal phagocytic activity of leucocytes decreased after yoghurt intake in the two groups. Exposure of monocytes and neutrophils ex vivo led to an increased cytokine response and free radical response, respectively. The normalisation of the various cytokine responses was more apparent in the group with positive breath test. In addition, the plasma levels of lipopolysaccharide binding protein and soluble CD14, lipopolysaccharide pattern recognition receptors and surrogate markers of lipopolysaccharide permeability were diminished by the end of the study. In conclusion, probiotic administration in the elderly normalises the response to endotoxin, and modulates activation markers in blood phagocytes, and therefore may help reduce low-grade chronic inflammation.
including malignancies, or had been subjected to any form of gastro-intestinal surgery. The prevalence of SIBO found with the H2 breath test was 15%\(^{18}\).

Twenty-three elderly subjects (eighteen women, five men) with positive breath test (SPH) and thirteen subjects (nine women, four men) with negative breath test (SNH) were included in a follow-up, where the yogurt was administrated (observational study with one treatment group).

Subjects repeated the breath test at the beginning of this study and were asked to consume probiotic yogurt (2 \(\times\) 150 g) \textit{Lactobacillus johnsonii} \textit{Lal} (daily dose of \(10^{10}\) colony-forming units) for 4 weeks with a previous restriction of 2 weeks for yoghurt, kefir or butter milk. During the 4-week consumption of the study subjects were not allowed to consume other fermented products. No other advice with regard to diet was given to the participants. They were allowed to continue routine treatment and medication for stable conditions.

**Hydrogen breath test**

Hydrogen concentration (parts per million; ppm) in exhaled air was determined after ingestion of 50 g glucose as described by Parlesak \textit{et al.} \(^{18}\). A positive result of the H2 test was taken as a rise of at least 10 ppm of expired H2 over baseline within 75 min. The breath test was performed at the baseline (prevalence study\(^{18}\)), before probiotic administration (1 week later) and after the 4 weeks of probiotic yoghurt consumption.

Intestinal permeability assessment was performed with polyethylene glycol of different molecular weights (400, 1500, 4000, 10 000) as previously described\(^{19}\).

Endotoxin determination was carried out before and after probiotic yoghurt consumption in peripheral venous blood as previously described\(^{10,11}\).

\textit{Ex vivo} phagocytic activity and cytokine production after stimulation with lipopolysaccharide (LPS) (\textit{Escherichia coli} 0111:B4) was performed on peripheral blood mononuclear cells.

Phagocytosis in human whole blood was performed using opsonised, fluorescein isothiocyanate-labelled \textit{E. coli} (PHAGOTEST\textsuperscript{TM}; Becton Dickinson, Basel, Switzerland) and flow cytometry as described previously\(^{12}\).

For the determination of cytokine release by monocytes peripheral blood mononuclear cells were separated from whole blood by gradient centrifugation with Ficoll\textsuperscript{6}.

Cytokine production by monocytes was determined as follows: monocytes were purified by adhesion to plastic dishes during 1.5 h. Cells were thereafter stimulated by the addition of 10 ng/ml endotoxin from \textit{E. coli} 0111:B4 (Sigma, Stuttgart, Germany). Cell culture supernatants were collected at 2.5 h for the determination of TNF-\(\alpha\) and IL-\(\beta\) and at 20 h for the determination of IL-10. Cytokine concentrations were measured with commercially available ELISA kits (Pharmingen, Heidelberg, Germany).

Release of reactive oxygen species by neutrophils procedure and evaluation are described in detail in a previous publication\(^{13}\). Neutrophils were isolated from whole blood by gradient centrifugation with Polymorphsphrep (Nycomed, Oslo, Norway). The chemiluminescence emitted by the LPS-stimulated neutrophils from SPH and SNH groups was assessed as the area under the curve in an assay that covers 1 h post-stimulation with different LPS concentrations.

**Statistical analysis**

The study presented has to be considered as an exploratory clinical trial. The design consists of one treatment group. The effect of the yogurt was investigated by a pre-post comparison. The observed differences may be due to treatment, but also due to ‘regression to the mean’ as well as due to unknown confounders. Measurements of H2 breath test are not approximately normally distributed. Also log transformation did not achieve the desired distributional characteristics. Consequently summary statistics are presented by median and quartiles and inferential statistics is performed by Wilcoxon sign-rank test. Treatment differences and CI are estimated according to Hodges & Lehmann\(^{14}\).

The other outcomes are approximately normally or log-normally distributed. Summary statistics are presented by means and their standard errors. Inferential statistics was performed by the \(t\) test. We are aware that our exploratory research may produce also false positive results. Therefore the \(P\) values presented throughout this report are interpreted as flags in order to indicate an interesting result. This interpretation is also in agreement with the international conference of harmonisation guideline, ICH E9.

**Results**

From the 294 subjects, only 279 subjects were further investigated in the cross-sectional screening study (data not shown). In this sample, thirty-eight elderly showed a positive hydrogen breath test (SPH) and 241 showed a negative hydrogen breath test (SNH). The cut point was 10 ppm. SPH elderly, showing compliance, were invited to participate in next stage of the study, resulting in twenty-three SPH participants (eighteen women, five men). Additionally thirteen SNH elderly were enrolled in the study (nine women, four men). The mean age was 78-6 (SD 7-8) and 77-5 (SD 5-8) years for the SPH and SNH subjects, respectively. Both groups were comparable in anthropometric data (weight 66 (SD 15) and 71 (SD 12) kg; BMI 24-0 (SD 3-8) and 25-8 (SD 3-6) kg/m\(^2\) for SPH and SNH elderly, respectively).

The H2 breath test was performed three times in each subject, on screening, before and on the last day of yogurt consumption, 4 weeks later.

Development over time of the H2 hydrogen breath test for each observation group (SPH, SNH) is presented in Fig. 1. The SPH subjects are showing a significant decrease (-18 ppm; 95% CI -34.5, -7.5; \(P=0.006\)) from screening until start of yogurt intake and then are remaining relatively stable until end of yogurt intake (+1 0 ppm; 95% CI -13.5, 12.0; \(P=0.87\)). SNH subjects were consistently negative (<10 ppm) in all three determinations and do not show relevant changes from screening until start (-0.5 ppm; 95% CI -3.0, 2.5; \(P=0.75\)) and until end of yogurt intake (± 0.0 ppm; 95% CI -2.5, 2.0; \(P=0.80\)).

**Intestinal permeability**

No significant association of increased intestinal permeability to macromolecules (polyethylene glycol) with small bowel intestinal overgrowth was observed, and no change was observed as a result of the probiotic yogurt intake (data not shown).
**Probiotic and immune function in elderly**

**Endotoxin concentration**

Before treatment no differences were observed for endotoxin concentrations in peripheral venous blood between subjects of the different groups (6.49 (SEM 8.1) pg/ml for SPH group; Fig. 2). After the 4 weeks of probiotic yoghurt consumption a trend towards a diminution of endotoxin concentration was observed in the SPH group (6.49 (SEM 8.1) v. 4.6 (SEM 5.6) pg/ml). While in the SNH group plasma endotoxin was significantly lower after probiotic yoghurt consumption (6.4 (SEM 6.3) v. 0.5 (SEM 0.0) pg/ml, \(P=0.043\), before v. after).

**Phagocytic activity of monocytes and neutrophils**

Before yogurt consumption, monocytes isolated from subjects of the SPH group produced significantly less IL-1\(\beta\) and IL-10 than subjects in the SNH group (199 (SEM 359) v. 586 (SEM 566) pg/ml, \(P=0.035\) for IL-1\(\beta\) and 759 (SEM 739) v. 1846 (SEM 1309) pg/ml, \(P=0.009\) for IL-10). After 4 weeks of yoghurt consumption monocytes from SPH participants showed an increased capacity to react to endotoxin challenge attaining similar levels to the SNH group.

**Cytokine release**

A trend towards a more elevated release of reactive oxygen species by neutrophils was observed after the yoghurt consumption in both groups. The differences were statistically significant at the highest LPS concentration in the SPH group (\(P=0.031\); see Fig. 3). Reactive oxygen species production by neutrophils from the SNH group (data not shown) showed a similar pattern, higher after probiotic yoghurt consumption, but in this case did not reach statistical significance.

**Discussion**

All participants had a good nutritional status and no apparent clinical manifestation of SIBO. Of the SPH subjects enrolled in the present study 43% did not reach the threshold increment of 10 ppm of \(\text{H}_2\) in the second breath test before the start of probiotic yoghurt consumption. On the other hand, none of...
the thirteen SNH subjects had a positive H₂ breath test in the second or third breath tests. The spontaneous normalization of a positive H₂ breath test in the SPH group may be due to various factors.

According to most authors, the sensitivity of glucose/H₂ breath test is approximately 70% and the specificity is about 80%. Thus the apparent discrepancy between the first and the second breath tests performed 1 week apart cannot be attributed to inherent limitations of the test alone(16).

The lack of reproducibility in the test may be due to physiological or pathophysiological variations such as carbohydrate malabsorption, gastrointestinal motor disorders with transit abnormalities(17). The stability of the intestinal bacterial population varies in both the number and type of colonising bacteria. The washout effect of diarrhoeal episodes, the loss of integrity and higher inflammatory response than subjects colonized by other types of microbiota(5). In the present study, the thirteen SNH subjects had a positive H₂ breath test in the second or third breath tests. The spontaneous normalization of a positive H₂ breath test in the SPH group may be due to various factors.

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SIBO can be associated with an increased immune-inflammatory activity of the mucosal immune-competent cells. Increased production of IL-6, higher numbers of intra-epithelial lymphocytes and of IgA-producing cells have been associated with the intestinal bacterial load(18). The inflammatory immune reaction seems to be mediated by a coliform predominant microbiota.

In addition to the local effect of SIBO in the mucosal immune compartment, leakage or translocation of bacteria or their components into the body can contribute to visceral toxicity, systemic inflammatory conditions and metabolic changes. SIBO and passage of LPS into the portal vein can be deleterious to the liver and contribute to non-alcoholic liver disease(19,20).

It has been postulated that the subclinical inflammatory status observed in a subgroup of elderly may be propagated from components in the intestinal environment as a consequence of a poor mucosal barrier and inadequate mucosal clearance of the abnormally high bacterial challenge(21). There is no strong evidence supporting an association between translocation bacteria or their products and increased intestinal permeability(22). Therefore, the unaltered permeability to polyethylene glycol does not rule out a closer interaction of the mucosal immune cells with the abnormally high bacterial load of the small bowel and a concomitant increase in lamina propria cellularity(23), due to macrophage and lymphocyte infiltration. Increased local IL-6 production is related to the activation of lamina propria mononuclear cells exposed to higher LPS levels. Local mucosal changes may not be detected systemically.

Positive glucose/H₂ breath test was not associated with higher levels of plasma LPS as both groups had similar concentrations at baseline. After the consumption of the probiotic yoghurt a significant decrease in plasma endotoxin was observed in the SNH group and a similar trend was present in the SPH group (Fig. 2). Since probiotic ingestion did not alter breath test results or permeability to polyethylene glycol, one can assume that the observed cellular changes are due to other mechanisms besides a change in the bacterial load of the small bowel or a compromise of mucosal barrier integrity. An altered small bowel microbiota with lower quantities of coliform endotoxin-producing bacteria may have resulted from probiotic administration. Alternatively probiotic administration may activate the innate macrophage system(12) in the mucosal compartment and thereby improve LPS clearance. Certainly, removal and clearance of LPS from the body is another of the many functions associated with the intestinal cellular compartment(24). In experimental conditions lamina propria macrophages and intestinal epithelial cells take up and clear LPS(24). These cells are probably also involved in the clearance of luminaly derived endotoxin and translocating bacteria. Long-term exposure to endotoxin may render phagocytes tolerant to LPS and incapable of mounting an energetic response to further challenge with bacterial products and endotoxin in particular. It is feasible that innate immune cells have a permanently low level of activation but paradoxically, lose their capacity to mount an effective response when needed. This can be clearly observed in the extreme case of...
liver cirrhosis\(^{(25)}\). In the present study the decreased \textit{ex vivo} phagocytic capacity of unstimulated individual cells after probiotic intake suggests a decrease in basal low-grade cellular activation in the elderly. In contrast, monocytes stimulated \textit{ex vivo} with endotoxin had a low response of cytokine production that was increased after the consumption of probiotic yoghurt. This change was significant in the SPH group.

The release of reactive oxygen species by neutrophils exposed to endotoxin was similar before and after probiotic intake, but a higher reactivity to increasing concentrations of endotoxin was observed after probiotic administration.

Although we have no direct proof of endotoxin leakage at the gut level there is some evidence to suggest that LBP, sCD14 and possibly other LPS pattern recognition receptors are important parameters for monitoring the host acute-phase reaction secondary to bacterial product leakage into the internal milieu. After the probiotic yoghurt administration a significant decrease in sCD14 and LBP plasma levels was observed.

In conclusion, monocytes and neutrophils exhibited a higher phagocytic capacity before probiotic consumption if no challenge with LPS was performed \textit{ex vivo}. In contrast, probiotic administration normalised responsiveness to LPS \textit{ex vivo} and resulted in an increased production of cytokines and free radicals.

A unifying explanation is that probiotic administration probably results in a lower exposure to endotoxin in the mucosal microenvironment and in the body. This lower exposure may be due to a qualitative switch from a Gram-negative endotoxin-producing microbiota to a Gram-positive (less pro-inflammatory) type of microbiota in the proximal small bowel\(^{(21)}\), and/or an activation of the innate immune system by probiotics that results in better clearance of endotoxin and improved immunocompetence.

Probiotics have been extensively studied for their health-promoting activities in a variety of human conditions where altered bacterial ecology seems to play a pathogenetic role. The distal small bowel and the colon are where most intestinal bacteria find their ecological niches and where probiotics may also find a more favourable environment. Here we explored the possibility that probiotics exert effects on abnormal bacterial communities along the entire length of the small bowel. The probiotic effect we observed may be mediated by different mechanisms: (1) competitive exclusion of colonising bacteria that are vying for the same ecological niches with subsequent qualitative changes in the composition of the microbiota; (2) stimulation of immune function and improvement of immune competence for controlling the levels of the bacterial populations; (3) increase in innate immune function and endotoxin clearance; and/or (4) the induction by probiotics of a homeostatic mucosal immune response that can compensate for the pro-inflammatory activity of the colonic-like bacteria. Any one of the aforementioned effects alone or in combination could be of benefit in subjects with SIBO.

The present clinical study suggests that the administration of supplements in the elderly may benefit from the addition of ingredients that improve the composition of the intestinal microbiota. Moreover, the present results indicate that an altered intestinal ecology underlies the low-grade inflammatory status that favours catabolism and loss of lean body mass in the elderly.

Acknowledgements

We are indebted to Mrs A. Bleher and S. Berhard for support in recording the diet histories and instructing the study participants during the breath tests. The work was generously supported by Nestec Ltd. The authors made the following contributions to the study: E. J. S. and A. P.: study concept and design, analysis and interpretation of data, preparation of the manuscript; C. B. and J. C. B.: study concept and design, subject recruitment, acquisition of data; M. A. v. H. and D. G.: analysis and interpretation of data; Y. G.: interpretation of data, preparation of the manuscript. A. P., C. B. and J. C. B. have no conflict of interest. E. J. S., M. A. v. H., D. G. and Y. G. are employees of Nestlé SA, Switzerland.

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