ISSUE 24 Spring 2015

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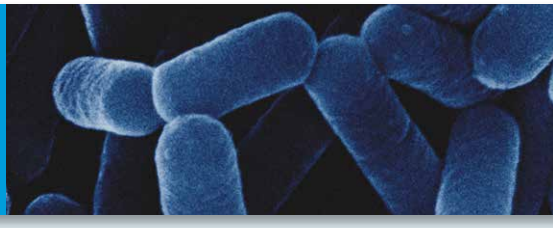
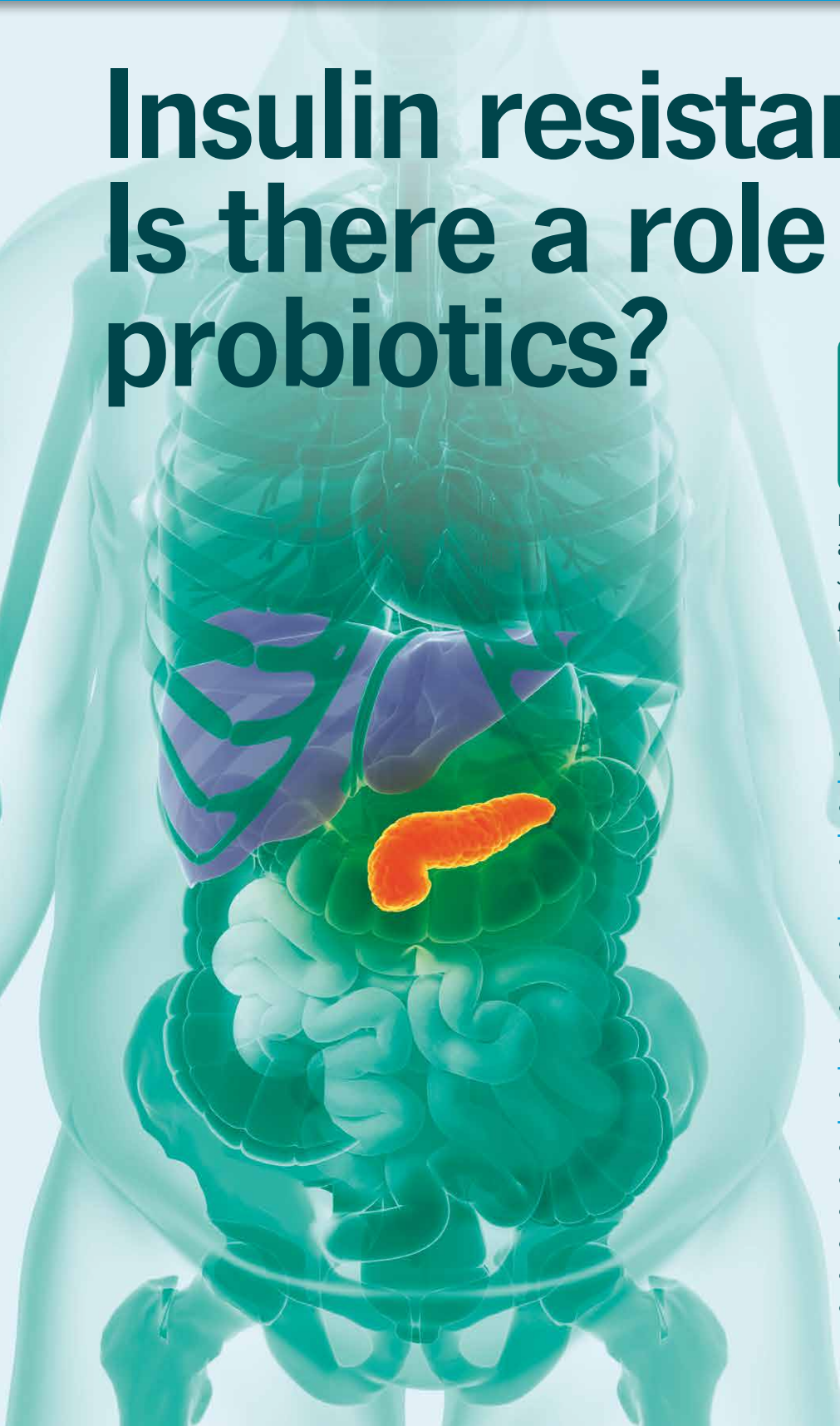
Probiotic Bulletin

A Newsletter for Healthcare Professionals



Insulin resistance: Is there a role for probiotics?



Dr Carl Hulston is a lecturer in the School of Sport, Exercise and Health at Loughborough University. His study investigating insulin resistance was published earlier this year, as an open access paper in the British Journal of Nutrition. A summary is given on page 3; Dr Hulston provided us with further insight.

In this issue:

Interview with an expert

(Dr Carl Hulston, continued)

Q What causes insulin resistance to develop?

A Insulin resistance is a complex metabolic disorder – a number of possible causes have been identified. Typically it has been attributed to impaired skeletal muscle glucose uptake, elevated liver glucose production, and/or impaired insulin secretion. Now it seems that the gut microbiota might also play an important role in the pathophysiology of insulin resistance, through a mechanism that is linked to increased gut permeability, metabolic endotoxaemia and systemic inflammation. Metabolic endotoxaemia is caused by the presence of lipopolysaccharides (LPS) in the blood; these endotoxins are key components of the outer membrane of Gram-negative bacteria.

Q Research on probiotics and diabetes is still in its infancy. What prompted your research?

A Animal data have shown that modification of the gut microbiota, through prebiotic or probiotic supplementation, can reduce the amount of LPS in the blood (ie, metabolic endotoxaemia) and improve glycaemic control in obese diabetic mouse models. We were interested in following this up in humans to see if probiotics might be useful in the prevention of insulin resistance and type 2 diabetes.

Q Can you explain the rationale for your study design?

A Short-term, high-fat diets have been shown to cause insulin resistance in both animal and human experiments. They have also been shown to alter the composition of the gut microbiota. We reasoned that if changes in the composition of the gut microbiota were responsible for the development of insulin resistance then supplementation with a probiotic such as *Lactobacillus casei* Shirota (LcS) might prevent high-fat, diet-induced insulin resistance.

Q What do you think were the key probiotic mechanisms of activity underlying the insulin effects seen in your study?

A The most likely mechanism is that the probiotic supplementation prevented systemic inflammation (which can cause insulin resistance in a number of tissues) by maintaining gut barrier integrity and preventing the translocation of LPS into the circulation, but this needs confirming in our follow-up work.

Q Could probiotics play a role in the treatment of obesity and/or type 2 diabetes?

A It's probably a little too early to make any bold claims surrounding this, but our preliminary data are very promising. I would, however, suggest taking probiotics as part of a generally healthy lifestyle in order to reduce the risk of developing insulin resistance and type 2 diabetes.

Q What impact does a high-fat diet have on the gut microbiota profile?

A Diets that are excessively high in fat have been shown to increase the ratio of Bacteroidetes [Gram-negative bacteria] to Firmicutes [Gram-positive bacteria] in the gut, when compared to a healthier, more moderate level of fat intake. An increase in Gram-negative bacteria in the gut is problematic because components of their cell membrane (like LPS) cause inflammation.

Q Why were the subjects in your study predominately male?

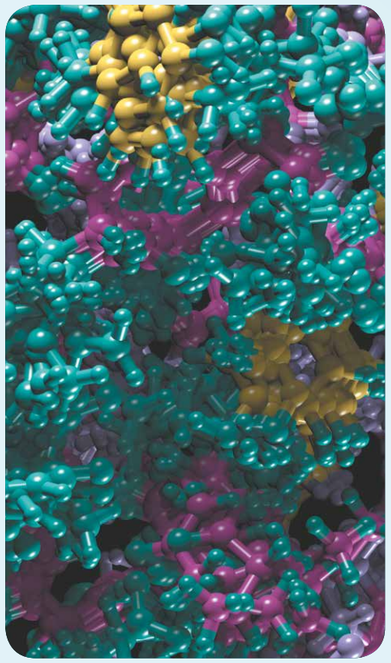
A This was simply because more men than women volunteered for the study, possibly because the idea of consuming a high-fat diet for seven days is off-putting for many women. I don't think this will have influenced the results as the male and female responses were similar in this study.

Q There is an increased risk of insulin resistance in certain people, for example sarcopenic patients, pregnant women and women with Polycystic Ovary Syndrome. Do you think your research has implications for such patients?

A In each of these patient groups, the mechanism of insulin resistance will be quite different. For example, reduced insulin sensitivity in sarcopenia is largely related to the loss of muscle mass, so it would be difficult to translate our results from healthy volunteers into advice for these patients.

Q What plans do you have for future research in this area?

A Our immediate plan is to follow-up this promising line of work with a much larger cohort of volunteers and with the addition of mechanistic measurements.



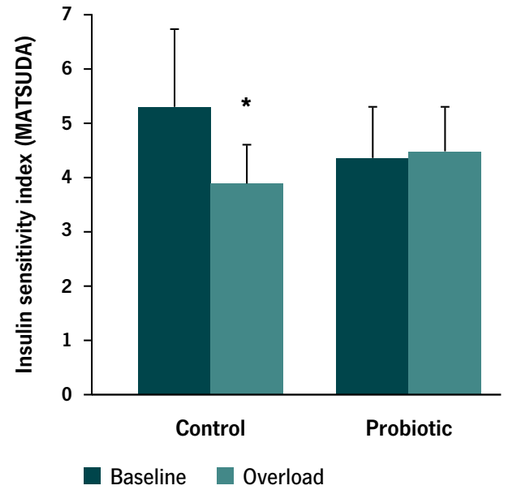
Hulston C *et al* (2015) Probiotic supplementation prevents high fat, overfeeding-induced insulin resistance in human subjects. *British Journal of Nutrition* 113(4):596-602

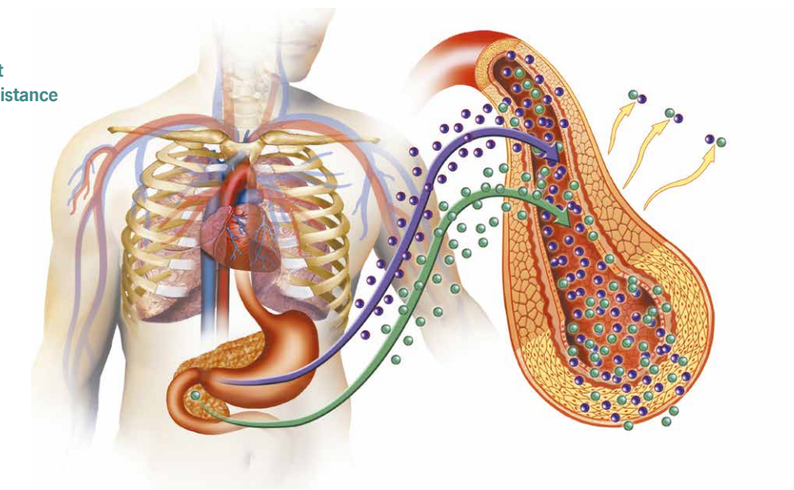
Seventeen healthy volunteers were randomly selected to receive either a probiotic (LcS; 2/day x four weeks) or no probiotic. All subjects consumed their habitual diet for three weeks, after which they all consumed a high-fat (65% of energy), high-energy (50% increase in energy intake) diet for seven days. Oral glucose tolerance tests (OGTT) were conducted on day 22 and 29 to measure the effect of the high-fat overfeeding, and to identify any differences between the two groups.

After the seven days of high-fat overfeeding, glucose AUC values in the control (non-probiotic) group increased by 10% ($P<0.05$) and insulin sensitivity decreased by 27% ($P<0.05$). In contrast, glycaemic control was preserved in the LcS group, and their insulin action was maintained throughout the overfeeding period ($P>0.05$).

The figure to the right shows the Matsuda insulin sensitivity index calculated during an OGTT conducted before and after the seven days of overeating. Values are means ± SE (n = 9, control group; n = 8 probiotic group).

*Significantly different from baseline, $P < 0.05$.





Did you know…

- The word diabetes comes from the Latin word diabētēs, which means 'to pass through'
- Insulin was first discovered by Sir Edward Albert Sharpey-Schafer in 1910 (diabetes.org)[1]
- In 2010 approximately 3.1 million people (16 years and over) in England were living with diabetes. This figure is expected to rise to 4.6 million by 2030 (90% of these have type 2 diabetes)[2]
- The UK's first recognised case of type 2 diabetes in white adolescents occurred in 2002 (Dyer, 2002)[3]
- 90% of people with diabetes have type 2 diabetes[4,5]
- Obesity is the most potent risk factor for type 2 diabetes[6]

A list of references is available at www.yakult.co.uk/hcp or from science@yakult.co.uk

Appetite regulation and the role of fermentable carbohydrates

By Claire Byrne, PhD student at Imperial College London

The recent shift in our dietary patterns towards consumption of high-energy dense convenience foods may play a role in augmenting the current obesity epidemic.[1] The average western-style diet differs greatly from the low-energy dense diet consumed by our ancestors, which had a much greater fermentable component.

Non-digestible carbohydrates reach the colon largely intact and are fermented by the resident gut microbiota. This results in the production of short-chain fatty acids – mainly acetate, propionate and butyrate – which are present in faecal samples in the ratio 3:1:1.[2] The consumption of fermentable carbohydrates has been associated with a wide range of health benefits including improvements in body composition, lipid and glucose metabolism, and reduction in colon cancer risk.[3,4,5] My current research is focused on investigating the effect of fermentable carbohydrate consumption on appetite regulation.

I have just completed my first human study, investigating the effect of propionate on anticipatory food reward. Oral propionate itself is not very palatable and is rapidly absorbed in the upper gastrointestinal tract, meaning small amounts reach the colon where it has its beneficial effects. In order to address this issue, our group has developed a novel system, inulin-propionate ester (IPE), which delivers propionate directly to the colon. Using the IPE, we have previously shown that propionate stimulates the release of the satiety hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), and reduces the amount of food consumed at a buffet meal.[3]

In my current study, my colleagues and I measured brain reward system activation during evaluation of food pictures after the consumption of IPE using functional magnetic resonance imaging (fMRI). We hypothesised that propionate would reduce anticipatory food reward responses. We recruited 20 healthy non-obese men and used a randomised, crossover design, so that each volunteer acted as their own control. Only men were recruited in order to rule out any effects of hormonal changes associated with the menstrual cycle on appetite measures. Each subject underwent an established fMRI food-picture evaluation task in which they rated the appeal of food (high-energy or low-energy) or control object pictures.[6,7] Subjects were scanned 300 minutes after consuming either IPE (treatment) or inulin (control). Activation during the fMRI task was examined in five regions of interest in the brain (amygdala; anterior insula; caudate nucleus; nucleus accumbens; orbitofrontal cortex), which are involved in reward processing. [6,7]

We found that propionate significantly and selectively reduced the response to high-energy foods (but not low-energy foods) in the caudate nucleus, as measured by a reduction in the blood oxygen level dependent (BOLD) signal compared to control.

We concluded that propionate reduces the brain response to anticipatory food reward. This effect may have been mediated by an increase in satiety hormones or by the direct action of propionate on brain reward systems. The results of our research so far suggest that the consumption of IPE may be effective in reducing food intake. Next, we plan to add IPE to common food products and conduct further human research studies to investigate their effect on appetite.

These results were presented at the Experimental Biology 2015 conference, which was held in Boston from 28th March to 1st April, 2015. I would like to thank Yakult UK Ltd for providing a grant to help cover my travel costs.

The mechanisms underlying the cytotoxic effects of probiotics for cancer cells

Shyu PT *et al* (2014) *BioMed Research International* 2014: Article ID 491740

Cancer incidence and associated mortality rates are on the increase in less developed countries. In the Philippines, colon cancer is now the third most frequent form of cancer in males and the fourth in females. This is also the third most common form of cancer for both men and women in the UK and Ireland (Cancer Research UK; Irish Cancer Society).

The unregulated growth that is characteristic of cancer cells is associated with an altered mitogen-activated protein kinase (MAPK) pathway. This pathway, which regulates cell division and cell death, results in the formation of the AP-1 transcription complex involved in the control of cellular processes such as differentiation, proliferation, apoptosis, organogenesis and response to stress. Two components of AP-1 are *cfos* and *cjun* genes.

Any impairment to the immune response (which can occur with cancer) can lead to chronic inflammation and alterations in cancer-related genes., Tumour necrosis factor – ☐ (TNF-☐) and interleukin -1☐ (IL-1☐) are two pro-inflammatory cytokines found at raised levels in some cancers, suggesting they may be involved in disease progression.

This study investigated the effects of supernatants from probiotic lactobacilli strains isolated from three dairy products available in the Philippines on two colon cancer cell lines, one leukaemia cell line and normal fibroblasts. All three probiotics showed significant and specific cytotoxicity against the colorectal cancer cell lines, at a level similar to the anti-cancer drug control (bleomycin). A significant increase in the expression of *cfos* and *cjun* early apoptosis genes was also seen in all colorectal cancer cell lines treated with probiotics. Furthermore, probiotic exposure was associated with decreased expression of IL-1☐ and TNF-☐ by lipopolysaccharide-treated macrophages.

The power of bacteria – can we harness it?

Breuer M *et al* (2015) Multi-haem cytochromes in *Shewanella oneidensis* MR-1: structures, functions and opportunities. *Journal of the Royal Society Interface.* 12(102): 20141117

Could we soon be running our mobile phones on electricity generated by bacteria? This may be a future possibility, according to the latest research into *Shewanella oneidensis* MR-1, a species of marine bacteria. Could these bacteria be 'tethered' directly to electrodes to produce electricity, perhaps leading to the development of efficient microbial 'bio batteries'?

Shewanella are facultative anaerobes: ie, they can switch to a different way of producing energy when oxygen is not available. In the right environment, instead they survive by 'breathing' minerals such as iron, which enables them to harness the energy needed to make their cellular fuel (adenosine triphosphate [ATP]). Electrons released in the cytoplasm by the oxidation process that drives ATP synthesis need to be transferred through a network of redox proteins to a terminal electron acceptor in the bacterial environment. In *Shewanella* this is achieved by the functionally diverse multi-haem cytochromes they contain: proteins that can transfer electrons over relatively large distances - at least in microbial terms (tens of nanometres).

With this species, a side product of anaerobic growth can be a flow of electricity from inside the bacteria across their outer membrane to rocks in the natural environment – or possibly to graphite electrodes in fuel cells, almost like the neutral wire in a household plug.

The story caught the attention of the national press *(Could POOP soon power smartphones?*). Lead researcher Professor Julea Butt (University of East Anglia) commented '*We hope that understanding how this natural process works will inspire the design of bespoke proteins which will underpin microbial fuel cells for sustainable energy production.'*

The British Geriatrics Society

This year we are pleased to announce our support of the British Geriatrics Society (BGS), the association of healthcare professionals specialising in caring for frail older people. BGS has an expanding membership of over 3,000 consisting of geriatricians in hospital and community settings, GPs, nurses, doctors and allied health professionals.

The society offers specialist medical expertise, promotes good practice, provides a forum for geriatricians and other professionals involved in the field, and encourages high quality research into older people's care.

For more details about the work of the BGS visit: **www.bgs.org.uk**

10th congress of the European Union Geriatric Medicine Society (EUGMS)

Held in Rotterdam last September, we were delighted to sponsor the session on 'Healthy ageing with gut microbiota and probiotics – cutting-edge research and practice'.

This was thoroughly informative, with a panel of eminent researchers describing the latest insights and research on the gut microbiota's interaction with age, diet and even happiness.

Your country needs poo!

Crowd-sourcing of human samples is the latest initiative from a research group at the Department of Twin Research at King's College London.

Headed by Professor Tim Spector, the team is working on a unique open collaboration called 'The British Gut Project'. The group urgently need a large-scale collection of human faecal samples. Their collaborators in the USA, who are working on the The American Gut Project, have already had over 7,000 people signing up. The aim of the UK project is to map the bacterial diversity of the 'Great British Gut' in order to learn more about the influence of the gut microbiome on health, and how lifestyle and diet affects its composition. And each contributor will be able to discover the bacterial profile of their own gut – an essential dinner party conversation piece!

To get involved visit: **http://www.britishgut.org**

Recent publications of studies, including those with *Lactobacillus casei* Shirota (LcS)

Constipation symptoms and haemorrhoids in women during puerperium

The effects of *L. casei* Shirota were investigated in a randomised, placebo-controlled study of 40 women in Belgium. Probiotic intake for six weeks after the women had given birth was associated with benefit as measured by questionnaires (better scores for total constipation symptoms, abdominal and rectal symptoms). There was also an indication that the probiotic aided recovery from haemorrhoids.

Sakai *et al* (2014) Beneficial Microbes Nov 6:1-10 [Epub ahead of print]

The behaviour of dendritic cells in ulcerative colitis

This UK study used gut biopsy samples from ulcerative colitis patients to show that intestinal dendritic cells (DC) can drive T cell dysfunction by skewing them towards a Th2 profile (increased IL-4 production, and loss of IL-22 and IFN□). Conditioning the gut DC with LcS, however, partially restored the normal stimulatory capacity of DC for T cells, restoring their ability to imprint skin-homing molecules on T cells and generating IL-22 production by stimulated T cells.

Mann *et al* (2014) Inflamm Bowel Dis. 2014 Dec;20(12):2299-307

Bifidobacterium bifidum YIT 1034: effects on gastric symptoms

A preliminary trial (n=305) and a subsequent randomised, placebo-controlled, crossover trial (n=28) were conducted with a fermented milk product containing this probiotic strain. Both trials showed two weeks' probiotic intervention to be associated with an improvement in the gastric symptoms of adults (who were not taking any medication).

Gomi *et al* (2015) J Dairy Sci Jan 30 [Epub ahead of print]

Probiotic milk and salivary *Streptococcus mutans*

This double-blind controlled crossover trial in India involved 31 children who were given a milk drink (with or without LcS) for 10 days. Analysis of saliva before and after this intervention revealed the probiotic to be significantly associated with lower *Streptococcus mutans* counts. There were also fewer children in the probiotic group with high *S. mutans* counts.

Yadav M *et al* (2014) J Clin Pediatr Dent 39(1):23-26.

Gut microbiota: people in Papua New Guinea

Reverse transcriptase real-time polymerase chain reaction (PCR) was used to show differences between the gut microbiota of those living in highland and lowland areas, and between adults and children. Most of the subjects (88%) were found to have higher levels of *Prevotella* compared to *Bacteroides*; this is probably linked to the traditional, subsistence type of lifestyle of these people. Yakult scientists were part of the research group.

Greenhill *et al* (2015) PLoS One Feb 6 [Epub ahead of print]

Improved detection method for toxigenic *Clostridium difficile*

Yakult scientists have helped develop a sensitive TaqMan-based quantitative PCR method with selective primers to detect toxigenic *C. difficile* in faecal samples. The method was used to investigate the carriage rate of *C. difficile* in 82 residents of a nursing home for older people. Over a six-month period, detection of toxigenic strain ranged from 1.2% to 3.8%.

Kubota *et al* (2014) PLoS One 9(10):e111684.

rRNA-targeted reverse transcriptome analysis of the vaginal microbiota

Yakult scientists have developed new specific primer sets to increase the sensitivity of this method, which has enabled them to detect and quantify a wider range of vaginal species. This has allowed detection of *Lactobacillus gasseri, L. crispatus, Atopobium vaginae, Gardnerella vaginalis, Mobiluncus curtisii, Chlamydia trachomatis/muridarum, Bifidobacterium longum subsp. longum, B. longum subsp. infantis, B. adolescentis* and *B. angulatum.*

Kurakawa *et al* (2015) J Microbiol Methods 111:93-104

Review: gut microbiota and gut permeability

This was the outcome of discussions by experts brought together in Germany by Yakult for this purpose. The review clarifies what is meant by 'intestinal permeability' and 'intestinal barrier', explains their key elements, and the effects of diet and the gut microbiota. Methods to measure intestinal permeability are also reviewed. Finally, the role of impaired intestinal permeability in various diseases (including obesity and metabolic diseases) is discussed. Such diseases are characterised by chronic inflammation, perhaps caused by leakage of luminal components.

Bischoff SC *et al* (2014) BMC Gastroenterol 14:189.

Exploring the Science of Digestion

Educating the public about the gut microbiota

Core is the national charity linked to the British Society of Gastroenterology (BSG), with the aim of raising awareness and funding research into gut, liver and pancreatic disease. ***Exploring the Science of Digestion*** was held in November last year at the Oval cricket ground. It was attended by over 600 people (many of whom were patients) all very keen to question the panel of experts. Slides and/or videos of the educational talks are available on the Core website: **http://www.corecharity.org.uk/video**


The ecologist will see you now

We're not individuals, we're ecosystems full of bacteria. Understanding how these microbes interact with our bodies is set to transform medicine.

"So from that point of view, you are only 10 per cent human"

It's a jungle in there

New resource - The ecologist will see you now

One of the hits of the day was the launch of an educational article for the general public about the gut microbiota, which was commissioned for Core by the Gut Microbiota for Health expert panel of the BSG and sponsored by Yakult. 'The ecologist will see you now' is authored by Claire Ainsworth, who has written articles for the New Scientist.

Download a copy at: **www.yakult.co.uk/hcp**
or to order hard copies, email: **science@yakult.co.uk** or call: **020 8842 7600**.

The International Yakult Symposium. Berlin, April 23-24, 2015.

'PROBIOTICS, A PROACTIVE APPROACH TO HEALTH – an expert insight into the latest research'

The symposium focused on probiotics and the importance of being proactive about health.
It provided an expert update on the role probiotics can play in strategies for health maintenance.

For further details visit: **http://yakultsymposium.com**

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We are celebrating our 80th anniversary!

- In 1930 the unique probiotic strain *Lactobacillus casei* Shirota was first selected and cultivated.
- 100 peer-reviewed papers describing human studies have been published on *L. casei* Shirota.

Yakult Study Day – educational videos and report now available!

Our popular study day, 'Current insights into the gut microbiota and its influence on health: An independent and expert review examining different patient groups', was held in October last year. All the presentations given on the day can be seen at **http://hcp.yakult.co.uk/symposia/uk-yakult-symposia**
The presentations have also been summarised in our new: 'LcS Insight: A study day report', which can be viewed online at: **www.yakult.co.uk/hcp.**
Hard copies are also available on request from: **science@yakult.co.uk** or **020 8842 7600.**

A ***FREE*** educational gut microbiota and probiotics talk for you and your colleagues in your place of work!
Contact a member of the Yakult science team today on: **020 8842 7600** or **science@yakult.co.uk**

We also offer:

- **Advice on probiotics**
- **Copies of our newsletter, reprints and other material**
- **Free trial period of product (subject to discussion)**

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