Investigating the gastrointestinal and systemic effects of apples *in vitro* and *in vivo*. By A. Koutsos[1,2], M. Lima[1], F. Fava[1], L. Conterno[1], A. Galvin[2], T. Braune[2], Julie A. Lovegrove[2], R. Viola[1] and K. Tuohy[1], [1]*Department of Food Quality and Nutrition, Fondazione Edmund Mach, IASMA Research and Innovation Centre, Via E. Mach 1, 38010 S. Michele (TN), Italy and* [2]*Hugh Sinclair Unit of Human Nutrition and Institute of Cardiovascular and Metabolic Research, University of Reading, Reading, RG6 6AP, United Kingdom*

There is now considerable scientific evidence that a diet rich in fruits and vegetables could improve human health (1). However, it is not clear whether different fruits and vegetables have different beneficial effects. Apples are a rich source of polyphenols and fiber and are widely consumed worldwide (2). An important proportion of these bioactive compounds in apples escape digestion in the upper intestinal tract and reach the colon where they can be fermented by the colonic microbiota (3). Preliminary results from our group have shown that apples - Gold Rush, Renetta Canada, Golden Delicious and Pink Lady - may beneficially increase the bifidobacteria levels in *in vitro* batch culture models with the Renetta Canada variety showing the most beneficial effects (Figure 1).

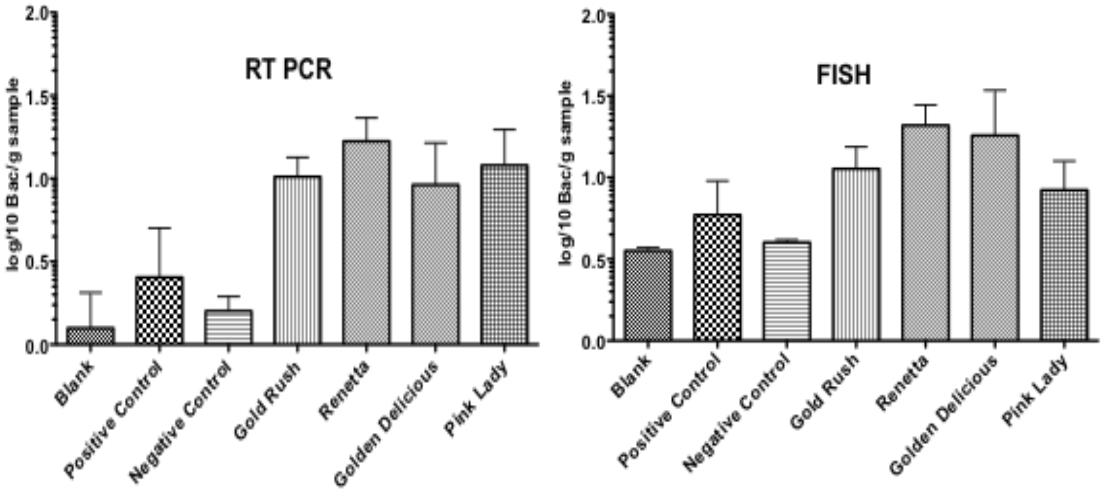

Figure 1. Bifidobacteria bacterial populations (log_{10} cells/ml batch culture fluid) determined by fluorescence in situ hybridisation (FISH) and quantitative polymerase chain reaction (qPCR) in anaerobic, stirred, pH and temperature controlled faecal batch cultures at 0, 5, 10 and 24 h using inulin as a positive control, cellulose as negative control and blank vessel (only faeces) as a baseline in order to compare the microbiota-modulating abilities of 4 different apple varieties (n=3 healthy adults). Results are the difference between T0 and T24. Bars represent the mean of triplicate, error bars represent SD.

Based on these results we are currently performing a randomized, controlled, crossover, dietary human intervention study -the AVAG study- which explores the hypothesis that a daily consumption of 2 apples for 8 weeks can decrease the risk of cardiovascular disease by reducing total cholesterol levels, improving vascular function and beneficially modulating gut microbiota in 40 mild hypercholesterolaemic subjects. The dietary intervention part of the study will finish in September, 2014 and the following analysis includes the determination of

the gut microbiota population using FISH and 454 pyrosequencing of community based 16-S ribosomal RNA genes and metabolomic analysis of the low molecular weight metabolite profiles in the biological fluids (blood, urine and faecal sample). Moreover, the determination of several markers of cardiovascular health including blood lipid, glucose and insulin levels, inflammatory and endothelial function markers and assessment of vascular function using Laser Doppler Iontophoresis and Pulse Wave Analysis will be performed.
This trial is registered at http://clinicaltrials.gov/show/NCT01988389.

References

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