Low-calorie sweeteners disrupt the gut microbiome in healthy subjects in association with impaired glycaemic control

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Background and aims: Epidemiological studies indicate that regular high intake of beverages sweetened with low-calorie sweeteners (LCS) increase the risk of developing type 2 diabetes mellitus (T2DM), but the underlying mechanisms are unknown. We recently showed that 2 week dietary supplementation with LCS in healthy non-diabetic subjects led to clinically relevant increases in glycaemic responses to enteral glucose. Augmented glucose absorption (serum 3-O-methyl glucose, 3-OMG) and attenuated release of glucagon-like peptide-1 (GLP-1) contribute to this dysglycaemia, however it is unclear whether gut dysbiosis due to LCS also contributes to dysglycaemia, as occurs in rodents.

Materials and methods: 29 non-diabetic subjects (age 30 ± 2 years, body mass index 24 ± 3 kg/m², HbA1c 32 ± 1 mmol/mol (5.2%), 16 male) were randomised, in double-blind fashion, to diet supplementation with a LCS combination (92 mg sucralose + 52 mg acesulfame-K, equivalent to ~1.5L of diet beverage consumption/day, N=14) or placebo (N=15); these were taken in capsules three times daily over 2 weeks. The gut microbiome was assessed by shotgun metagenomic sequencing in stool collected before and after treatment. Differences in taxonomic and functional microbiome characteristics were determined using MetaPhlAn2 and HUMAnN2 abundance, respectively.

Results: LCS-treated subjects exhibited a greater variation in faecal microbiota composition, along with a significant reduction in the health-associated bacterium *Eubacterium cylindroides* (-11 log2 fold change, FC) and an increased abundance of 11 opportunistic gut pathogens, including *Klebsiella* (17 FC), *Porphyromonas* (15 FC) and *Finegoldia* (12 FC; all P ≤ 0.001). A decrease in beneficial and fermentative *Bifidobacterium, Lactobacillus* and *Bacteroides* populations correlated with augmented glucose absorption (3-OMG), while a decrease in *Butyrivibrio* populations correlated with attenuated GLP-1 release (Spearman correlation: $\rho \geq ±0.37; P \leq 0.05$). Finally, shifts in the abundance of microbial genes involved in sucrose degradation and pyruvate metabolism correlated with a deterioration in glucose regulation in LCS-treated subjects.

Conclusion: In healthy non-diabetic subjects 2 weeks of LCS supplementation (i) causes gut dysbiosis and (ii) increases the abundance of gut pathogens normally absent in health. Moreover, a decrease in fermentative microbial populations and shifts in bacterial energy harvesting pathways due to LCS predict a deterioration in glucose regulation. Our findings support the concept that LCS disrupt glycaemic responses in healthy humans via dysregulation of glucose uptake and disposal, and secondary to dysbiosis of gut commensal bacteria. This highlights the clinical relevance of dietary LCS patterns to overall glycaemic control.

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