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Research Article

Effect of Dietary Carbohydrate Restriction on an Obesity-Related *Prevotella*-Dominated Human Fecal Microbiota

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Abstract Relatively few studies have been conducted on the effect of diet on the intestinal microbiota. We followed the response of the fecal microbiota to a carbohydrate-restricted diet in an obese volunteer. The community composition was estimated through 16S rRNA gene sequence analysis. Bacteria of the *Prevotella* genus dominated the microbiota. Elimination of carbohydrates from diet triggered an immediate decrease in *Prevotella*, compensated by the increase in all other bacteria, and higher fluctuations in the community composition. Fecal microbiota changed back to its previous structure no later than 4 weeks after reintroducing carbohydrates in diet. Our results show the dependence of *Prevotella* on dietary carbohydrates and confirm the resilience of the microbiota to short-term dietary interventions. The potential link between *Prevotella* and obesity warrants further research.

Keywords diet; gut microbiota; metagenomics; obesity; *Prevotella*

1. Introduction

Recent evidence suggests that dysbiosis of the gut microbiota and its effects on host metabolism play an important role in the pathogenesis of obesity [15]. In a previous study, we found several obese subjects with a low bacterial diversity in feces due to an unusually high prevalence of the *Prevotella* genus [7]. A decrease in the level of diversity had been previously associated with obesity [13], and higher levels of the Prevotellaceae family had been found in a small cohort of obese individuals compared to normal-weight individuals [19]. Also, one of the main types of microbial assemblies identified in the human gut, the so-called enterotypes, is driven by a relatively high level of *Prevotella* [2]. Our analysis of the 16S rRNA samples obtained in the study of Turnbaugh et al. [13], classified into enterotypes, plus the samples from [7], a total of 157 individuals, gave a statistically significant association between the *Prevotella*-enriched enterotype and some type of obesity [2, 7, 13].

Bacteria of the *Prevotella* genus are known to degrade insoluble plant fiber and ferment soluble carbohydrates (CHOs) to short-chain fatty acids [9]. Fecal communities rich in *Prevotella* were also found in a cohort of African children, which ate a low-calorie diet rich in fiber [5]. It was hypothesized that *Prevotella* co-evolved with that diet because they are more efficient in extracting energy from complex polysaccharides. In fact, higher amounts of short-chain fatty acids were found in the feces of the African children compared to those of European children [5]. Going beyond, we postulate that this capability of *Prevotella* may be useful on a low-calorie diet to survive, but it may lead to obesity on the rather high-calorie Western diet.

The *Prevotella*-enriched enterotype was linked to a long-term dietary pattern consisting in high intake of CHOs and simple sugars, and low intake of proteins, amino acids, and saturated fats [18]. In this study, we monitored the *Prevotella*-dominated bacterial community of an obese subject, individual B in our previous study [7], fed on a diet rich in protein and fat and very low in CHOs.

2. Materials and methods

2.1. Sample collection

A volunteer donated fecal samples before, during, and after a 24-day ketogenic diet (high in fat and protein and virtually without CHOs). He was 40 years old, obese class I ($30 \leq$ BMI < 35), and otherwise healthy. Feces were collected in tubes containing phosphate-buffered saline (PBS) and kept at 4 °C for 1–2 hours before being stored at −80 °C. A total of 22 samples, taken at days −6, −5, −2, −1 (pre-diet), 1, 2, 3, 4, 7, 8, 9, 13, 14, 15, 19, 24 (during diet), and +1, +2, +3, +4, +27, +57 (post-diet), were analyzed. The daily food intake and weight loss were registered during the follow-up.

2.2. DNA extraction

Fecal samples were resuspended in PBS and centrifuged at 4,000 rpm to remove large particles. Supernatants were centrifuged at 14,000 rpm to pellet cells. DNA was extracted using the AllPrep DNA/RNA Mini Kit (QIAGEN).

2.3. Sequencing of 16S rRNA genes

The 16S rRNA genes were amplified using the broad range bacterial primers 8F and 357R [3], with 8-nucleotide barcodes added to the forward primer to tag each PCR product. The PCR conditions were 5 min of initial denaturation at 95 °C followed by 20 cycles of denaturation (30 s at 95 °C), annealing (30 s at 52 °C), and elongation (30 s at 72 °C). The PCR products were purified by vacuum filtration, and equal amounts of the PCR products from different samples were pooled. The mixtures were sent for pyrosequencing on a Genome Sequencer FLX system (454 Life Sciences, Roche).

2.4. Sequence analyses

Sequences with low average quality scores (< 20) and/or short read length (< 250 nt) were discarded. The remaining sequences were checked for potential chimeras using the *chimera.slayer* tool incorporated into the *mothur v1.13.0* package [11]. The taxonomic affiliation of sequences was determined using the *Classifier* tool of the Ribosomal Database Project (RDP)-II [4, 17], with a bootstrap threshold of 70%. Clustering at 97% of sequence identity was carried out using the cluster tool of the *usearch v5.0* package [8]. The closest species of those phylotypes were determined with a *BLASTN* search [1] against the 16S rRNA genes of bacterial isolates in the RDP-II [4]. The Shannon biodiversity index was computed for each sample [12]. The similarity between samples according to bacterial composition was assessed with detrended correspondence analysis (DCA) [10].

2.5. Sequence submission

The entire dataset has been deposited in the Sequence Read Archive of the European Bioinformatics Institute under the study accession number ERP002337.

3. Results and discussion

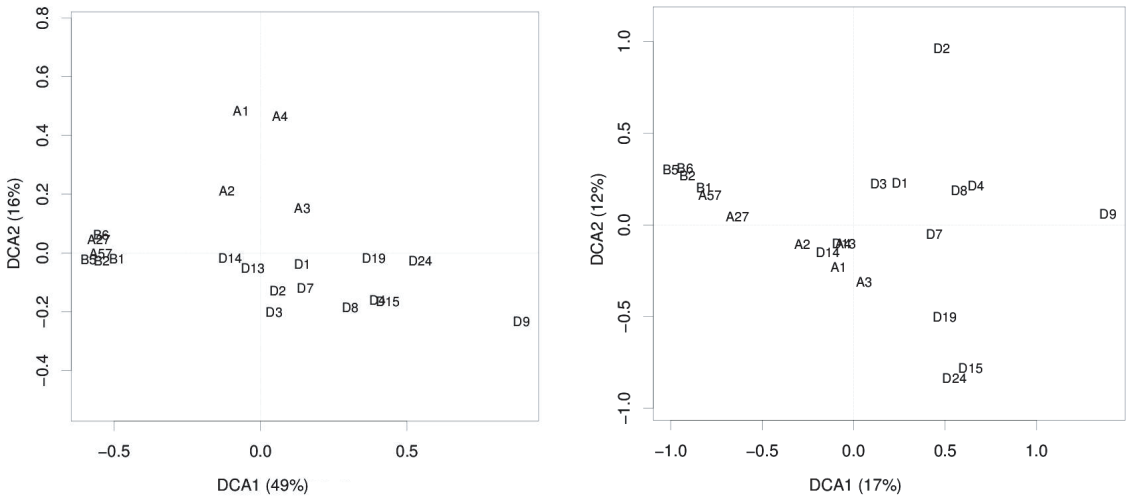
The normal profile of the fecal bacterial community in this subject (based on the samples taken before initiating the dietary intervention) was dominated by bacteria of the Bacteroidetes phylum, mostly (85%) within *Prevotella*. *Bacteroides* included 4.5% of the sequences, and other taxa within Bacteroidetes, less than 1%. Apart from those genera, only *Sutterella* (Proteobacteria), *Roseburia* (Firmicutes), and *Faecalibacterium* (Firmicutes) had a prevalence greater than 1%. The community structure experienced marked alterations immediately after the introduction of a CHO-restricted diet. Within 24 hours, the prevalence of *Prevotella* was reduced by half. This space was filled mainly by the increase in *Bacteroides* and *Sutterella*, although most of the genera also increased their abundance. These changes were rejected in the increase in the bacterial diversity of the community (as measured by the Shannon index) produced by the change in diet. No later than 4 weeks after restoring the normal diet, the composition of the fecal microbiota moved back to its original structure.

This dynamics was consistent at the phylotype level. The same phylotypes dominated within each genus and period (before, during, and after the ketogenic diet), but their relative abundances were altered: phylotypes within *Prevotella* decreased in abundance while those within *Bacteroides*, *Sutterella*, etc., increased in abundance during the CHO-restricted diet, and then returned to their previous levels.

CHO elimination from diet increased the temporal instability in the structure of the community; that is, fecal bacteria showed large fluctuations in their relative abundances. This instability persisted the first days after the subject switched back to his former feeding. DCA plots clearly showed the similarity between the samples taken before changing the diet and those of a few weeks after restoring the normal diet, and the strong shifts and instability in the community composition introduced by CHO restriction (Figure 1).

Our results add to the increasing evidence of the impact of diet on the composition of the gut microbiota. Wu et al. [18] already showed short-term effects of diet on the gut microbiota [18]. They monitored subjects of the *Bacteroides* enterotype when fed on a low- or high-fiber diet. Both studies revealed rapid and reversible changes in the composition of the microbiota in response to change in diet. In our study, the decrease in *Prevotella* under CHO restriction was mainly compensated by the increase in *Bacteroides*, which is consistent with the nutrient preferences reported for these genera [18]. In turn, Wu et al. [18] found greater compositional changes in the high-fiber diet group, as could be expected since it is the diet that correlated negatively with *Bacteroides*, but it was not reported whether the high-fiber diet led to an increase in *Prevotella* [18]. Both studies confirm the resilience of the gut microbiota. The rapid restoration of the community composition is indicative of selective forces imposed by the environment (composition of the diet) and intrinsic to the community (ecological interactions between the gut microbes). Non-selective forces could also be implied, such as recolonization of the gut lumen from the outmost of the mucus layer [14].

The effect of low-CHO diets on fecal bacterial populations was previously evaluated in obese and overweight humans. Duncan et al. [6] found a reduction in *Roseburia*, the *Eubacterium rectale* group, and bifidobacteria as CHO intake decreased [6], whereas Walker et al. [16] found a



(a) (b)

Figure 1: DCA at genus (a) and phylotype (b) levels. Percentages correspond to the fraction of inertia explained by each axis. Samples were taken before (days B6–B1), during (days D1–D24), and after (days A1–A57) a CHO-restricted diet.

reduction in *Collinsella aerofaciens* and an increase in the *Oscillibacter* group [16]. They reported no significant change in the proportion of Bacteroidetes. However, it is unknown whether changes within Bacteroidetes similar to the ones observed in this study occurred in those trials too, since they used a probe targeting the *Bacteroides-Prevotella* group. In any case, it is likely that the responses to dietary changes depend on the initial composition of the microbiota, as it is suggested by the clustering of samples by individual rather than by diet observed by Walker et al. [16].

4. Conclusions

Our study shows the strong dependence of *Prevotella* on carbohydrates in diet, the instability introduced in the gut microbiota by dietary change, and the ability of the gut microbiota in adult humans to return to its original composition after short-term dietary perturbation. Although the initial factors that shape the *Prevotella*-enriched communities are difficult to define, the causal relationship between high levels of *Prevotella* in the gut and subsequent development of obesity should be investigated.

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References

[1] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, *Basic local alignment search tool*, J Mol Biol, 215 (1990), 403–410.

[2] M. Arumugam, J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, et al., *Enterotypes of the human gut microbiome*, Nature, 473 (2011), 174–180.

[3] G. C. Baker, J. J. Smith, and D. A. Cowan, *Review and re-analysis of domain-specific 16S primers*, J Microbiol Methods, 55 (2003), 541–555.

[4] J. R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, et al., *The Ribosomal Database Project: improved alignments and new tools for rRNA analysis*, Nucleic Acids Res, 37 (2009), D141–D145.

[5] C. De Filippo, D. Cavalieri, M. Di Paola, M. Ramazzotti, J. B. Poullet, S. Massart, et al., *Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa*, Proc Natl Acad Sci U S A, 107 (2010), 14691–14696.

[6] S. H. Duncan, A. Belenguer, G. Holtrop, A. M. Johnstone, H. J. Flint, and G. E. Lobley, *Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces*, Appl Environ Microbiol, 73 (2007), 1073–1078.

[7] A. Durbán, J. J. Abellán, N. Jiménez-Hernández, A. Latorre, and A. Moya, *Daily follow-up of bacterial communities in the human gut reveals stable composition and host-specific patterns of interaction*, FEMS Microbiol Ecol, 81 (2012), 427–437.

[8] R. C. Edgar, *Search and clustering orders of magnitude faster than BLAST*, Bioinformatics, 26 (2010), 2460–2461.

[9] H. J. Flint, E. A. Bayer, M. T. Rincon, R. Lamed, and B. A. White, *Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis*, Nat Rev Microbiol, 6 (2008), 121–131.

[10] G. P. Quinn and M. J. Keough, *Experimental Design and Data Analysis for Biologists*, Cambridge University Press, 2002.

[11] P. D. Schloss, S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, et al., *Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities*, Appl Environ Microbiol, 75 (2009), 7537–7541.

[12] C. E. Shannon, *A mathematical theory of communication*, Bell Syst Tech J, 27 (1948), 379–423.

[13] P. J. Turnbaugh, M. Hamady, T. Yatsunenko, B. L. Cantarel, A. Duncan, R. E. Ley, et al., *A core gut microbiome in obese and lean twins*, Nature, 457 (2009), 480–484.

[14] P. Van den Abbeele, T. Van de Wiele, W. Verstraete, and S. Possemiers, *The host selects mucosal and luminal associations*

of coevolved gut microorganisms: a novel concept, FEMS Microbiol Rev, 35 (2011), 681–704.

[15] A. Vrieze, F. Holleman, E. G. Zoetendal, W. M. de Vos, J. B. Hoekstra, and M. Nieuwdorp, *The environment within: how gut microbiota may influence metabolism and body composition*, Diabetologia, 53 (2010), 606–613.

[16] A. W. Walker, J. Ince, S. H. Duncan, L. M. Webster, G. Holtrop, X. Ze, et al., *Dominant and diet-responsive groups of bacteria within the human colonic microbiota*, ISME J, 5 (2011), 220–230.

[17] Q. Wang, G. M. Garrity, J. M. Tiedje, and J. R. Cole, *Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy*, Appl Environ Microbiol, 73 (2007), 5261–5267.

[18] G. D. Wu, J. Chen, C. Hoffmann, K. Bittinger, Y. Y. Chen, S. A. Keilbaugh, et al., *Linking long-term dietary patterns with gut microbial enterotypes*, Science, 334 (2011), 105–108.

[19] H. Zhang, J. K. DiBaise, A. Zuccolo, D. Kudrna, M. Braidotti, Y. Yu, et al., *Human gut microbiota in obesity and after gastric bypass*, Proc Natl Acad Sci U S A, 106 (2009), 2365–2370.