

Manipulation of Nutrient Absorption by Diet and Antibiotics

Preview for “Basolo et al. (2020) Effects of underfeeding and oral vancomycin on gut microbiome and nutrient absorption in humans. *Nat. Med.* 26, 589-598”

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INTRODUCTION

At the current time we face a severe global health problem of overweight, with the prevalence of obesity as high as 42.4% in 2018 in the U.S. alone. Thus, studying nutrient absorption and energy balance in the human is greatly related to the well-being of taxpayers. As the proverb goes, you are what you eat. The food we eat undergoes digestion and absorption prior to the utilization in the body. Gut microbiota, the collective community of microorganisms in our gut, acts as an inalienable facilitator of nutrient absorption. Emerging evidence indicates that microbiota participate in a broad range of physiological events, hence modulates nutrition homeostasis and contributes to disease susceptibility¹. As for nutrient processing, gut microbiota contribute directly to the processing of dietary polysaccharides and fibers through fermentation, meanwhile yielding short-chain fatty acids¹. Moreover, they can modify gene expression related to metabolism and nutrient absorption in the gut. For instance, compared with germ-free mice, those with gut microbiota show suppressed expression of a circulating lipoprotein lipase inhibitor in the gut, which may contribute to the adiposity². Moreover, transplantation of microbiota from obesity model mice to germ-free mice leads to a greater increase in body fat than the lean counterpart³, substantiating microbiota as a direct contribution of host nutrient status.

Given the abundant evidence in the rodent model and significant translational potential of microbiota treatment on human obesity, there have been several clinical trials evaluating the functional role of the human gut microbiome in nutrient absorption. During clinical trials, researchers face more difficulties compared with animal models, including but not restricted to the limited number of patient or healthy individual

volunteers, ethically limited pharmaceutical or genetic perturbation, and restricted measurements on samples.

One exact absorption measurement that researchers rely on is the loss of energy in the stools and urine in the total caloric intake. A clinical trial of 12 lean and 9 obese individuals revealed that lean individuals lost relatively less energy in stools with a 3400-kcal per day diet than with 2400 kcal per day diet⁴. This difference, albeit marginal, is absent in the obese group. This change is also associated with phylum-level changes in the fecal bacterial community structure, with 20% increase in Firmicutes and a corresponding decrease in Bacteroidetes. However, another trial with 57 obese individuals conclude no change in the energy harvest (feces calories) or other parameters including adipocyte size, and whole-body insulin sensitivity after microbiota perturbation by oral antibiotic vancomycin⁵.

Given such complexity, Basolo *et al.* carefully designed this inpatient clinical trial to clarify the causal role of randomized dietary and antibiotic interventions on nutrient absorption in the human gut⁶. They found that underfeeding and vancomycin administration alter microbial composition in the gut and indeed affect nutrient absorption.

RESULTS

Basolo *et al.* conducted an inpatient study with high BMI people that have impaired glucose tolerance and obesity. To carefully measure how nutrient absorption in the gut is altered under different feeding conditions (calorically distinct diets) and by gut microbiota manipulation using oral antibiotic administration. They composed their research in two phases where Phase I was composed of overfeeding (OF) vs underfeeding (UF) of the patients for 3 days and Phase II contained oral vancomycin vs placebo administration. To examine the percent calorie loss by stool (calories excreted in stool), they regularly examined the stool samples of patients in the beginning, midpoint, and end of each trial. They found that both UF and vancomycin administration

lead to reduced nutrient absorption and increased percentage of stool calorie loss which is assessed by the ratio of calories lost in the stool compared to initial caloric intake.

They then examined whether reduced nutrient absorption was due to a shift in the gut microbiome. Using amplicon and metagenomic sequencing, they analyzed the gut microbial composition to identify candidate effector microorganisms. qPCR-based quantification of 16S rRNA gene copies showed a significant increase in overall gut microbial colonization during UF vs OF. Four 16S ribosomal RNA sequence variants that were altered between diet arms (UF to OF or OF to UF) were identified as *A. muciniphila*, *Bacteroides coprocola*, a *Lachnospiraceae* sp. and a *Ruminococcus* sp, suggesting that these diet-responsive bacterial species can contribute to the nutrient absorption in the gut. They also performed metagenomic sequencing both for diet arms and vancomycin vs placebo intervention. Sequencing results identified nine bacterial species that showed significant differences between the two diet arms. Consistent with their finding by qPCR-based quantification of 16S rRNA, they identified an increase in *A. muciniphila*, but not the other three species, upon UF. On the other hand, oral vancomycin vs placebo administration resulted in a significant and reproducible reduction in microbial diversity, as assessed by both 16S rRNA gene sequencing and metagenomic sequencing. Interestingly, they identified multiple bacteria species that are increased upon vancomycin treatment. However, although UF vs OF and vancomycin vs placebo displayed similar results regarding stool calorie loss, these interventions had different effects on gut microbiota. While vancomycin administration led to widespread alterations in the gut microbial community, UF and OF resulted in changes in only a few bacterial species.

Next, to identify bacterial factors and metabolic pathways that are involved in nutrient absorption, they utilized HUMAnN2 (the HMP Unified Metabolic Analysis Network), a search strategy that enables functional profiling of presence and abundance of microbial pathways. They found that vancomycin treatment compared to placebo causes a reduction in 3 out of 290 metabolic pathways. Two of the three metabolic pathways were both associated with the fermentation of sugars to short chain fatty acid butyrate, an end-product of bacterial metabolism in the gut. Intriguingly, they found that butyrate levels were decreased during both UF and vancomycin treatment, suggesting a reduced

capacity of gut microbiome metabolism. During both interventions, they also observed a reduction in bile acid, deoxycholic acid levels, which may implicate preservation of gut barrier function. To test whether gut barrier permeability is altered upon these interventions, they examined the levels of zonulin and lipopolysaccharide (LPS), that are known to be increased upon increased gut permeability. They found OF causes slightly increased zonulin but unchanged LPS levels. On the other hand, vancomycin did not alter levels of zonulin and LPS. Finally, they did not find significant differences in substrate oxidation rates, fat storage or glucose metabolism during vancomycin administration.

SIGNIFICANCE AND FUTURE DIRECTIONS

In this paper, the authors demonstrated that the comparable stool calorie loss increased when UF versus OF and oral vancomycin versus placebo. This increase affects the gut microbiota community. UF has modest perturbation of gut microbial community structure compared to OF, while oral vancomycin reduced the diversity and marked shifts in gut bacterial relative abundances. After sequencing, they found out that the gut microbiota reduced butyrate production or bacterial metabolism during vancomycin treatment.

Significance

- 1) Previous literature indicates that *A. muciniphila* is increased in both UF and vancomycin condition, which may reduce gut permeability by increasing turnover of the mucous layer^{7,8}. This study provides a mechanistic explanation to the increased stool calorie loss.
- 2) Their results showed that both deoxycholic acid and butyrate reduced after UF or oral vancomycin treatment. Studies showed that deoxycholic acid interferes with gut barrier function⁹, while butyrate and other short-chain fatty acids stimulate intestinal barrier formation. Taken together, this paper suggests that caloric intake and antibiotics may disrupt the balance between these, hence altering nutrient absorption.

3) In the field of energy balance, the role of human gut microbiome in nutrient absorption has been a hot topic. This paper provides a strong evidence for the involvement of the human gut microbiome in nutrient metabolism.

Discussion

4) The authors had conflicting results regarding phylum-level bacterial relative abundance, compared to their previous report, which indicates that there are other factors like food composition that may affect bacterial diversity at the phylum level.

5) Other studies didn't show oral vancomycin has an impact on stool calories¹⁰, which may be due to their overlook on the exact measurement of ingested calories. The stool calories absorbed percentage of ingested calories reflect "metabolizable" calories. This is critical in energy balance studies.

6) This paper did not find evidence that gut permeability decrease led to reduced nutrient absorption. However, colonic transit time may also affect energy content in stool. Under UF condition, rapid transit could have decreased contact time with epithelium, thus, decreasing absorption.

7) The authors mentioned that calorie loss in the urine showed similar results with stool calorie loss, which suggests a cross-tissue coordination of energy balance under a currently unknown mechanism.

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