A computational framework for human-microbial co-metabolism. By Almut Heinken and Ines Thiele, *Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belval, Luxembourg*.

Introduction

The human gut microbiota is well known to influence host health and wellbeing (1). Due to metagenomic analysis of the human gut microbiome, our understanding of "who is there" is steadily increasing (2). However, it remains unclear what microbial players perform important metabolic functions (3). A common approach is the comparison of the metabolome for germfree and ex-germfree or conventional animals (4), however, such approaches are impossible to perform for humans. Furthermore, the effect of the microbiota on host whole body metabolism has rarely been studied (3). The constraint-based reconstruction and analysis (COBRA) approach allows the metabolic modeling of host-microbe interaction by setting well-defined species boundaries between host and microbes while allowing interspecies metabolic exchange (5). Here, we present the first constraint-based model of the human host and a community of up to 11 gut microbial species including commensals, probiotics, opportunistic pathogens, and pathogens. We apply the modeling framework to predict the host body fluid metabolome in "germfree" human, and in the presence and absence of commensal and pathogenic species. Our modeling approach reveals significant differences in the metabolic potential of commensal and pathogenic microbes to affect host metabolism.

Results

To establish a constraint-based host-microbe modeling framework, the currently most extensive global human reconstruction Recon2 (6) was joined with 11 manually curated and validated gut microbe reconstructions (7-15). The "germfree" condition (GF) included only Recon2. Furthermore, Recon2 was joined with a commensal community including *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, *E. coli* strain MG1655, *Lactobacillus plantarum* and *Streptococcus thermophilus* (condition HS/5CM), and with a pathogenic community including *Helicobacter pylori*, *Klebsiella pneumoniae*, *Samonella enterica* subsp. typhimurium, E. coli O157:H7 strain Sakai and *E. coli* O157:H7 strain EDL933 (condition HS/5PM). Finally, Recon2 was joined with the five commensals, the five pathogens and additionally *Lactococcus lactis* subsp. cremoris (condition HS/All).

The host body fluid metabolome was predicted for the constructed conditions while simulating a "Western" diet consisting of 50% carbohydrates, 35% fat, and 15% protein. A total of 342 metabolites was secreted under the given dietary regime, of which only 11 could not be secreted by the germfree host. However, the quantitative secretion of a variety of metabolic products, including amino acids, carbohydrates, fatty acids, hormones and nucleosides, varied significantly with and without the presence of the microbes. The impact of the microbes on body fluid secretion was quantified as follows: The maximally possible secretion flux for all 342 secreted metabolites for all conditions was calculated. The achieved percentage of this maximal value was

compared for conditions GF, HS/5CM, HS/5PM and HS/All. To further elucidate the influence of individual microbes on the body fluid metabolome, the achieved percentage was calculated for Recon2 joined with one microbe at a time.

The results revealed that the five commensals alone increased the secretion of 173 body fluid metabolites from less than 90% of the maximally possible absolute value to >99% (saturation) on the simulated Western diet and had a larger impact on the predicted body fluid metabolome than all 11 microbes combined (Figure 1). In presence of all microbes, only 109 metabolites were increased from less than 90% of the maximally possible absolute value to >99%. The decrease was due to enforcing a low level of microbe growth, which consumed resources that were thus no longer available for host biosynthesis of the secreted metabolites. Furthermore, condition HS/5PM was unable to saturate the secretion of any metabolites. In fact, the commensals *B. thetaiotaomicron* (condition HS/BT) and *E. coli* MG1655 (condition HS/EC) were able to affect the host body fluid metabolome more strongly than the five pathogens combined (Figure 1). The difference in the predicted impact on the host body fluid metabolome was due to the significantly lower range of metabolites usable by host synthesized by the pathogens compared with the commensals (data not shown). Thus, the pathogenic community was poorer in the metabolic capabilities encoded in its collective genome. This led to a lower capability to produce microbial products that could be consumed by the host and influence the predicted host body fluid metabolome.

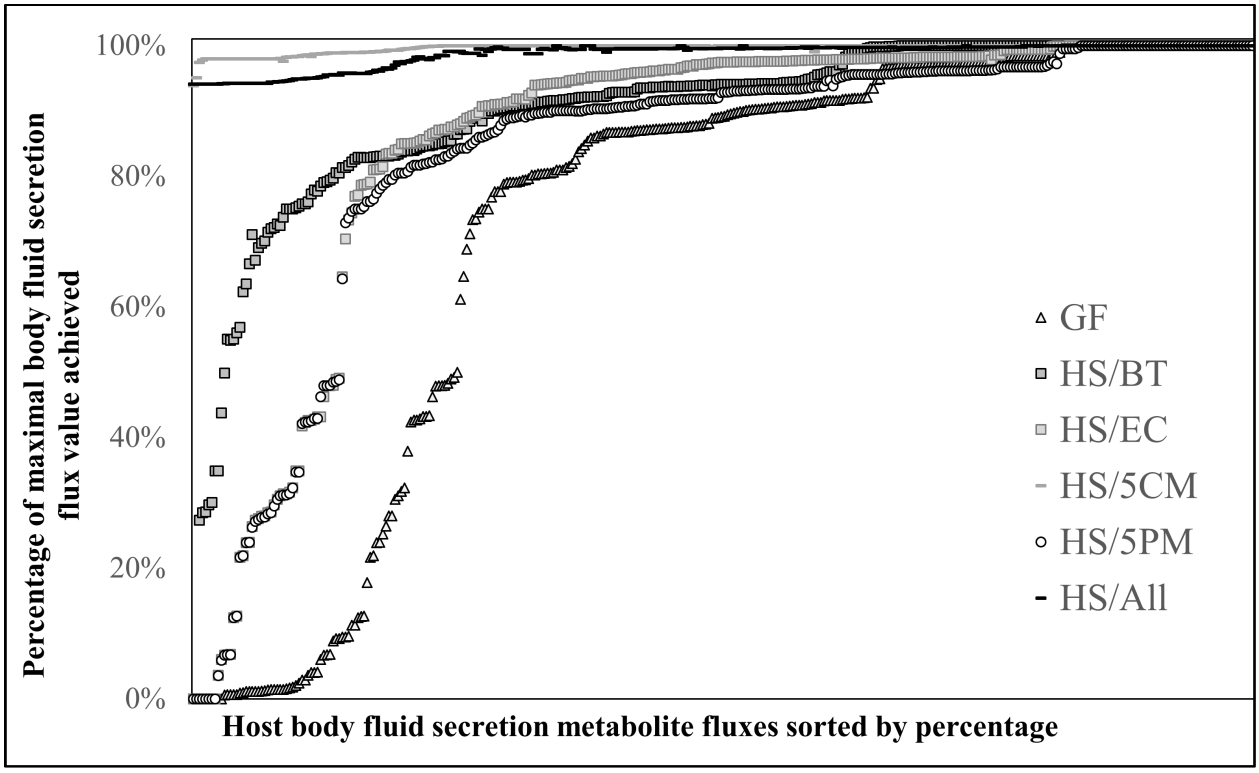

Figure 1: **Host body fluid metabolite secretion achieved on a Western diet in germfree host and the host-microbe models.** For all 342 metabolites which could be synthesized and secreted into the body fluid compartment under the given dietary constraints, the maximal body fluid secretion value achieved out of all models was computed. Subsequently, the percentage of the maximal value achieved in each model was calculated. The achieved percentages for the 342 metabolites were plotted sorted by achieved percentage of the maximal value. GF = germfree human (Recon2), HS/BT = Recon2 + *B. thetaiotaomicron*, HS/EC = Recon2 + *E. coli* MG1655, HS/5CM = Recon2 + five commensal microbes, HS/5PM = Recon2 + five pathogenic microbes, HS/All = Recon2 + all 11 microbes.

Discussion

We demonstrate here that a computational framework can predict the complex metabolic effects of the human gut microbiota on its host. The framework enables the prediction of the host body fluid metabolome (e.g., blood, urine), which is well known to be influenced by the microbiota (4). Using metabolic modeling, high-throughput metabolomic data could be put in context. We showed that a commensal microbial community had a significantly higher potential to affect host metabolic functions than a pathogenic community due to possessing more encoded functions in its collective genome. As a result, a wider range of microbe-derived compounds was available to the host in the presence of the commensals, which caused the described global effect on the predicted host body fluid metabolome (Figure 1). This suggests that an increase in pathogenic microbes and a loss of commensal microbes would result in the depletion of important metabolic functions able to influence whole host body metabolism. Our framework can be readily

expanded by any number of additional gut microbial reconstructions, enabling further insight into the impact of key species and the collective gut microbiota on human metabolism.

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