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LETTER TO THE EDITOR

The use of fecal samples for studying human obesity

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The impact of colonic bacteria on obesity in humans should not be underestimated. The breakdown of otherwise indigestible compounds by the colonic microbiota is an important function accomplished by syntrophic interactions, the products of which (mainly short chain fatty acids—SCFA—) may account for more than a 10 % of the human energy requirement. It is estimated that a slight increase of just 1 % in the microbial metabolic activity may increase an input of energy of 20 kcal day^{-1} to the host (based on a diet of 2000 kcal day^{-1}) which could lead to a weight gain of approximately 1 kg per year [1]. On the other hand, production of SCFA may lead to satiety by inducing the secretion of gut hormones [2]. Therefore, it is crucial to understand factors that elicit changes in the microbiota and its fermentation efficiency since it may impact host energy balance as well.

Accurate in situ measurement of production of SCFA in humans is restricted not only due to the fact that such measurements in the portal vein are not representative when compared to the systemic circulation or in the lumen of the colon, but also because of the ethical constraints and the high costs of studies in humans. Furthermore, the analysis of a specific dietary test compound in humans cannot be easily discriminated from other sources of fibers present in the host diet, unless a labelled compound is used. Therefore, most studies evaluating the impact of the intestinal microbiota and its metabolic products on host health have been conducted in animal models, human feces, in vitro batch cultures or continuous culture systems simulating the human gastrointestinal tract.

Determining the fermentability of indigestible compounds by the colonic microbiota is relatively easy in in vitro batch cultures and continuous culture systems simulating the human gastrointestinal tract. Earlier studies indicate that the use of these tools offers a rapid approach for the estimation of the energy input from specific substrates to the host. Furthermore, these techniques are faster, cheaper and ethically superior than in vivo studies. Fecal samples are used to conduct research in this area. As previously observed, the analysis of feces allows the estimation of a wide catalogue of information from individuals, their unique genetic fingerprinting, pathogens and food habits. Furthermore, numerous metabolites can be analyzed from fecal material. Non-invasive molecular techniques increase the resolution of the analysis of feces. Therefore, considering fecal matter as waste is called into question. However, the analysis of feces has been considered as an indirect and non optimal method to study the role that microbiota plays in obesity. Raoult and Henrissat [3]

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suggest, instead, to study the small intestine to overcome the bias that studying feces implies. However, the small intestinal community is much simpler compared to the colonic microbiota with respect to diversity, number of different species present, metabolic activity and microbial density. In contrast, the fecal microbiota has been considered representative to the luminal microbiota of the colon [4].

Therefore, despite that the analysis of the small intestine microbiota and its nutritional modulation unveils an important impact of this microbiota on host health, including obesity, there are some observations that need to be taken into account before underestimating the potential of fecal samples as effective tools to study part of the aetiology of human obesity [3]. Physiologically, the small intestine is the part of the human body that is thought to be key in the development of obesity due to that most of the ingested calories are absorbed. Since the diet of obese subjects includes simple sugars and fats which are mainly absorbed in the small intestine, the impact of the colonic microbiota could be considered very little due to the fact that they do not metabolize such compounds [3]. Indeed, the place for the main absorption of dietary sugars and fats is the small intestine. As a consequence, it is very likely that the microbiota from this region may have a considerable impact on host physiology. However, fats are not fully absorbed in the small intestine. Contrary to specified by Raoult and Henrissat [3] there is evidence that fats may escape absorption in the small intestine and can reach the large intestine where they could be metabolized by colonic bacteria, which may have a profound impact on the gut homeostasis [5]. In addition, the authors' arguments consider obesity as mainly associated with an imbalance in energy consumed when compared to energy expenditure, which seems to be incomplete given the recent mechanisms proposed underlying obesity and the role of the colonic microbiota in this. Accumulating evidence from animals, in vitro and human studies indicates that there is an overactive and aberrant composition and metabolic activity in the colonic microbiota from obese subjects when compared to lean [2]. Furthermore, it is believed that the additional energy input to the host from its microbiota could be used for gluconeogenesis and de novo hepatic triglyceride synthesis [1].

The fact that there is a relatively low concentration of bacteria in the small intestine compared to the feces (10^2–10^7 compared to 10^{12} CFU/ml, respectively) [6] also supports the impact of the colonic microbiota in human obesity. The low density of the microbiota found in the small intestine is the result of the secretion of bactericidal substances and the rapid luminal flow in this region [6]. In addition, such shorter transit time is believed to exhibit a minor contribution to bioconversions of metabolites [4]. In contrast, more than 5 million genes resulting from the combination of the proteins/enzymes encoded by the genomes of the highly dense population of bacteria found in the human colon naturally confers additional molecules and special functions. It has been estimated that this can be greater than the host's own genetic potential by two orders of magnitude [7]. In addition, shotgun metaproteomics highlights an enriched community in carbohydrate metabolism, translation and energy production in feces [8]. This, naturally, alters the (human) homeostasis which is probably more likely to affect energy balance.

The observation that the antibiotics that cause weight gain do not dramatically modify the fecal composition, may not provide sufficient evidence about the impact of colonic microbiota in obesity. Despite the controversial results, some studies in children suggest that the impact of antibiotics in obesity consist of altering the diversity and proper establishment of the microbiota. Furthermore, the fact that the composition dynamics and activity of the colonic microbiota respond to antibiotics and that such effects are reflected either in weight gain or loss clearly suggest the implications of colonic microbiota in health. Furthermore, the promising results of fecal microbial therapies in subjects with metabolic disorders or certain pathologies demonstrate the value of a fecal donation and its utility for studying the role that the gut microbiota plays in human obesity.

Though interesting, the suggestion of studying the microbiota in the small intestine carries the inconvenience of the availability of samples and the high invasive procedures to obtain them. The small intestine in healthy individuals is poorly accessible and volunteers from whom samples are taken via intraluminal naso-ileal catheters are fasted and they often require luminal flushing which represents a bias for the analysis of a representative community from the small intestine [6]. Furthermore, despite that in some cases it is possible to obtain (non-invasively) effluent from the ileum (stoma), patients from whom samples are derived have previously suffered from a critical clinical condition such as colon cancer and it is very likely that these subjects require stoma-related medication. In addition, previous studies emphasize: (1) the fluctuation over time in the composition of the microbial communities from ileostomy effluent in contrast to a more stable community observed in the colon [9] and (2) the possibility that some oxygen may penetrate the stoma which alters the composition of the small intestinal microbiota benefiting the growth of facultative anaerobe populations.

We, however, also acknowledge the limitations of using feces for studying human obesity. In some circumstances, the use of fresh human microbiota is not possible because donors live far away from the laboratory or because they are not continuously available to repeatedly participate at various times during the study. In addition, the use of feces for fermentation studies requires the strict anaerobic handling of the samples, although this is also the case for samples from the small intestine. However, collecting feces is a fast and harmless method which could be standardized. To guarantee a constant inoculum over time for different studies, most studies use prepared and stored inocula. In these cases, the use of frozen feces provides more flexibility for this type of experiments.

Therefore, we argue that the analysis of feces supported by the revolutionary molecular techniques available can incorporate (non-invasively) a complete picture of which microbes play a crucial role in our health and how, metabolically speaking, they impact our lives, also in obesity. Restricting the view to small intestinal microbes [3] is being oblivious of the role of colonic bacteria, and hence feces, in obesity research. This should not be underestimated.

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Conflict of interest The authors declare that they have no conflict of interest.

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