

Gnotobiotic mice housing conditions makes the difference in the context of obesity!

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Faecal microbiota transplantation (FMT) has been initially used in medical centres to successfully treat recurrent or refractory intestinal infections with *Clostridioides difficile*. Eisman *et al* published the first scientific paper describing the successful treatment using FMT to treat pseudomembranous enterocolitis.¹ Nowadays, FMT is widely used in humans with the attempt to treat many non-gastrointestinal diseases (eg, obesity, diabetes, liver diseases, autism, neurodegenerative diseases) but still with controversial outcomes.

In preclinical studies, FMT is extensively used because it is possible to determine the contribution of the gut microbiota to a given pathology or condition since the composition of the transplanted microbiota is known. Interestingly, the development of germ-free (GF) animals started in the 1940s² and in 1964, host energy metabolism has been comprehensively studied using GF mice, when studies on GF and conventional rats suggested that the gut microbiota was interfering with the absorption of fats.³

In the field of obesity and metabolic disorders, a 2006 seminal study by Turnbaugh *et al* unequivocally demonstrated that colonising GF mice with the gut microbiota obtained from *ob/ob* mice (ie, genetically obese mice) induced higher fat mass gain as compared with the gut microbes from lean mice and this effect was independent of food intake.⁴ Nowadays, this technique is widely used to allow researchers to better mimic human physiology by transplanting human faecal samples to GF mice or in conventional

mice depleted of their gut microbiota using antibiotics and/or laxatives.⁵ However, it is also largely debated that FMT techniques lack standardisation and are suffering from careful experimental design in view of obtaining optimal results.⁶

Although several recommendations exist, in Gut, Daoust *et al* elegantly studied and highlighted how FMT experiments should be carefully designed.⁷ Their excellent study demonstrates the need to standardise the condition of experimentation of GF mice in order to ensure reproducibility of experiments. They discovered that different gnotobiotic housing conditions such as GF versus specific pathogen free (SPF) critically influence the liver phenotype associated with transfer of faecal microbiota in the context of obesity.⁷ In their study, the authors aimed to determine the impact of distinct housing conditions (SPF vs GF) on the metabolic phenotype of GF mice fed a high-fat high-sucrose (HFHS) diet and colonised with the gut microbiota of donors treated with HFHS alone or with specific polyphenols (cranberry proanthocyanidins—PAC), a condition known to improve metabolic health and liver health in obese mice.

The striking discovery of Daoust *et al* is that FMT using same donors and same faecal slurries induce opposite changes in GF recipient mice if they are living in GF versus SPF conditions. When housed in the GF sector, GF mice that received FMT from HFHS-PAC mice exhibited a significant decrease in liver weight and hepatic triglycerides and cholesterol accumulation as compared with those colonised with faeces from vehicle-treated HFHS mice. In contrast, mice kept in SPF conditions receiving an FMT HFHS+PAC showed a tendency for increased liver weight and significantly greater liver triglycerides and cholesterol levels as compared with their FMT HFHS counterparts. Faecal slurries from HFHS-PAC mice did not impact body weight gain or food intake, regardless of housing conditions. However, although this was not discussed in the paper, it is also striking to see that both HFHS and HFHS-PAC mice housed in

GF conditions had higher basal fasting glycaemia and insulinaemia, thereby leading to a basal insulin resistance state twice higher than the mice housed in SPF conditions. This suggests that mice in GF housing are more insulin resistant than in SPF conditions. Whether this could have contributed to the liver phenotype is not explored. In addition, and surprisingly, the liver seems to be the only metabolic tissue affected by the experimental conditions. Further experiments might investigate other tissues such as for instance the pancreas, the adipose tissue or the brain.

To further explore the reasons of such important discrepancies on the liver phenotype, the authors analysed the composition of the gut microbiota and metabolites from the donors and recipient mice. As expected, the PAC induced specific changes in the gut microbiota of the HFHS, but the housing conditions exerted the strongest influence on the colonisation of the gut microbiota. Although, 43 bacterial taxa were shared between the inoculum and the colonised mice housed in both GF and SPF conditions, few taxa were shared only between the inoculum and mice housed in the GF or the SPF sector. Therefore, several changes in microbiota composition were dependent on housing conditions. Surprisingly, although the phenotype is stronger in the SPF sector, the authors could not detect specific functional changes and concluded that 'the absence of significant changes in the SPF sector suggests the compositional changes of the gut microbiota in the SPF sector were not robust enough to induce functional alterations in the gut microbiota'. We are not fully aligned with this conclusion because other specific metabolites associated with liver metabolism could have been targeted. For instance, bile acids and oxysterols are derived from hepatic cholesterol and are known to strongly influence liver and metabolic health.⁸ But many others potential metabolites and factors produced by the gut microbiota could be part of the overall phenotypic differences.⁹ In their study, the authors found specific changes in liver cholesterol and triglyceride levels, but no specific molecular or mechanistic aspects were investigated in the liver or in the intestinal tract to further elucidate the exact causes of discrepancies between the SPF and the GF condition.

In addition to these points, it is also important to mention that the studies have been done using a pool of faecal material from the different groups, whether this could also be a potential confounding factor is still a matter of debate. Indeed,

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a wide range of experimental designs are seen in the literature, with some studies using one recipient per donor, whereas others are using a pool of faecal samples from several donors for FMT into one recipient animal.⁶ To account for experimental and individual variability, one should use several recipients per donor or per pooled samples, but this is exactly what it was done in the current study. Moreover, whether such kind of distinct phenotype is limited to the use of GF mice or would also be observed in conventional mice depleted of their gut microbiota using antibiotics and/or laxatives remains to be proven. Finally, the gut microbiota is not limited to the gut bacteria but other major representative such as viruses, phages and fungi may have contributed to the current divergences of phenotype.¹⁰

In conclusion, this very elegant and well-designed study unequivocally shows that a simultaneous colonisation of GF mice in distinct environments from the same animal facility (GF vs SPF sector) can lead to differential metabolic phenotypes in obese mice, such as an improvement *vs* exacerbated liver steatosis. This study shows the importance of preclinical models such as gnotobiotic mice to assess causality of specific bacterium or complex microbiota, but also highlights the key impact of environmental pressure.

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