Childhood stunting model induced by chronic undernutrition in gnotobiotic mice

Marion Darnaud[1], Pascaline Bogeat[1], Hélène Dugua[1], Martin Schwarzer[2,3], François Leulier[2], Andrea Tamellini[1]

[1]BIOASTER, Preclinical Models & Imaging Unit, 69007 Lyon, France

[2]Institut de Génomique Fonctionnelle de Lyon (IGFL), Université de Lyon, Ecole Normale Supérieure de Lyon, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, Unité Mixte de Recherche 5242, 69364 Lyon, Cedex 07, France

[3]Present address: Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Nový Hrádek, Czech Republic

INTRODUCTION

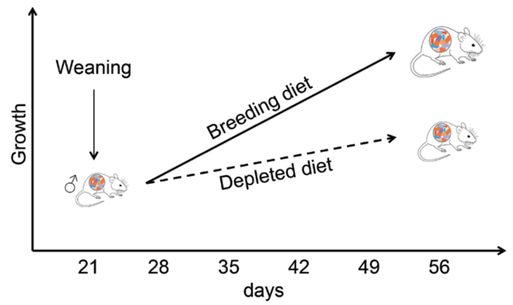
- Childhood chronic undernutrition affects metabolism and hormonal signaling leading to stunting syndrome, which is associated with higher juvenile mortality rate and correlate with immune, cognitive and gut mucosal barrier deficits in adulthood (1, 2).
- Recent studies have highlighted the role of gut microbiota on juvenile growth (3). Germ-free mice colonized with undernourished child microbiota recapitulate the impaired growth phenotype (4). Besides, monocolonized mouse models have been used to demonstrate that specific bacterial strains can promote juvenile growth, interact with the somatotropic hormone axis and buffer the adverse effects of childhood chronic undernutrition (5). Moreover, another preclinical study has shown that gut microbiota, and possibly short-chain fatty acids produced by bacteria, restores bone mass, which is likely mediated by the insulin-like growth factor 1 (IGF-1) (6). Taken together, these findings suggest that specific bacterial strains can reshape undernourished children microbiota and offer opportunities to improve juvenile growth and health.
- Here we introduce a representative **preclinical model of childhood stunting induced by chronic undernutrition in gnotobiotic mice featuring a simplified and controlled murine microbiota,** which recapitulates the phenotype observed with a complex murine microbiota. We believe this gnotobiotic mouse model is of interest **to further decode the impact of gut microbiota and specific bacterial strains on juvenile growth, and develop novel microbiota-directed therapeutics.**

MATERIALS & METHODS

- Animal experiments were approved by the committee for protection and use of experimental animals of the ANSES Laboratory of Lyon. **C57Bl/6J mice were housed in isolators, supplied with autoclaved tap water and irradiated sterile feed ad libitum.** Germ-free (GF), gnotobiotic (GNOTO) and specific opportunistic pathogen free (SOPF) male pups were weaned at day 21 and randomly distributed in subgroups either fed with depleted diet **(DD; 4% proteins by weight, 2.5% fat, 3.5 kcal/g)** or control breeding diet **(BD; 21% proteins, 5% fat, 3.4 kcal/g).** Body weight and size were recorded weekly using isoflurane anesthesia, as well as feed and water intake. Animals were sacrificed at D56 for the collection of blood, cecum, femur and tibia. **GF mice were monitored weekly to confirm the absence of microbes, and GNOTO mice controlled microbiota was assessed by strains specific qPCR.**
- Serum IGF-1 levels were measured in triplicate by ELISA kits (R&D systems) according to manufacturer's instructions.
- Statistical analysis was performed with GraphPad Prism 7.0 software using Mann-Whitney test for comparison between two groups.

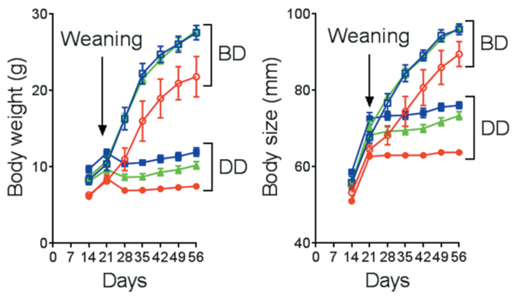
Experimental design

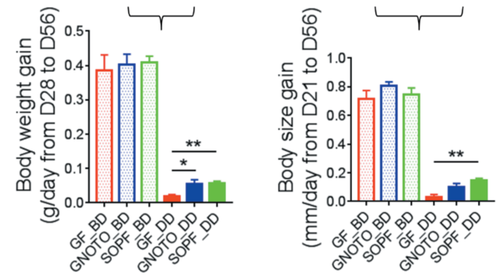
3 conditions \ 6 isolators, 2 diets, N mice	Breeding diet (**BD**)	Depleted diet (**DD**)
Germ-free (**GF**)	4	4
Gnotobiotic (**GNOTO**)	6	8
Specific opportunistic pathogen free (**SOPF**)	10	10



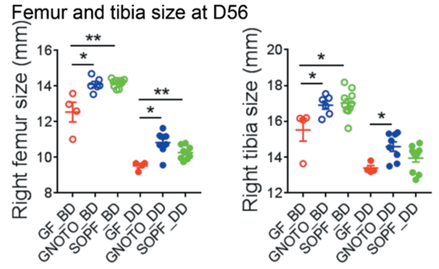
Results

- Body weight and size growth curves during the juvenile period and associated daily gain

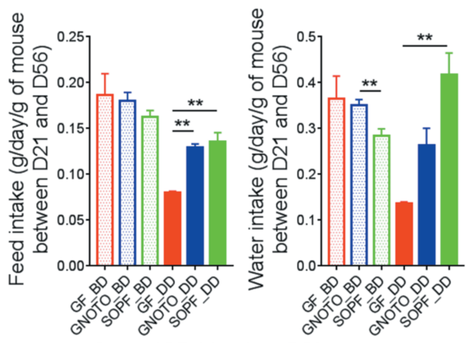




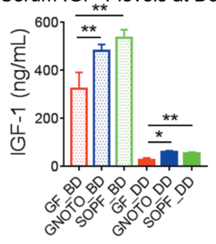
- Femur and tibia size at D56



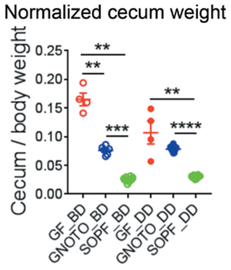
- Normalized daily feed and water intake



- Serum IGF-1 levels at D56



- Normalized cecum weight at D56



All data are expressed as the mean ± standard error of the mean
*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

CONCLUSION

- Our results confirm that GF mice are more altered by chronic undernutrition than SOPF mice. Gut microbiota is therefore beneficial to buffer the deleterious effect of chronic undernutrition on juvenile growth.
- GNOTO mice recapitulate the stunting phenotype observed in SOPF mice, especially for:
  - Body weight
  - Body size
  - Bone size
  - IGF-1 levels
- **GNOTO mice, featuring a simplified murine gut microbiota, are thus necessary and sufficient to reproduce the stunting model obtained with a complex microbiota.**
- **This new gnotobiotic mouse model offers the potential to study the mechanistic basis of the interactions between diet, microbiota and the host during chronic undernutrition, and allows the development of novel microbiota-directed therapeutics (such as probiotics, metabolites produced by bacteria, vaccines, etc.) to target childhood stunting.**

REFERENCES

1- Prendergast et al. The stunting syndrome in developing countries. Paediatr Int Child Health 2014.
2- Blanton et al. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science 2016
3- Schwarzer. Gut microbiota: puppeteer of the host juvenile growth. Curr Opin Clin Nutr Metab Care 2018.
4- Blanton et al. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science 2016
5- Schwarzer et al. Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition. Science 2016.
6- Yan et al. Gut microbiota induce IGF-1 and promote bone formation and growth. PNAS 2016.

ACKNOWLEDGMENTS

BIOASTER is supported by the Programme of Investments for the Future (PIA). We also would like to acknowledge Stéphanie Geoffroy and Anne-Laure Bulteau from IGFL for performing the IGF-1 titration.

Keystone Symposia Conference, Manipulation of the Gut Microbiota for Metabolic Health / Microbiome, Host Resistance and Disease, March 4-8. 2018, Banff, Canada

marion.darnaud@bioaster.org | bioaster.org