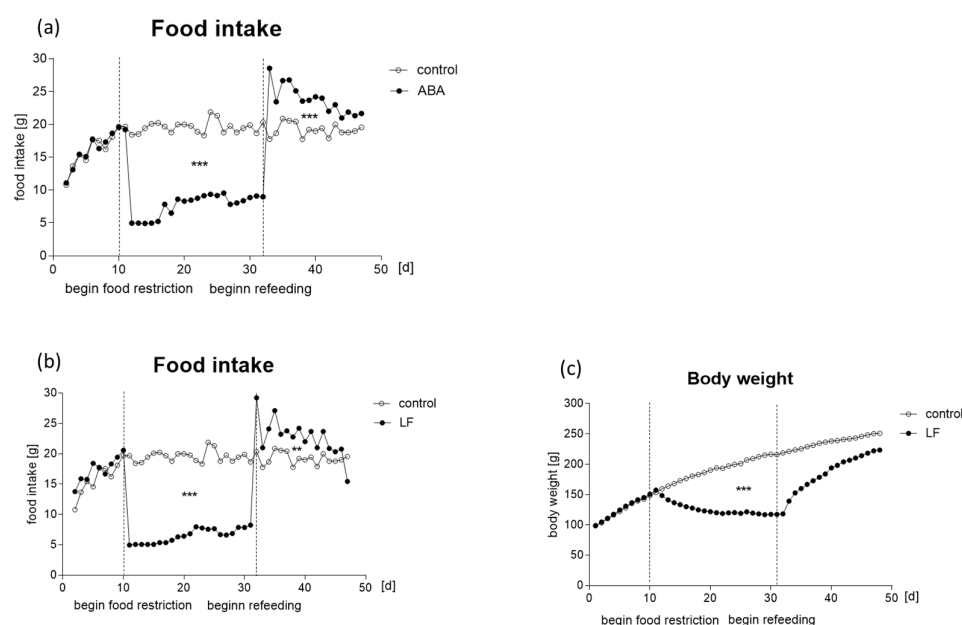


Article

Gut Microbiota and Brain Alterations after Refeeding in a Translational Anorexia Nervosa Rat Model

Stefanie Trinh ¹, Vanessa Kogel ¹, Lilly Kneisel ¹, Elena Müller-Limberger ¹, Beate Herpertz-Dahlmann ², Cordian Beyer ^{1,*} and Jochen Seitz ²

- ¹ Institute of Neuroanatomy, RWTH Aachen University, Wendlingweg 2, 52074 Aachen, Germany; ntrinh@ukaachen.de (S.T.); vanessa.kogel@rwth-aachen.de (V.K.); lilly.kneisel@rwth-aachen.de (L.K.); elena.mueller-limberger@uk-koeln.de (E.M.-L.)
² Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, RWTH Aachen University, Neuenhofer Weg 21, 52074 Aachen, Germany; bherpertz@ukaachen.de (B.H.-D.); jseitz@ukaachen.de (J.S.)
* Correspondence: cbeyer@ukaachen.de



Citation: Trinh, S.; Kogel, V.; Kneisel, L.; Müller-Limberger, E.; Herpertz-Dahlmann, B.; Beyer, C.; Seitz, J. Gut Microbiota and Brain Alterations after Refeeding in a Translational Anorexia Nervosa Rat Model. *Int. J. Mol. Sci.* **2023**, *24*, 9496. <https://doi.org/10.3390/ijms24119496>

Academic Editors: Dan Cristian Vodnar and Ashu Johri

Received: 29 April 2023

Revised: 23 May 2023

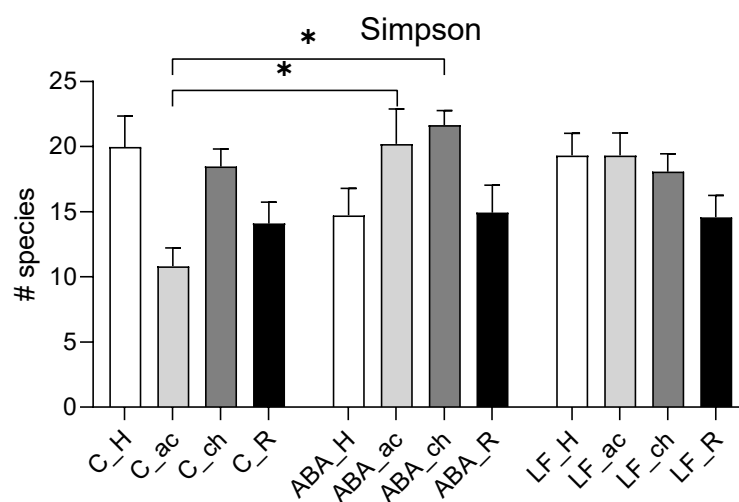
Accepted: 27 May 2023

Published: 30 May 2023

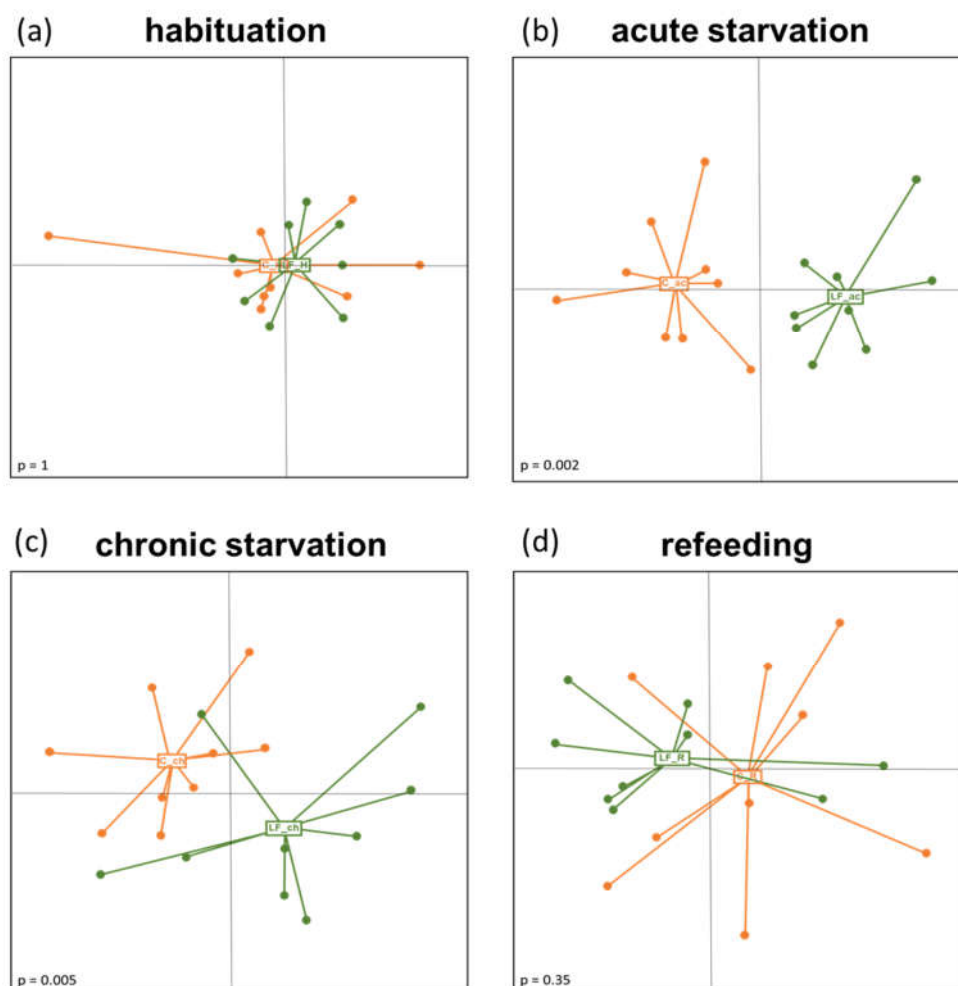


Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Supplementary Figure S1: Food intake and body weight profile. (a) mean food intake in grams per day for ABA group, (b) mean food intake in grams per day for LF group, (c) Mean body weight in grams per day for LF group. C = control, ABA = activity-based anorexia, LF = limited food ** $p \leq 0.01$, *** $p \leq 0.001$.



Supplementary Figure S2: Simpson effective count as α -diversity measurement. C = control, LF = limited food, H = habituation, ac = acute starvation, ch = chronic starvation, R = refeeding. * $p \leq 0.05$.



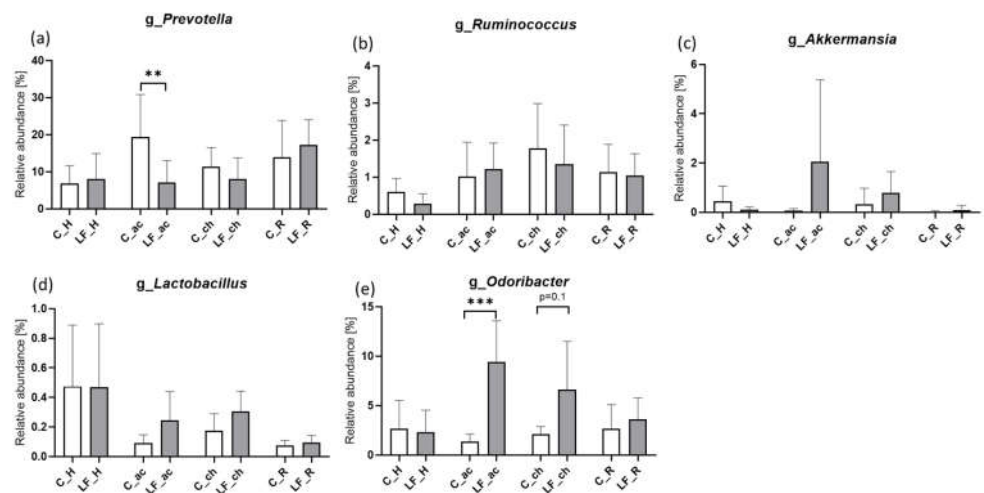
Supplementary Figure S3: β -diversity metrics in limited food vs. control group.

(a), (b), (c), and (d) generalized UniFrac distances as β -diversity measurement in a multidimensional non-scaling plot at different time points (habituation, acute starvation, chronic starvation, refeed-ing). C = control, LF = limited food, H = habituation, ac = acute starvation, ch = chronic starvation, R = refeeding.

Supplementary Table S1: All genera meeting inclusion criteria

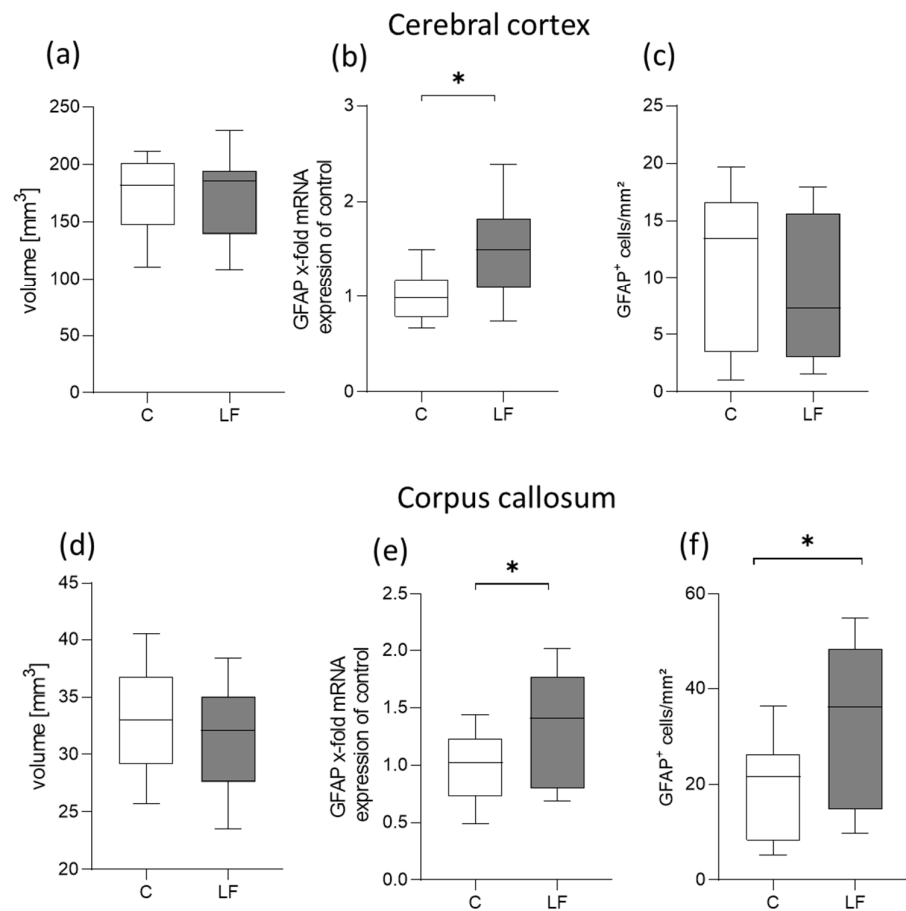
21 genera that meet inclusion criteria for analysis described in the microbiome analysis section.

Akkermansia
Alistipes
Alloprevotella
Bacteroides
Blautia
Butyricicoccus
Clostridium_IV
Clostridium_XI
Clostridium_XIVa
Clostridium_XIVb
Clostridium_XVIII
Flavonifractor
Hydrogenoanaerobacterium
Lactobacillus
Odoribacter
Oscillibacter
Prevotella
Pseudoflavonifractor
Ruminococcus
Sporobacter
Turicibacter



Supplementary Figure S4: Relative abundance of specific genera in limited food group

Relative abundance in % of the genus (a) *Prevotella*, (b) *Ruminococcus*, (c) *Akkermansia*, (d) *Lactobacillus*, and (e) *Odoribacter* at different measure time points (habituation, acute starvation, chronic starvation, refeeding). C = control, LF = limited food, H = habituation, ac = acute starvation, ch = chronic starvation, R = refeeding. ** $p \leq 0.01$, *** $p \leq 0.001$.



Supplementary Figure S5: Brain alterations after refeeding in limited food group

(a) Mean brain volume in mm³ in the cerebral cortex, (b) mRNA expression of GFAP in the cerebral cortex, (c) mean number of GFAP⁺ cells per mm² in the cerebral cortex, (d) mean brain volume in mm³ in the corpus callosum, (e) mRNA expression of GFAP in the corpus callosum, (f) mean number of GFAP⁺ cells per mm² in the corpus callosum. GFAP = glial fibrillary acidic protein, C = control, LF = limited food. * $p \leq 0.05$.