

UNNECESSARY GLUTEN-FREE DIET IMPACTS ON THE GUT MICROBIOTA

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Objectives: The use of gluten-free diet (GFD) in healthy people shows a steady increase worldwide based on the unsupported assumption that gluten avoidance can be beneficial to body wellness. Conversely, voluntary GFD impacts on living costs, psychosocial profile and may cause malnutrition in the long-term. Since dietary changes may significantly affect the gut microbiota composition, we tested whether a 30-day unnecessary GFD in healthy subjects impact diversity and richness of the gut microbiota.

Methods: 116 fecal samples were collected from 30 thoroughly examined healthy subjects (21 females, 9 males; 34±14 years) recruited at St. Anna University Hospital in Ferrara, Italy. All subjects had no demonstrable metabolic, cardiovascular, respiratory, rheumatic and neurological illnesses and were asymptomatic for gastrointestinal symptom (established by GSRC). Stool samples, collected at day 0, and 3, 7, 30 days from GFD were shotgun metagenomic sequenced and computationally profiled (bioBAkery 3). Data were analyzed on MetaPhlAn 4.0 and HUMAnN 3.0 for taxonomic and functional profiling, respectively and relative abundance transformed via arcsin-sqrt. Only species present at any time point in at least 5 subjects were considered (600 species). Statistical analysis

was performed using a linear mixed model and Friedman's ANOVA. p values were combined and corrected for multiple hypothesis testing (q value).

Results: Species alpha-diversity did not significantly change during 30 days of GFD ($p > 0.05$). However, during the GFD, 14 species significantly decreased ($q < 0.05$) in relative abundance. Most of the decreased bacteria belonged to *Bifidobacterium* genus (e.g. *Bifidobacterium longum* $p=0.000000003$; $q=0.000001$) or those involved in complex carbohydrate metabolism.

Conclusions: In healthy subjects, a 30-day GFD did not significantly affect gut eubiosis. However, consistent changes in the relative abundance of gut microbiota species showed a reduction of *Bifidobacterium* genus (known to contribute to host physiology) which may impact health status. Studies, evaluating the impact of a planned GFD (i.e. using non-gluten containing carbohydrates), are eagerly awaited to determine gut microbiota changes of healthy subjects strictly caused by gluten exclusion.