It is not who they are but what they do. By Alicia Ruiz, 1, Tomás Cerdó 2, Ester Hernández, 3 Rafael Bargiela, 3 María Suárez Diez, 4 Anette Friedrichs, 5,6 Ana Elena Pérez-Cobas, 7,8,9 María José Gosalbes, 7,8,9 Henrik Knecht, 5 Mónica Martínez-Martínez, 3 Jana Seifert, 10,11 Martin von Bergen, 10,12 Alejandro Artacho, 7,8,9 Amparo Latorre, 7,8,9 Stephan J. Ott, 5,6 Andrés Moya, 7,8,9 Vitor A.P. Martins dos Santos 4,13, N. Chueca 14, F. García 14 and Manuel Ferrer 3, Cristina Campoy, 2 and Antonio Suárez,1

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The gut microbiota has recently emerged as an important, and previously unappreciated, player in host physiology [(1)]. In particular, the gut microbiota contributes to a variety of physiological and pathophysiological processes in the host as inflammatory bowel disease[(2, 3)], type 2 diabetes [(4, 5)] or obesity [(6-8)].

During the past few years, with the improvement of high-throughput technologies and culture-independent genomic methods, it has become possible to accurately characterize the composition of microbial ecology [(9)].

Focused on obesity, many studies have been done to link this pathology with alterations in the composition of the intestinal microbiota An increased ratio of *Firmicutes/Bacteroidetes* has been observed in genetically obese mice (ob/ob) as well as obese humans. However, a number of other studies have failed to confirm these findings and have shown variable patterns in phylum level changes measured in the composition of the microbiota of obese humans. Although it is clear from the studies described above that the gut microbiota is likely to play some role in obesity and metabolic disease, it is difficult to draw definite conclusions on the importance of particular bacterial groups[(10)].

Besides, bionformatics tools considers organism to be the same species if they have >97% identity in the 16S ribosomal RNA gene. However, genomes from the same species can have large differences in DNA sequences outside the 16S ribosomal RNA gene. Importantly, they often have different sets of gene clusters that regulate production of specialized metabolites (eg, antibiotics, virulence factors, siderophores, and so on)[(11)].

These findings are changing the focus from “who”to “what”, namely, the interest is moving from the microbial composition to microbial functionality. Based on this, metabolomics studies have been applied to human gut microbiota as reviewed Ursell et al. [(11)].

Some microbial metabolites have well defined interactions with the human host, including short chain fatty acids (SCFAs; acetate, propionate, and butyrate that are absorbed by the epithelial cells and regulate the energy supply, control the pH in the colon and provide resistance to growth of pathogens) Abnormalities in the metabolism of SCFAs lead to the occurrence of obesity, type 2 diabetes, and colorectal cancer. Including also choline that has a key role in lipid metabolism, and is implicated in liver and cardiovascular diseases and other as bile acids, phenolic and aromatic acids, fatty acids, and phospholipids[12] .

Another option for studying the microbial functionality in the gut is to measure microbial enzymatic activities. There is a significant challenge in categorizing gut-specific and gut-enriched microbial activities functionally to determine the relevance in physiological or pathological context.

Therefore, we set to assess the functional differences and consequences of microbial shifts in the human gastrointestinal tract in relation to obesity. A number of reports suggest that the relative contribution of glycoside-hydrolases (GH) is indicative of the capacity for sugar metabolism and energy production and conversion in the gut microbiome [13, 14], and that its alteration may stimulate weight gain [15].To this end, we systematically collected glycosidase activity data in faecal bacterial proteins from obese (n = 7) and lean (n = 5) adolescents who did not present any intestinal disorders and had not taken antibiotics. The variations in faecal bacterial glycosidase activities were complemented with a comparative analysis between activity levels, community analysis based on 16S rRNA pyrosequencing and anthropometric and biochemical parameters to find presumptive correlation variables.

We further evaluated the glycosidase activity profiles in the fecal microbiota of lean (n = 5) and obese (n = 7) adolescents aged 13–16 y. As regards to the activity values per each of the sugars, no statistically significant differences ($p < 0.05$; Student's t-test) were found between the subsets of each of the two groups; results in Figure 1 are presented as mean values ± standard errors. From the data shown in Figure 1, obese subjects were characterized by a higher total sugar metabolism capacity, with a net global increase for the 23 carbohydrate tested from 38.0 ± 0.6 to 426.5 ± 3.2 units/g total protein. In addition, in comparison with lean subjects, obese had a less balanced gut biochemical environment.

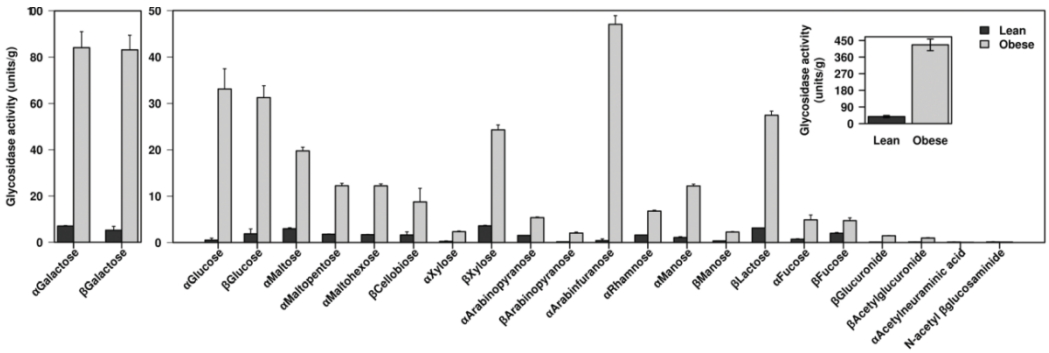

Figure 1. Shifts in carbohydrate turnover profile. Enzyme activities (units per gram total protein) from the total faecal microbiota against 23 different sugar substrates were quantified by measuring the release of *p*NP in triplicates

We further evaluated whether a relation between the gut glycosidase activity (Fig. 1) and BMI could be established. Results shown in Figure 2 provide evidences of the goodness of a sigmoid (r^2 = 0.98; BMI_{50} value of 24) model to adequately describe this dependence for all subjects investigated.

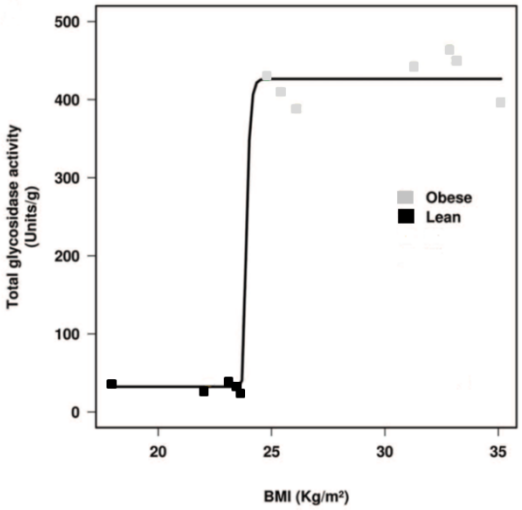

Figure 2. Correlation between metabolic GIT activity and anthropometric and biochemical parameters. Association between the BMI and total glycosidase activity.

And finally, we analysed the microbial composition by 16S rRNA pyrosequencing with 454 platform. Figure 3 shows an unsupervised PCA that indicates difference between the microbiota composition of lean and obese subjects. Samples belonging to each group cluster together but with a percentage of explanation of 41%.

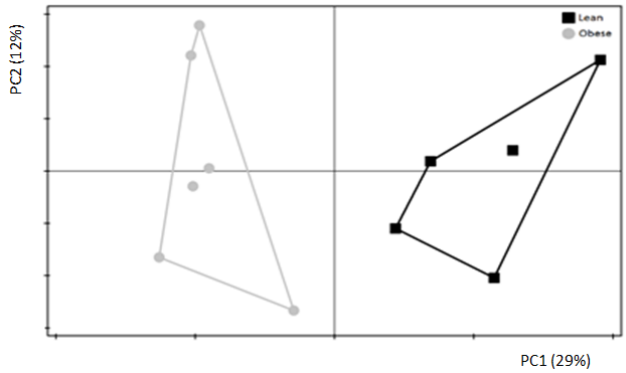

Figure 3. Unsupervised PCA of the microbial gut communities from lean and obese subjects.

The results presented here enabled us to gain valuable insights into the biochemical and metabolic-wide effects of obesity in the gut microbiota and their links. Interesting result of this study was the high anabolic capacity toward dietary sugars in obese patients. In addition,compared with lean, obese microbiota have a preferential capacity to anabolize α-polyglucoses, present in the "western diet" while having a relative lower capacity to metabolize α-rhamnose, β-fucose, α-arabinopyranose and β-glucose (an essential plant component). Accordingly, it is plausible,that a mechanism that promotes weight gain

may be the increased level of enzymes aimed at the digestion of highly refined carbohydrates.

Our data provide a metabolic connection between gut microbiota and host glucose metabolism as we observed a positive correlation between gut glycosidase activities and biochemical parameters such as BMI and fasting blood glucose level and HOMA-IR index in obese subjects (data not shown). The sigmoid model proposed for BMI and GH activity pose an intriguing question on the causes that trigger gut GH anabolic capacity at BMI higher than 24, an event that significantly enhance energy extraction from dietary α-polyglucoses. Food choices, social components, physical performance and many other possible reasons may underlie this effect.

On the other hand, community analysis results based on the 16S rRNA pyrosequencing explain the differences between samples in a 41%, that is a low percentage considering the low number of samples. This leads us to hypotesize that gut communities should be classified by functionalities rather than by 16S-assigned members. What nowdays is known as metabotypes, different metabolizing phenotypes.

In conclusion, we believe that the correlations observed support our hypothesis reasonably well and that it is plausible that the changes in the GIT microbiota capacity to metabolize carbohydrates may be an origin factor to develop obesity. Altogether, lay a foundation for subsequent, systematic research that will design personalized diets based on actual potential digestibility of dietary polysaccharides to regulate weight gain. Obviously,we are aware that these correlations were determined for a very small number of subjects and that thus require further confirmation with a higher set of individuals and conditions.

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