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Project acronym: MyNewGut

Project title: Microbiome Influence on Energy Balance and Brain Development/Function Put into Action to Tackle Diet-Related Diseases and Behaviour.

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"Microbiome Influence on Energy Balance and Brain Development- Function Put into Action to Tackle Diet-Related Diseases and Behavior" – MyNewGut
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MyNewGut
FINAL PUBLISHABLE SUMMARY

Final report

Period covered - start date: 01/12/2013
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"Microbiome Influence on Energy balance and Brain Development - Function Put into Action to tackle Diet-related Diseases and behavior" - MyNewGut has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement nº 613979
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"Microbiome Influence on Energy balance and Brain Development - Function Put into Action to tackle Diet-related Diseases and behavior"– MyNewGut has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement nº 613979
**1. Final publishable summary**

This section must be of suitable quality to enable direct publication by the Commission and should preferably not exceed 40 pages. This report should address a wide audience, including the general public. The publishable summary has to include **5 distinct parts** described below:

- **An executive summary** (not exceeding 1 page).

MyNewGut was a multidisciplinary project that provided advanced knowledge on the role of the interactions between the gut microbiota and the lifestyle in energy balance and brain development and function via immune, neural and endocrine routes. This initiative also contributed to accelerating the translation of this knowledge into practical solutions to tackle more effectively diet-related metabolic and mental disorders linked to stress.

Obesity and the associated cardio-metabolic (metabolic syndrome, diabetes, etc.) and mental (eating behavioural and mood disorders, like depression) comorbidities constitute leading causes of morbidity and disability worldwide. Nevertheless, effective preventive strategies for these conditions have barely been developed. The gut microbiota constitutes a modifiable factor that can influence the disease risk, and be integrated in future multi-faceted strategies to effectively reduce the prevalence of those disorders. MyNewGut filed important knowledge gaps and progressed towards this ultimate goal, through the following achievements. (1) We have shed light into the role of the gut microbiota and different dietary components (proteins, PUFAS, fibres) in nutrient metabolism and energy balance through intervention studies. Specifically, we proved that not only the amount of dietary protein but also the source (animal or plant origin) influences the generation of toxic microbiota-derived metabolites and gut mucosa homeostasis. We also identified which dietary fibres could induce microbiota-mediated effects in glucose metabolism. In addition, we demonstrated that the gut microbiota and their metabolites regulate the gut-brain axis impacting food intake in humans. In observational studies, (2) we identified specific microbiota configurations and dietary patterns that altogether contribute to the development of obesity and metabolic inflammation in children and (3) key factors (maternal obesity, delivery by caesarean section, antibiotic intake) that influence the gut microbiota in the perinatal and early postnatal periods affecting the nervous, immune and endocrine systems and later health outcomes, like the response to stress (a risk factor for depression). (4) In pre-clinical trials, we disentangled new players and molecular mechanisms behind the effects of the diet and the gut microbiota on obesity and comorbidities; for example, we identified new human bacteria with efficacy on obesity, the influence of dipeptidyl-peptidase IV on gut microbiota and intestinal homeostasis and the role of the gut microbiota-leptin-brain network in depressive behaviour associated with obesity. (5) Intervention trials provided evidence of the ability of microbiome-directed dietary strategies to reduce disease risk. Specifically, we proved the effectiveness of a fibre combination that reduces cardio-metabolic disease risk markers and a probiotic strain that improves stress-related outcomes in humans.

In this 5-year project, the MyNewGut consortium has generated rich data sets and an important body of evidence supporting not only associations but also causation between the gut microbiota, the diet, and metabolic and mental health-related outcomes. This evidence was based on the integration of data from four observational and six intervention studies in humans, using multi-omics approaches and...
systems biology, coupled with studies in advanced experimental models. Altogether the exploitation, dissemination and communication activities of the MyNewGut consortium include, 93 papers (original scientific papers, opinion papers and reviews) with more coming; about 200 contributions to conferences and workshops; development of three patents, creation of a start-up and active presence in massive media (Twitter, Facebook, press releases, TV, radio).

- **A summary description of project context and objectives** (not exceeding 4 pages).

The MyNewGut project was based on emerging evidence supporting the notion that the gut microbiota and its genome (microbiome) play important roles in human physiology and, thereby, can contribute to determining the risk of developing metabolic and mental disorders. These microbiome-related functions are the result of interactions with lifestyle (e.g. diet, eating habits, mode of delivery at birth, etc.) and host factors, which jointly influence the bidirectional communication and function of the gut, the brain and the peripheral tissues that determine our health status. The proposal was also grounded on the hypothesis that developing microbiome-based dietary recommendations and interventions can provide cost-effective measures to reduce the socioeconomic burden of diet-related diseases and, in particular, obesity and chronic-metabolic and mental disorders.

Obesity and associated cardio-metabolic co-morbidities (metabolic syndrome, diabetes, cardiovascular disease, etc.) constitute a major health concern worldwide and in particular in the EU. The worldwide prevalence of obesity nearly tripled between 1975 and 2016, with 39% of adults with overweight and 13% with obesity in 2016. In the WHO European region obesity affects up to 30% of the population aged 40–65 years, which is similar to the situation in the US. This unprecedented obesity pandemic is going to have a strong impact on future morbidity rates and reduce life expectancy by 5-20 years. Alterations in eating behaviour related to obesity, stress and other psycho-social factors are also increasing, already affecting about 1% of the population. Furthermore, stress and obesity are associated with the risk of developing mood disorders, like depression, which represents the leading cause of disability worldwide (WHO, 2017). Yet effective management and, specially, preventive strategies for these metabolic and mental conditions have barely been developed, urging the MyNewGut project to tackle these societal challenges.

The gut microbiota structure and function has emerged as a novel modifiable factor that can influence the risk of developing metabolic and mental disorders, and which must therefore be considered in future multi-faceted strategies to effectively reverse their prevalence trends.

To fill the existing knowledge gaps and progress in this direction, the MyNewGut project has addressed the following objectives:

1. Shed light on the response of the gut microbiota to the diet and the contribution of specific microbiota components/pathways to nutrient metabolism and energy balance in human subjects in order to identify targets for dietary intervention that help reduce the incidence of obesity and associated metabolic disorders (WP3; WP9).

2. Identify microbiome-related features that contribute to or predict obesity and associated metabolic and behavioural disorders by investigating their interactions with lifestyle, host factors (epigenome, immune and neuroendocrine systems) and social determinants of eating behaviour in epidemiological studies, including specific population groups at critical stages of life and representing different metabolic profiles and behavioural patterns (WP4, WP9).
3. Understand how environmental factors influence the gut microbiota in the perinatal and early postnatal periods and their effects on the nervous, immune and endocrine systems and in later health in childhood and adulthood (WP6, WP9).

4. Disentangle the mechanism behind the effects mediated by the diet and the gut microbiota in obesity and related metabolic and behavioural disorders in pre-clinical trials and select a new generation of probiotics (WP5).

5. Provide proof-of-concept of the potential of dietary interventions with new food prototypes and ingredients, targeting the gut microbiome, to tackle obesity and metabolic disease as well as stress-related disorders in humans (WP7, WP8, WP10).

6. Disseminate information about the project to the scientific community, stakeholders and the general public, inform policies and recommendations, and promote the exploitation of project’s results (WP11, WP12).

To achieve the aforementioned objectives, the MyNewGut project has applied a multidisciplinary and translational research approach, using functional omics-technologies (metagenomics, transcriptomics, metabolomics, lipidomics, epigenetics) combined with robust clinical biomarkers and systems biology, in well-controlled observational and interventional trials in humans. In addition, these studies have been complemented with trials in advanced experimental in vitro and in vivo models to confirm findings in humans (e.g. causality of gut microbiota or specific bacteria), screen functional ingredients (prebiotic fibres, potential probiotics), and deepen our mechanistic understanding of the diet-microbe interactions in obesity and associated mood disorders. This has enabled us to establish both robust associations and causality between the microbiota, the diet and the host phenotype (health/disease), and to progress in the translation of the new knowledge into solutions and recommendations to improve the EU position in disease prevention via dietary and lifestyle strategies.

To make this possible, the MyNewGut consortium brought together leading world experts in all the required disciplinary fields, including microbiology, omics-technologies, physiology, nutrition, immunology, psychiatry and neuroscience, and experimental and computational modelling from 15 EU (CSIC, UNIBO, UCC, UCPH, INRA, UCL, TUM, TNO, AMC, UHR, UREAD, MUG, BIPS, UGR) and non-EU countries (CSIRO, UQ, QU-KGH, UOA, BCM, UM). Translation of results into practice was also possible thanks to the collaborative work between academic and industrial partners (CARG, CAPSA, LAL, ADMS) and SMEs (AH, NC) and the support of NGOs and associations (FINS, ICC, EFFoST, EUFIC). 

**Objective 1 (WP3).** To address the first objective, we conducted two randomized-controlled intervention trials with different macronutrients (proteins, PUFAS, fibre). This allowed us to shed light on the influence of the interactions between different dietary components and the gut microbiota in the pool of metabolites generated by microbes and host-microbe co-metabolic processes, to identify the gut microbiome components responsive to dietary intervention and their effects on different aspects of energy balance in overweight subjects. Furthermore, a third intervention trial was conducted to evaluate whether the gut microbiota or its metabolites (butyrate) play a role in the regulation of the gut-brain axis and metabolic syndrome via faecal transplantation experiments in humans.
Objectives 2 and 3 (WP4&WP6). To investigate the stages critical for the latter development of obesity and mental-related disorders and their complexity, the MyNewGut project selected a series of relevant study cohorts to specifically investigate links between the gut microbiome, the lifestyle and the health status. These study populations constituted models of general population and encompassed an extended period of the lifespan representing several lifestyles (sedentary/sleep habits, dietary preferences, etc.) and behaviours (food addiction in women, stress vulnerability) as well as different developmental process (from perinatal and early postnatal period to childhood and adulthood), where a profound and complex interplay takes place between the gut microbiota and the host. MyNewGut conducted observations studies capitalizing on the following existing cohorts:

The FP6 IDEFICS and the FP7 I-Family projects cohort of children, who were followed longitudinally for 4 years to investigate the role of the microbiota, the lifestyle and behaviour in the development of obesity/insulin resistance in the WP4.

The FP7 NEUROFAST cohort of obese women, with and without diagnosed food addiction and alteration in eating behaviour, included in WP4 to specifically investigated the possible role of the microbiota in eating behavioural alterations and obesity.

Two infant cohorts: one including mothers and infant pairs (PREOBE project) and another including only infants (INFANTMET project). These cohorts were followed-up longitudinally to deepen our understanding of the role of early perinatal and postnatal lifestyle factors in establishment and development of gut microbiota and its impact on programming metabolic, neural and immune functions and latter health in childhood and adulthood in WP6.

In all cases, new subjects were recruited and data from multiple readouts (omics-, physiological and clinical data) were added and integrated in the context of MyNewGut.

Objective 4 (WP5). Experimental models (in vitro and in vivo) have also been used to mechanistically and causally prove the role of the specific dietary components, key bacteria and bacterially produced metabolites (previously identified in humans) in obesity and associated disorders, like depression. These trials have also enabled us to screening different fibres to identify those with beneficial effects on the obesity phenotype, through microbiota-mediated mechanisms, latter tested in humans in WP3. The use of models has also allowed us to evaluated the effects of specific bacteria associated with a healthy metabolic phenotype in humans, progressing from associations to causality and identifying a new generation of potential probiotics and their mechanisms of action.

Objective 5 (WP8, WP7 & WP10). In the WP8 the MyNewGut consortium has conducted three additional randomized, controlled intervention trials with new food prototypes and probiotics to provide proof-of-concept of the possibility of reducing metabolic disease risk and regulating stress-related disorders in humans. This has been achieved through cooperative work with the industrial partners (CAPSA, AH) that optimized and scale-up the production of food prototypes and ingredients and the corresponding placebos for three independent intervention trials (WP7, WP10).

Objective 6. (WP11 and WP12).

MyNewGut’s six review-type position papers (to be) published in Clinical Nutrition provided gut microbiome related information and insights relevant for public health policy and dietary guidelines. This information was translated for multiple categories of stakeholders in widely disseminated semi-scientific articles and summarized in concise recommendations to inform future dietary guidelines.
and public health policies. Exploitation of project results was promoted by providing a IPR management plan and a market analysis, identifying opportunities and barriers.

MyNewGut’s communication strategy has been to adapt its various communication channels and messages, accordingly, targeting audiences online through articles, podcasts and videos, as well as offline through leaflets and presentations. Scientific results have been published in international peer-reviewed journals, as well as non-peer reviewed/popular specialised journals. The results were also presented by partners at many scientific conferences and events to promote the project’s outcomes. Project news reached a large audience including the general public via the project’s website, newsletters, developed social media channels and through press coverage. To promote the exploitation of the results across Europe, a summary of the results and a press release was translated to multiple languages.

The main results and achievements as well as their impact are described in detail in the following sections, subdivided in the different work packages (WP).

- **A description of the main S&T results/foregrounds (not exceeding 25 pages).**

**Human Intervention Trials to Understand the Ability of the Gut Microbiome to Influence the Metabolism of Nutrients and Energy Balance (WP3)**

In WP3 we have investigated how different macronutrients influence the gut microbiota composition and function, and the pool of metabolites generated by gut microbes and host-microbe co-metabolic processes, and their relationships to obesity and metabolic disease risk markers.

**Dietary intervention with varying content and sources of proteins**

Knowledge of the impact of dietary proteins on the gut microbiota and derived metabolites and its potential health effects on humans was limited and evaluations of the effects of the specific protein source were lacking by the time MyNewGut studies were designed (*Portune et al. 2016; 2017*). In MyNewGut, a 3-week randomized double-blind, placebo-controlled, parallel-design intervention trial was performed to investigate the effects of the amount and types of proteins on healthy overweight volunteers at INRA (Dr. F. Blancher). The diet was supplemented with either casein, as an animal source of proteins, soy protein as a plant source, or maltodextrin used as a placebo, in the context of a diet with similar energy and fibre intake. The compliance of volunteers (assessed by measurement of uremia) was excellent. This study showed that such supplementation does not change body weight and biochemical/physiological parameters, with the notable exception of the systolic blood pressure which was markedly decreased for the volunteers consuming soy protein as supplement, but not for those consuming casein. However, the respective part played by the soy protein and/or isoflavones contained in the soy extract on such parameters remains unknown. The absence of effects on body weight following high-protein diet (HPD) consumption indicates that the consumption of such diets, often used for their effect on satiety in order to decrease body weight, does not exert measurable slimming effects when energy consumption is not decreased. Focusing on the impact of HPD on the intestinal tract, we found that both HPDs decrease butyrate concentration in the faeces, an effect that is considered unfavourable given the role of this bacterial metabolite as an energy substrate and regulator of gene expression in the colonic epithelial cells. Both HPDs increased the concentration of indoxylsulfate in urine, an unfavourable effect as this bacterial co-metabolite is well known to act as a...
uremic toxin. Casein, but not soy protein consumption, resulted in addition in an increase of the urinary concentration of p-cresylsulfate, another uremic toxin; and of p-cresol. Since p-cresol has been shown by us (in the WP5) to have genotoxic effects on human colonocytes, this indicates another unfavourable effects of casein supplementation on the colonic epithelial cells. The gut microbiota analyses showed specific correlations among different taxonomic units and potentially toxic metabolites derived from amino acid metabolism, which should be investigated further. However, the composition of the faecal and colonic mucosa associated microbiota was not modified significantly by the HPDs. This indicates that substrate availability (i.e. the fraction of protein products reaching the large intestine) influences bacterial metabolite production even in the absence of significant changes in its composition and that the metabolic output may be a better predictor of microbiota-mediated effects. Both HPDs were found to modify the expression of genes involved in the intestinal mucosa homeostasis in the rectal biopsies recovered at the end of the nutritional intervention. Interestingly, although some modifications of gene expression were common to both HPD, some of them were differently modified according to the source of protein, likely because of different digestibility and amino acid composition. However, no sign of intestinal inflammation was recorded, indicating that HPDs per se do not induce mucosal inflammation in short-term. The results are published in Am J Clin Nutr (Beaumont et al., 2017).

Dietary intervention with varying content of fibres and fat composition

Gut microbiota composition has been linked to obesity and the metabolic syndrome. Knowledge of specific nutrients and doses required to obtain a dietary modulation of gut microbiota that beneficially influence body weight and components of the metabolic syndrome is limited (Benítez-Páez et al., 2016). The aim of this second intervention trial was to investigate diet-induced effects on the gut microbiota and metabolic markers in 30 overweight individuals with indices of the metabolic syndrome. We designed a twelve-week study that was conducted as a randomized cross-over trial with two diet intervention periods separated by a washout period (3×4 weeks). The two diet interventions were (i) intake of a wheat bran extract (WBE), rich in arabinosylan oligosaccharides (AXOS) (10.4 g/d AXOS) and (ii) intake of polyunsaturated fatty acids (PUFA) provided in fish oil capsules (3.6 g/d n-3 PUFA). Data including faecal and blood samples were collected four times during the study: baseline (week 0), after first dietary intervention period (week 4), after washout (before second diet period) (week 8), and after the second dietary intervention period (week 12). The study was conducted at the UCPH (Prof. A. Astrup and L. Hingstrup). No changes in human metabolic markers were observed after any of the interventions. Gut microbiota composition (taxonomy) and function were analysed by CSIC (Prof. Y. Sanz) and metabolites by UREAD (Prof. S. Clause) and UHR (Prof. G. Liebisch). The taxonomic analysis by 16S ribosomal RNA gene amplification of the V3-V4 hypervariable region showed that intake of AXOS increased the abundance of bifidobacteria and butyrate producing bacterial species. Beta-diversity analysis indicated that the structure of the gut microbiota changed as a result of the AXOS intervention (Kjølbaek et al. Clin Nutr 2018. In press). Metagenomic analysis indicated that AXOS intake increased the capability of the gut microbiota to produce vitamins, cofactors, neurotransmitters and its precursors and reduced the plasma levels of ceramides and increases organic acids (short-chain fatty acids [SCFAs], like propionate and succinate). These metabolites together with the compositional changes of the microbiota could, theoretically, contribute to improve glucose homeostasis in longer interventions (Benitez-Páez et al., Microbiome. Under review). PUFA intake did not affected the gut microbiota composition. Main results are described in Kjølbaek et al. (Clin Nutr 2018. In press) and detail omics analyses in (Benitez-Páez et al., Microbiome. Under review).
**Evaluation of the respective role of a “healthy” microbiome and microbiome-related metabolites (butyrate) on components of the metabolic syndrome in a human intervention study.**

In total, 24 male and female treatment naïve metabolic syndrome subjects completed the study as well as 6 faecal microbial transfer (FMT) donors. This study was led by AMC (Dr. M. Nieuwdorp). Primary endpoint analyses (brain SPECT scans for dopamine/serotonin receptor expression and hyperinsulinemic clamp data upon intervention) revealed that 4 weeks after FMT + placebo tablets (n=12) caused a significant increase in striatal dopamine receptor expression (as determined by SPECT scan after Radiotracer 100 MBq ¹²³I-FP-CIT infusion), which is known to be associated with improved impulse control and appetite/intake in comparison to 4 weeks of butyrate + autologous FMT (n=12) which had an opposite effect. Hypothalamic serotonin receptor expression was not affected by either intervention. In contrast to our previous studies with lean donors, postbariatric donor FMT did not affect peripheral and hepatic insulin sensitivity. In line, no effect of oral butyrate was seen on these parameters. Upon secondary endpoints analyses, we found specific changes in both metabolites (Prof. Claus, UREAD) and faecal microbiota (Prof. Sanz, CISC). Subsequent machine learning data analyses revealed that a decrease in the amount of *Prevotella* strains was significantly associated with decreased brain dopamine receptor expression (Spearman correlation r = -0.5), whereas increased *Bacteroides uniformis* was significantly associated with an increased dopamine receptor expression in brain (Spearman correlation r = 0.7). In line, plasma metabolites like glycine and betaine were significantly associated with these gut microbiota changes as well as increased striatum dopamine receptor expression upon the intervention. In conclusion, our small randomized controlled intervention trial (yet with deeply phenoptyped human subjects) suggests that also in human metabolic syndrome subjects a gut brain axis seems to be present, which is driven by specific metabolites and faecal bacterial strains. The corresponding manuscript is in preparation.

**Epidemiologic Studies to Identify the Influence of Lifestyle and Other Factors on the Gut Microbiome and Its Role in the Development of Obesity and in Eating Behaviour (WP4)**

The main goal of the MyNewGut WP4 was to fully characterize the microbiota of two different groups of subjects (242 samples), and correlate the microbiome data with physiological, metabolic, and psychometric parameters. The first group of subjects included children of the IDEFICS/I.FAMILY cohort, which showed different body weight gain trajectories, comprising normal weight and overweight/obese individuals (Prof. W. Ahrens from BIPS,). The second group was composed by normal weight and obese women, recruited within the NEUROFAST project (led by the University of Bologna), and characterized for their metabolic, emotional-affective and hormonal levels. The first step of the WP foresaw the generation of a comprehensive database, including all the variables collected during the exploitation of the IDEFICS/I.FAMILY and NEUROFAST projects and other extra-EU projects. This database has been used during the microbiome analyses, for integrating all the results of 16S rRNA sequencing, metagenomics and metatranscriptomics (Prof. P. Brigidi from UNIBO). In the frame of the project, we successfully analysed, by 16S rRNA sequencing, the entire pool of samples and we performed the metagenomic and metatranscriptomic analysis on a representative sub-group of samples. We also assessed the epigenetic profile from blood samples by characterizing the DNA methylation profile of all the available samples. Finally, we performed the lipidomics (Prof. G. Liebisch from UHR) and metabolomics (Prof. S Claus from UREAD,) analysis from stool (metabolomics and lipidomics), urine (metabolomics) and blood (metabolomics and lipidomics), using LC/GC-MS/MS methods or 1H NMR spectroscopy, respectively.

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Dietary habits were associated with specific differences in the intestinal microbiome profiles and health metadata. In particular, unhealthy eating behaviour of obese children (including high consumption of simple sugar and fat), supported a low-diverse microbiome and a higher level of inflammation. Interestingly, the characterization of a prospective cohort of children showed that this specific fingerprint of “diet, inflammation and microbiome” was present before the onset of obesity, suggesting a predictive potential of the diet-microbiome-host configurations. These results strengthen the concept of obesity as a complex mosaic, in which several endogenous and exogenous variables, including host genetics, physical activity and food intake, contribute to health decline and to the obesity onset. Among these variables, the gut microbiome must be considered an important mosaic tile that can worsen or improve the overall picture. The results have been published (Rampelli et al., Commun Biol. 2018).

The role of the microbiome in obesity was also supported also by the metagenomic and metatranscriptomic results obtained in a comparative study of normal weight women vs obese women, which confirmed significantly different phylogenetic and functional microbiome profiles.

No difference among the epigenetic profiles of obese and normal weight subjects (both children and women) was found.

A reliable correlation between subjects’ age and metabolic profile of urine has been found in the children of the IDEFICS/I.FAMILY cohort, independently from obesity. In particular, succinate and hippurate were associated to younger children, and creatinine to older children. It is important to note that creatinine has been previously associated with muscle mass, in accordance to the pubertal stage of the children. On the other hand, succinate is a well-known endogenous intermediate of the TCA (tricarboxylic acid cycle), largely produced by gut bacteria, and hippurate is a microbial co-metabolite derived from bacterial degradation of phenolic compounds (for example, found in fruit juices and vegetables and is therefore often associated with a healthy diet) and with a more diverse diet richer in fibres.

NMR-based metabolomics analysis and multivariate statistical analysis were applied to urine, faecal and blood serum samples collected from obese/overweight females with low and high food addiction and normal weight women as part of the NEUROFAST cohort. Even if the primary aim of these analyses was to identify correlation between the metabolic profiles and food addiction, supervised and unsupervised analysis failed to reveal any correlation with food addiction.

**Preclinical Trials to Understand the Mechanism of the Relationship Among Diet, Behaviour and Gut Microbiome in the Control of Obesity and Related Disorders (WP5)**

The main goal of the MyNewGut WP5 was to investigate the mechanisms by which the intestinal microbiota can influence the development of obesity and related disorders using preclinical models. To this end, the WP5 partners initially reviewed the literature and their previous studies to select the best experimental animal model to test the different hypothesis of the WP5 (collaboration TUM, CSIRO and UCL; Neyrinck et al., Trends in Food Science & Technology, 2016).
Mode of action of new intestinal human bacteria with a potential protective role in obesity and associated metabolic and behavioural disorders in diet-induced obese mice

CSIC (Prof. Y. Sanz) developed different strategies to isolate fastidious intestinal human bacteria of taxonomic units associated with a healthy metabolic phenotype in laboratory conditions. This has enabled us to enlarge our culture collection with 200 additional isolates, representing about 50 different bacterial species, in addition to those existing in our collection before the initiation of this project. The identity of all the isolates was confirmed by 16S rRNA gene sequencing. In preclinical trials conducted in diet-induced obese (DIO) mice, it was demonstrated that the strain *B. uniformis* CECT 7771 (1x10⁸ CFU) reduces body weight gain and weight of epididymal adipose tissue, improves glucose tolerance and plasma levels of biochemical markers (glucose, cholesterol and triglycerides). These improvements in metabolic parameters were associated with a reduction in the inflammatory tone, involving increases in the proportions of Tregs and reductions in the macrophage balance (M1/M2) in different tissues. These effects could be mediated through TLR5 signalling since the expression of this receptor was drastically reduced in obese mice but restored by the *B. uniformis* CECT 7771 administration. The effects in TLR5 could be elicited directly by *B. uniformis* CECT 7771 as proven in in vitro cell cultures. The administration of lower levels of *B. uniformis* CECT 7771 (1x10⁷ CFU) together with fibres (AXOS used also in human trials in WP3) led to complementary and synergistic effects, helping in the maintenance of the energy balance in obesity. CSIC also identified two new strains with different ability to modify the metabolic and immune phenotype in DIO mice. While one of them improved the impaired glucose tolerance, the other prevented both body weight gain and glucose intolerance associated. Their mode of action via endocrine and immune routes has been investigated. The strains have been protected under patent. Drafting of the corresponding papers is ongoing, after IPR protection.

MUG (Prof. P. Holzer) has examined the gut-brain axis mechanisms underlying the behavioural effects of an obesogenic diet in collaboration with UCL (Prof. N. Delzenne) and UREAD (Prof. S. Claus). High fat diet (HFD; 60 kJ% from fat) induced a distinct depression-like phenotype characterized by anhedonia, reduced sociability and self-care, lethargy and a disturbed circadian pattern of ingestion. The HFD-evoked depression-like behaviour was associated with distinct alterations in the gut microbiota (enhanced Firmicutes/Bacteroidetes ratio), enhanced plasma DPP-4-like activity, alterations in the metabolome of distinct brain regions (molecules related to energy metabolism and neuronal signalling) and a decrease in neuropeptide Y expression in hypothalamus and hippocampus. These findings indicate that specific mechanisms of the gut microbiota-brain axis cause metabolic impairment of the brain and disturb emotional-affective behaviour (*Hassan et al., Nutr Neurosci. 2018*).

MUG (Prof. D. Haller) has also investigated the role of specific key bacteria in metabolic impairment of the brain. First, MUG observed that the depletion of the gut microbiota by treatment of mice with a mixture of non-absorbable antibiotics (meropenem, neomycin and vancomycin) blunted the HFD-induced weight gain, obesity and depression-like behaviour. This finding provides direct evidence that the obesity-related impairment of brain function involves the gut microbiota as an essential component in the interaction between unfavourable nutrition and emotional-affective disease. *Christensenella minuta*, a component of the human gut microbiota associated with low body mass, was evaluated in this mouse model in collaboration with CSIC. The bacterium failed to counteract the effect of HFD in inducing obesity and depression-like behaviour in mice in a 4-week intervention, which could be partly due to the short study duration. Further measurements revealed that antibiotic treatment of mice suppressed the HFD-induced increase in plasma leptin. In line with this result, HFD...
failed to induce depression-like behaviour in leptin-deficient ob/ob mice. These data provide direct evidence that leptin mediates the HFD-induced microbiota-dependent depression-like behaviour. Leptin, thus, represents an important molecular marker of the microbiota-related impairment of the brain in obese mice.

**Influence of bacterial metabolites produced from amino acids in the colonic homeostasis and liver inflammation**

INRA has focused its studies on the effects on the colonic epithelium of several bacterial metabolites produced from the amino acids released by the intestinal microbiota from undigested or not fully-digested dietary and endogenous proteins. INRA concluded from the data that p-cresol acts as a metabolic troublemaker and a genotoxic agent towards human colonocytes (Andriamihaja et al., *Free Radic Biol Med, 2015*). Then, INRA studied the effects of hydrogen sulphide (H$_2$S), a gas partially dissolved in the luminal fluid facing the colonic epithelium, and that is produced by the intestinal microbiota mainly from L-cysteine. INRA implemented the new technic of luminal instillation of bacterial metabolite *in vivo* in anesthetized rats and found that H$_2$S increases in colonocytes the expression of several genes related to the inflammatory process. Furthermore, INRA found that NaHS was able to stimulate at low micromolar concentrations oxygen consumption in the rat colonocytes, while inhibiting it at higher concentrations. These observations motivated the study of the effects of high-protein diet (HPD) on the concentration of H$_2$S in the large intestine. INRA concluded that although HPD increases the H$_2$S content in colonocytes, thus interfering with energy metabolism in colonocytes, colonic adaptive responses allow to limit the epithelial exposure to this deleterious bacterial metabolite (Beaumont et al., *Free Radic Biol Med, 2016*). INRA concluded from other experiments that modifications of the luminal environment provoked by HPD consumption was associated with the maintenance of the colonic homeostasis that might be the result of adaptive processes in the epithelium related to the observed transcriptional regulations (Beaumont et al., *BMC Genomics, 2017*). In addition, INRA highlighted the potential impact of increased luminal osmolarity on the intestinal epithelium renewal and barrier function, and pointed out some cellular adaptive capacities towards luminal hyperosmolar environment (Grauso et al., *submitted 2018*). Lastly, because we found in the clinical study testing the effects of HPDs on the colon ecosystem (see WP3) that the most cytotoxic faecal water recovered from the volunteers were those containing the highest concentration of the bacterial metabolite hydroxypenylvacetate, INRA tested this compound for its effect on human colonocytes. The data reinforced the view that the effects of a given bacterial metabolites is different when tested individually, or in a mixture of compound (Armand et al., *Biochim Biophys Acta, under revision*).

Besides influencing the intestinal homeostasis, the gut microbiota has recently emerged as a critical regulator of liver homeostasis. In this sense, UCL has studied the influence of intestinal bacteria on liver physiology mainly occurs through the “gut-liver axis,” referring to the intimate communication between these two organs. The microbiota produces a wide variety of metabolites that can potentially reach the liver, but only very few of them have been studied. UCL has identified that indole and p-cresol, two bacterial metabolites produced from aromatic amino acids, can dose-dependently inhibit the LPS-induced overexpression of pro-inflammatory genes in a process that is partly independent of Kupffer cells. *In vivo* experiments confirmed that indole has anti-inflammatory effects in the liver. Targeting the production of indole by the gut microbiota could, therefore, be a promising strategy to limit liver inflammation (Beaumont et al., *FASEB J, 2018*).
Proteolytic activities coded by the microbiome as novel mechanisms to control the metabolic, immune and behavioural responses

The enzymatic activity dipeptidyl-peptidase 4 (DPP-4) produced by the eukaryotic cells had been previously proposed as a potential target in metabolic and behavioural disorders since it modulates the functionally of several gut peptides and neuropeptides. In the frame of the MyNewGut project, the DPP-4 activity of the gut microbiota was proposed as a novel mechanism to control the host metabolism and behaviour. Through the collaboration between several groups of WP5 (TUM, CSIC, MUG & UCL) it has been proven for the first time that DPP-4 like activity also originates from the gut microbiota (Olivares et al., Front Microbiol. 2018). Besides, UCL demonstrated that the DPP-4 inhibitor vildagliptin impacted the intestinal homeostasis, influencing the gut microbiota composition and activity, and improved the gastrointestinal function as a result. If confirmed in humans, this could open new therapeutic uses of DPP-4 inhibition to tackle gut dysfunctions in different pathophysiological contexts (Olivares et al., Diabetologia, 2018).

UCL has also investigated the influence of the addition of other macronutrients, like gluten, into the Western diet (45 kJ% from fat and 17% from sucrose) and has observed that gluten potentiates the obesogenic effect of the diet. In parallel, UCL has studied in this murine model (obesogenic diet with gluten) if the proteolytic activity of the gut microbiota (DPP-4 and prolyl endopeptidase activities) could be increased by prebiotics (arabinoxyloligosacarides (AXOS), or fructans) to promote the digestion of gluten and prevent its detrimental effects consequently. AXOS protects from the increase in the body weight and the expansion of the adipose tissue. In agreement with the initial hypothesis, AXOS caused an increase in the proteolytic activities involved in gluten digestion associated with a reduction of gluten immunogenic peptides in the caecal content. The beneficial effect of AXOS occurred linked to profound changes in the gut microbiota composition, favouring the bloom of beneficial bacteria negatively correlated to gluten immunogenic peptides (Olivares et al., to be submitted).

Causality studies based on transplants of the gut microbiota of obese and insulin-resistant patients into in germ-free mice

The causal relationship between diet-induced obesity and metabolic disorders is not clear. One of the objectives of TUM (Prof. D. Haller) was to establish a humanized mouse model for obesity and metabolic dysfunction using patient-derived faecal microbiota. TUM also investigated the effect of human microbiota transplants showing different clinical outcomes, on mouse metabolism as well as liver, gut and fat structure and function at various colonization periods. For this purpose, germ-free male wild type C57BL/6 mice were colonized once with faecal microbiota from an obese and an insulin resistant patient receiving exclusively control diet during the experiment. Additionally, the impact of high fat feeding on the mouse organism was investigated. Therefore, mice were fed a palm-oil based high fat diet based for four weeks in order to provoke diet-induced obesity. As an additional control one set of mice was colonised with microbiota from a lean patient. Transferring microbiota from obese and insulin resistant patients did not induce respective phenotype in gnotobiotic mice compared to animals colonized with lean microbiota. Only mice on control diet revealed normal body development; fat pad weights and unaffected fasting blood glucose levels independently of human donor. However, diet-induced obesity in colonized mice provoked insulin resistance, inflammation independent liver steatosis and low-grade fat inflammation combined with adipocyte hypertrophy, but no gut barrier impairment and intestinal inflammation regardless of human donor. Analysis of microbiota profiles showed an incomplete transfer of human donor microbiota after transplantation to germ-free mice. This was characterized by a loss in number of bacterial species and dramatic changes in community
structure. Interestingly, transfer efficiency was constant over time independent of the colonization period.

**Epidemiologic Studies to Identify the Effects of Environmental Factors on Gut Microbiome and Its Influence on Brain, Immune, and Metabolic Programming, Development and Function (WP6)**

**Study of the microbiome and biomarkers of immune and stress in infants with altered microbiota composition**

With regard to the objective to assess the consequences of altered microbiota composition in early life, 50 babies delivered by Caesarean section (C-section) and treated with antibiotics during the first 4 days of life were recruited into the study coordinated by the UCC (Prof. C. Stanton). The full cohort of 50 babies did not reach the age of 2 years within the time period, and as a result, only a sub-set of the data was analysed by the end of the project (November 2018). However, it is clear that there are differences in the profile of gut microbiota present in the stool samples in these babies compared with vaginally-delivered babies (samples from INFANTMET). MyNewGut babies had decreased levels of bifidobacteria and lactobacilli at the week 1 sampling time point, and there was an increase of both from week 1 to year 1, as the profile becomes more normalised to vaginally-delivered babies.

As regards neurodevelopment, the MyNewGut babies which had been delivered by C-section and treated with antibiotics during the first 4 days of life were assessed using the Bayley Developmental Assessment (at approx. age 2 years, N=17). The MyNewGut babies were compared with a cohort of similarly aged babies delivered by C-section but who had not received any antibiotics in the first 4 days of life (N=23). Antibiotic-treated infants born by C-section (MyNewGut cohort) performed significantly worse than non-antibiotic treated infants born by C-section (INFANTMET cohort) on the Language, Motor, and Adaptive composite scales of the Bayley-III. Furthermore, antibiotic treated infants born by C-section (MyNewGut cohort) performed significantly worse than non-antibiotic treated infants born by C-section (INFANTMET cohort) on the Receptive and Expressive Language subscales, as well as the Gross Motor subscale. These findings have significant implications for neonatology practice. A more in-depth analysis will determine whether the neurodevelopmental anomalies relate to the antibiotic impact on the gut microbiota or the underlying condition that required antibiotic therapy.

**Study of stress, endocrine and immune parameters in adults born via caesarean section delivery to assess the impact of altered gut microbiota during early life in the adulthood**

The MyNewGut project also included a study on young adult males born either by C-section or naturally delivered (UCC, Prof. T. Dinan). The study recruited 36 C-section and 40 natural-born male adults, aged 18-24 years. The impact of an altered microbiota during early life (due to mode of delivery at birth) on the cognitive, endocrine and immune response to laboratory-based public speaking acute stress (Trier Social Stress Test, TSST) and following a period of naturalistic (university examination) stress, compared with a non-stress period, was assessed. C-section participants did not exhibit a differential salivary cortisol response to the TSST. Measures of positive affect were significantly lower throughout the procedure, although negative affect was not. Interestingly, participants born by C-
section reported greater psychological stress in response to the TSST when compared to vaginally born participants.

To further examine the effect of exam stress on hypothalamic pituitary adrenal (HPA) axis function, the salivary cortisol awakening response (CAR) was measured at each time point (non-stress and exam-stress), but this was not significantly different between C-section and vaginally-born participants. When comparing psychological distress levels during the Non-Stress and Exam-Stress periods, participants born by C-section reported significantly greater levels of trait anxiety, perceived stress, but not depression when compared to vaginally born participants, during the Exam Stress period but not during the Non-Stress period. To our knowledge this is the first time differences in stress response have been reported in adults who had been born by C-section.

To determine the effect of stress on cognitive function, tasks from the CANTAB battery of cognitive tests were used at each time point (non-stress and exam stress). Interestingly, we identified no difference in cognitive performance on tests of visuospatial memory, response inhibition attentional flexibility or reversal learning between C-section and vaginally-born participants during the Non-Stress or Exam Stress period. Overall, we found that birth by C-section leads to greater psychological vulnerability to both acute and naturalistic stress in young adulthood.

Furthermore, mode of delivery at birth had an enduring effect on host immune system. Higher plasma levels of IL-1β and IL-10 were observed in the young adults born by C-section when compared to vaginally-born participants, supporting a dysregulation of immune-brain signalling in regulating behaviour. Again this is an immune observation that has not been demonstrated previously. With regard to gut microbiota, overall, the microbiota of both adults born via C-section or by natural delivery, is indistinguishable in adulthood, even though the negative behavioural effects of C-section endure. Nonetheless, effects of C-section on gut microbiota early in life could have programmed the differential response to stress seen in adulthood.

The UCC-APC MYNEWGUT Culture Collection now houses 500+ putative *Lactobacillus* spp. and 550+ putative *Bifidobacterium* spp., collected from the adult participants and infant MYNEWGUT cohort at 1, 4, 8, 24, 52 and 104 weeks of life. Screening of these strains for psychobiotic potential - probiotic bacteria which may have the potential as a novel therapeutic strategy in the prevention and/or treatment of certain neurological and neuropsychiatric conditions will continue. All strains are currently being assessed for psychobiotic potential with a particular focus on serotonin and GABA. We have published a strategy paper for the identification of beneficial microbes with psychobiotic potential ([Bambury et al. Br J Pharmacol, 2018](#)) and we are currently following this approach.

In summary, it is clear from the research undertaken in WP6, that mode-of-delivery in conjunction with antibiotic use during the first few days of life has an adverse impact on neurodevelopmental and outcomes in early childhood, and on psychological stress responses in early adulthood. In order to mitigate this effect, it is clear that minimising unnecessary provision of C-section and antibiotic use in the first few days of life is a priority.

Study of growth and neurodevelopment and their relationship with gut microbiota in children, who were born to mothers with different metabolic phenotypes, up to the age of 6.5 years.

The study performed in the PREOBE cohort within MyNewGut framework was coordinated by UGR (Prof. C. Campoy). This included the re-call and examination of 188 children at 6.5 years of age, who
were born to normal weight (NW), overweight (OV), obese (OB) or mothers with gestational diabetes (GD). Children were examined including growth, neurodevelopment and brain imaging assessment and gut microbiota analysis. All previous data obtained in this cohort from pregnancy till 6.5 years old have been used in the different statistical models to fulfil our objectives and answer the hypothesis. Initially, we studied the microbial structure and function in infants at 6 and 18 months (UGR) and in children at 3.5 and 6.5 years old (UNIBO).

At 6 months old, bacteria that best differentiated the groups (NW, OV, OB, GD) were Parasutterella, unclassified Bradyrhizobiaceae and Haemophilus. At 18 months old, bacteria that best differentiated the groups were unclassified Alphaproteobacteria, Fusobacterium and Leuconostoc. Lastly, at 6.5 years, bacteria that best differentiated the study groups were Clostridia and Negativicutes, followed by Erysipelotrichia, Actinobacteria, Bacilli, Coriobacteria and Bacteroidea.

At 6.5 years, children born to OB mothers had significantly higher Z-score weight for age, Z-score body mass index (BMI), hip circumference and mid-thigh circumference than those born to NW women. Clostridia and Verrucomicrobiae and Bacilli and Negativicutes, followed by Coriobacteria were the bacteria that best differentiated the PREOBE study groups at phylum and class level. Furthermore, anthropometric parameters and phylum level abundance were correlated. In children born to NW mothers, Proteobacteria were negatively correlated to fat-free mass and total body water. By contrast, in children born to OV/OB mothers, Proteobacteria abundance was associated with BMI after being adjusted by confounding factors. In the offspring of NW mothers, Firmicutes was negatively correlated to head circumference, while in those born to OV/OB mothers, the correlation between these two parameters was positive. Furthermore, in OV/OB and GD groups, Bacteroidetes were negatively correlated to head circumference, but not in NW mothers.

Besides, we observed that Lactococcus, Lachnospiraceae_incertae_sedis and Bacteroides were able to differentiate between infants of 6 months of age above and below the median of composite cognitive score (CCS). Furthermore, metaproteomic analyses suggested mechanisms that might underlie microbial effects on infant neurodevelopment. In fact, proteins involved in intracellular trafficking were more abundant in infants below the median in the CCS, while those involved in carbohydrate transport were enriched in children with above the median CCS. At 6.5 years of age, Firmicutes abundance in the gut microbiota of children born to OV/OB and GD mothers was associated with better vocabulary, matrix typical and IQ typical composite scores. Instead, children born to GD mothers, Firmicutes and Proteobacteria abundances were associated with a better vocabulary and matrix typical scores. After adjusting different variables (maternal age, maternal pre-gestational BMI, maternal intelligence quotient (IQ), type of delivery, children sex, birth weight and type of infant feeding) children born to OV mothers presented a lower volume of the right temporal inferior gyrus and higher left gyrus rectus cortical thickness compared to the other three groups. Right inferior temporal volume showed significant positive correlation with the number of errors during a memory verbal task. That means that the higher volume, the higher number of errors. Children born to OB mothers presented a higher right superior insula and parieto-occipital cortices compared to those born to NW and OV mothers. The right superior insula thickness showed a negative correlation with the scores of a working memory task \( r = -0.176, p=0.041 \). So, the smaller thickness is related with better performance in this task.

We also investigated the possible links between the gut microbiota of 6 and 18 months-old children and the neuroimaging variables assessed later in life (at 6.5 years old). At 6 months, Proteobacteria...
and Firmicutes were the taxa most strongly associated with long-term influences on brain structure. At 18 months of age, the greater diversity of the gut microbiota made more difficult to establish clear correlations. Several correlations between gut microbiota and brain structure were observed in all groups, but in the offspring of the OB mothers, the correlations between gut microbiota abundance and total brain volumes and surfaces were more numerous and stronger compared to the rest of the groups.

Phyla levels were associated with different brain structural areas according to maternal metabolic condition in all groups. Almost all phyla were associated with different brain structural volumes or surfaces, in some cases negatively and in others positively. However, the associations only remained after adjustment in those children born to OV/OB or to GD mothers. These results suggested a role for the microbial structure and metabolic functionality in the balanced structural development of the brain that is influenced by maternal metabolic condition. Again, after adjusting to the different variables considered (maternal age, maternal pre-gestational BMI, maternal, type of delivery, children sex, birth weight, type of infant feeding) we analysed the associations between gut microbiota at 6 and 18 months of life and brain connectome. At 6 months of age, higher Proteobacteria, Firmicutes and Bacteroidetes abundances were associated with brain motor and default mode networks. Furthermore, Proteobacteria and Firmicutes were negatively correlated to salience network, and Bacteroidetes were correlated positively. Regarding executive control network, Proteobacteria and Bacteroidetes correlated negatively with prefrontal cortex and Firmicutes were correlated positively. At 18 months of age, a higher Bacteroidetes and Firmicutes abundance were associated with brain salience and executive control network and default mode network, respectively. By contrast, Firmicutes abundance was correlated negatively with salience network. It is noted that motor network was only positively correlated to Proteobacteria abundance.

In conclusion, this study has demonstrated a link between gut microbiota and children body composition, neurodevelopment and brain structure and function. Furthermore, we were able to confirm that maternal metabolic condition has a strong impact on the establishment and functionality of the offspring gut microbiota during the first 18 months of life, which persist during childhood up to 6.5 years of age.

Innovative Food Ingredients and Products Targeting the Human Gut Microbiome (WP7)

In WP7, we have developed and evaluated new approaches for the modulation of the intestinal microbiota based on prebiotics or probiotics and their incorporation to innovative food products as a mean to control metabolic health.

Develop innovative prebiotic strategies

Cargill, together with Barilla and CAPSA, worked on the selection of fibre-rich ingredients used in the development of innovative food prototypes targeting the human gut microbiome. The selection was based on ingredients used, market insights and consumer understanding. The selected compounds included WBE rich in AXOS (WBE-AXOS), Ultrafine milled wheat bran (heat-treated) and Resistant starch type 3 from tapioca (C*Actistar 11700). Characterization of in vitro digestibility analysis of the compounds was performed to analyse the non-digestibility properties of the fibre component of these products.
Afterwards, UCL evaluated the protective roles of the three types of candidate prebiotic compounds selected in a murine model of diet-induced obesity and metabolic dysfunction (developed in WP5) to establish the basis for possible human studies (in WP8). Based on the first results obtained on body weight and adiposity, UCL performed an additional experiment with the best prebiotic candidate produced by Cargill (WBE-AXOS) in order to evaluate its efficacy on several key parameters in the context of obesity and associated metabolic disorders: gut immune and endocrine function, gut barrier function, glucose and lipid homeostasis. It was demonstrated that WBE-AXOS decreased obesity and fat mass expansion. This effect was associated with higher number of bifidobacteria in the caecal content and lower inflammation in the subcutaneous adipose tissue (Suriano et al., Sci Rep, 2017). It was concluded that WBE is a good prebiotic candidate for a human study in the context of obesity (which was tested in WP3 in humans). In the last step of the project, the partners of WP7 revisited the concept of dietary fibres, taking into account their interaction with the gut microbiota. A Position Paper has been elaborated by the partners of the WP7 among other to summarize the main effects of dietary fibres with prebiotic properties in intervention studies in humans, with a particular emphasis on the effects of arabinoolxylans and AXOS on metabolic alterations associated with obesity (Delzenne et al., Clin Nutr, 2018. Under revision).

**Develop innovative probiotic strategies**
Several strategies have been followed to advance in the field of the probiotics. On one hand, novel functions for existing probiotics (one strain of *L. paracasei*) were evaluated. On the other hand, new bacteria that might constitute the "next generation of probiotics" were isolated from humans (see WP5). The safety of some of these bacterial stains, the feasibility of scaling-up their production and their stabilization through encapsulation strategies were also investigated.

For investigating the efficacy of existing probiotics on obesity and associated disorders, TUM colonized germ-free mice at the age of 4 weeks for 12 weeks with a human inoculum from a patient that was obese, insulin resistant and high in CRP levels. In the first 8 weeks of colonization, mice were fed the control diet. Then, mice were switched to 48 kJ% palm oil-based HFD and simultaneously be challenged with a single dose of *L. paracasei* strain via gavage. Afterwards, mice were followed for 4 weeks. A dose of *L. paracasei* administered to mice fed a HFD did not result in improved metabolic parameters compared to only HFD fed mice, but rather in slight increase in body and fat weight. In addition, inflammatory status in *L. paracasei* -treated mice was not changed.

Regarding new potential probiotic bacteria, CSIC isolated the strain *Bacteroides uniformis* CECT 7771 from a healthy breastfed infant and demonstrated to ameliorate metabolic disorders in a pre-clinical mouse model of obesity in WP5. In WP7, the safety of this strain was confirmed preliminarily with an acute toxicity study in mice from which adverse effects could not be observed (Fernández-Murga and Sanz, PLoS ONE 2016). The safety of this strain was also evaluated by whole-genome sequencing and in silico analysis of possible virulence factors and antibiotic resistance genes. The genome of *B. uniformis* CECT 7771 was sequenced with second and third generation massive sequencing technologies. The genome assembly was based on long DNA reads generated by nanopore-based platform (Oxford Nanopore Technologies) and short DNA reads generated by the Illumina HiSeq2500 platform. The presence of a plasmid (pBU7771) was detected, but no evidence of potential biological risk was found. This assumption was based on the following facts: (i) this was a very low copy number plasmid (1.86 plasmids per cell), (ii) no specific function was identified for the four coding genes.
detected and (iii) these genes do not encode proteins related to antibiotic resistance or virulence factors by comparing their amino acid sequences to specialised databases (ARDB, VFDB and MvirDB). In the chromosome, of more than 5,200 genes analysed, we found that ORFs BUNIF7771_3570 and BUNIF7771_4507 are homologous to the genes cbla and tetQ encoding for a β-lactamase of B. uniformis ATCC 8492 and for the enzyme TetQ of B. fragilis YCH46, respectively. These genes would potentially confer resistance to β-lactamics and tetracyclines, respectively. However, these genes are constituents of the chromosome not encoded or flanked by mobile or transposable elements and, therefore, likely constitute intrinsic antibiotic resistances. This study has been published (Benítez-Páez et al., Front Microbiol. 2017).

The safety of B. uniformis CECT 7771 was also confirmed in a sub-chronic animal trial of 90-days (Gomez Del Pulgar et al., 2018, submitted). This study has been conducted in Wistar rats (males and females) divided into 5 experimental groups (N = 10), each were administered either a dose of B. uniformis CECT 7771 (10⁶ CFU/day, 10⁹ CFU/day or 10¹⁰ CFU/day) or B. longum ATCC 15707T (10¹⁰ CFU/day) used as a control strain with qualified presumption of safety (QPS) status or placebo (vehicle) for 90 days. Signs of toxicity, morbidity and mortality were recorded daily while weight and food intake were recorded weekly. Biochemical parameters related to pancreatic, liver and kidney functions were analysed as well as inflammatory immune markers. Bacterial translocation to peripheral tissues was assessed and histological/histometric analysis was performed in colon sections. Adverse effects were not observed regarding the general health status and food intake at any of the doses tested. The biochemical parameters showed no differences related to the treatment, except for alanine aminotransferase, whose levels were reduced as the dose of B. uniformis CECT 7771 was increased, indicating a potentially beneficial role in liver function. The ratio of anti-inflammatory to pro-inflammatory cytokines suggested that B. longum ATCC 15707T at 10¹⁰ CFU/day and B. uniformis CECT 7771 at 10⁹ CFU/day could exert anti-inflammatory effects after long-term administration. The faecal microbiota analysis showed that B. uniformis CECT 7771 successfully colonize the intestine tract of rats after oral administration and modulates the gut microbiota with no adverse effects, like reducing the richness and diversity.

The results indicate that the oral consumption of B. uniformis CECT 7771 during a sub-chronic 90-day study in rats does not raise safety concerns. The effects of the bacterial strain on markers of liver function and inflammation suggest that it can confer potential benefits. However, further studies are required to confirm the safety of this strain and its products thereof in humans.

Our industrial partner Lallemand (LAL) has investigated the feasibility of the production in lab-scale bioreactors of two obligatory anaerobic intestinal bacterial strains. These strains were previously isolated by CSIC from human samples and proven to have efficacy to ameliorate metabolic dysfunction in DIO mice (WP5). LAL succeeded in cultivating pure cultures of the two strains, but further modifications of the growth conditions (e.g. regarding media composition, gas composition, temperature and pH regulation) could be investigated to maximize the bacterial production.

CSIC (Dr. A. López) has also developed a couple of strategies to improve the stability of sensitive intestinal bacterial strains through encapsulation approaches. The first one was based on the use of no-purified agar fractions (as cheaper gelling agents), showing that the presence of other compounds (mainly proteins and polyphenols) in the non-purified agar fractions significantly improved the viability of these sensitive bacteria both at ambient and refrigerated storage conditions. Furthermore, the presence of impurities allowed the increase of solids content in the formulation giving raise to...
stronger gel particles, which could contribute to limited oxygen diffusion, thus, partly explaining the improved protection. The second strategy was the formation of agarose-based hydrogel particles using a newly developed method named “oil-induced biphasic hydrogel particle formation”. The protection ability of this method versus directly freeze-drying the bacteria-containing solutions was demonstrated during storage and simulated in-vitro digestion. Both, the formation of a continuous layer surrounding the bacteria and the optimal combination of materials (agarose providing suitable oxygen barrier and WPC with proven probiotic affinity) rendered encapsulation systems keeping viability levels required for commercial applications.

**Develop innovative food prototypes**

The partners FINS and ADMS prepared a new fermented milk product (‘probiotic yogurt’) with 1.5% milk fat, made from milk, yogurt cultures and probiotic bacterial cultures. Milk was inoculated with a blend of probiotic bacteria provided by LAL (Bifidobacterium B94 and Lactobacillus HA119) as well as with the yogurt cultures Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Different aspects of the new probiotic yogurt were analysed. First, the typical chemical parameters were determined (milk fat, dry matter, fat in dry matter, pH and acidity as well as its microbiological status in the presence of Enterobacteriaceae, Staphilococcus aureus, yeast and mould). Also sensory analysis of new yogurt was performed. Values for physico-chemical tests were in the range typical for this type of product. The microbiological analysis of the product obtained was carried out in accordance with Regulation (EC) No 2073/2005. The microbiological analysis found no presence of Enterobacteriaceae, S. aureus, yeast and mould above the maximum permissible level. The sensory evaluation of five samples of yogurt (new probiotic yoghurt plus four samples of commercial yogurts) was performed with a panel of ten trained evaluators, in two sessions, using quantitative descriptive analysis (QDA) and 10 cm intensity scale. All yogurt samples, except newly-made yogurt, are commercially available and are selected based on fat content (1-2% interval). The results indicated that new probiotic yogurt has statistically significant different values for total yogurt flavour (least expressed), acidic taste (least pronounced), density (largest in relation to other samples) and lining of the mouth (most pronounced). The new probiotic yogurt is low fat, neutral, non-acidic, dense and lines the mouth very well. These characteristics make it very good for commercialization as a novel type of probiotic yoghurt with positive sensory traits.

The activities of CAPSA were related to the objective of developing a new dairy product that incorporates the prebiotic fibre selected in the previous WP7 tasks. Different trials at laboratory level were made to assess the impact of WBE-AXOS addition in a dairy matrix, fixing the preliminary conditions of mixing the fibre with the milk, and trying different options of flavouring. Two different doses of WBE-AXOS (20 and 40 g/l) were assayed and tried different vanilla flavours and doses. After several trials of optimization, four combinations of WBE-AXOS and flavour were selected to be fully analysed. In addition to the samples of trials, an additional sample of milk without WBE (as reference) was sent to Cargill to analyse the WBE-AXOS content. The results were in accordance with the calculated AXOS content of the WBE containing products (1.4% and 2.8% for 20 g WBE/l, and 40 g WBE/l), meaning that the WBE was stable in the milk matrix and that the losses due to heat treatment were limited. The developmental work was finished at pilot scale by studying the behaviour of the mix to the heat treatment and establishing the best conditions of mixing, heat treatment and homogenization for this type of product. These selected conditions of the process were used in the up-scaling phase (WP10) and production for the intervention trial (WP8). However, due to identification of risk posed by Bacillus cereus in some of the batches of the fibre ingredient produced by Cargill, it
was decided to not use WBE but use a mixture of inulin-type fructans and resistant maltodextrin for the human intervention conducted in WP8.

**Human Intervention Trials to Evaluate the Efficacy of Innovative Food Prototypes and Ingredients on Brain and Diet-Related Disorders (WP8)**

In the WP8, we have carried out two intervention trials with potential probiotic strains led by UCC and one intervention with a food product supplemented with fibre led by UCPH to provide evidence of their possible efficacy in humans

**Intervention with new probiotics to determine their efficacy on brain-related disorders**

The aim of the intervention studies conducted by UCC-APC (Prof. T. Dinan) was to translate successful pre-clinical findings of efficacy of the probiotics in promotion of anxiolytic phenotypes. The first study, a double-blind placebo-controlled crossover study of a *Lactobacillus rhamnosus* strain (JB1™) intervention in young healthy volunteers (mean age 24.59 years; N=29) examined the effect of the probiotic on measures of acute stress, inflammatory profile and cognition. The probiotic failed to show any clear benefit on these measures (*Kelly et al., Brain Behav Immun. 2016*), and highlights the difficulties with translating promising pre-clinical work into human studies successfully. There are several reasons as to why this study failed to show efficacy: perhaps the dosing was too low, the study population was inappropriate, anxious patients might have responded but the answer may lie in the fact that rodents respond to this bacteria and humans do not.

A second probiotic intervention study, a double-blind, placebo-controlled study using a *Bifidobacterium longum* strain (1714™) intervention, was undertaken in the final year of the MyNewGut project. Preliminary results from this study (N=20) show positive effects of the probiotic in ameliorating some sleep deficits in young university students undergoing a naturalistic stressor (exam stress). Stress is associated with poor sleep quality. Therefore, probiotic supplementation, which can impact upon and improve sleep quality during stress or in people suffering from stress-related disorders, presents an exciting potential therapeutic strategy for such populations in the future.

**Intervention with a new food prototype supplemented with fibre to determine its efficacy on obesity and components of the metabolic syndrome**

Cargill developed food prototypes for three types of bakery products with the selected prebiotic fibre WBE. Bread, crisp bread and rusk were selected as having the highest potential for incorporation of high levels of fibres and showed a good fit with the intent of developing bakery product low in calories for use in the intervention trial of WP8. It was decided to focus the development work towards an EFSA high fibre nutritional claim, i.e. 6 g fibre/100g product. Bakery specialists developed recipes and food prototypes that were evaluated for physical and sensorial properties in comparison to reference products. Addition of WBE in bread provided a fresh and good crumb of which the structure was slightly denser and the colour more yellow with a nice golden brown colour to the crust. The addition of fibres in the crisp bread resulted in a slightly softer dough and a lower hardness compared to the reference product. The addition of WBE seems to result in a higher retention of moisture in comparison to the reference, which was reflected in the water activity. This might influence the crispiness over time. Moreover, 75% of the sugar in the reference products was replaced by WBE. Sugar reduction contributes to decreased hardness. The crisp breads with WBE have in general a nicer golden brown and very bright colour. The hardness of rusks with WBE was very similar to the
reference and did not change after 1 week. The moisture content and water activity of the rusks with WBE were higher in comparison to the reference products. In general, development work on the addition of WBE to product types bread, crisp bread and rusk at dosages of 6 g fibre/100g final product (high fibre nutritional claim) was found to be very successful and promising both in terms of physical and sensorial properties.

As state in the WP7, it was finally decided to not use WBE but use inulin-type fructans and resistant maltodextrin for the intervention study of WP8, due to the risk posed by the identification of the presence of Bacillus cereus in some of the batches of the WBE.

UCPH (Prof. T.M. Larsen) conducted the intervention trial. The aim of this study was to investigate whether a milk product supplemented with fibres (inulin-type fructans [oligofructose] and resistant maltodextrin) would induce a more significant change in body weight compared to placebo (maltodextrin) during 12 weeks of energy restriction in an obese/overweight population. Secondary objectives were to investigate the effects of the fibre-containing product on body composition, the gut microbiome, glucose metabolism, metabolomics, and lipidomics.

The participants were randomized to one of the two dietary products (fibre or placebo) before initiation of a 12-week energy-restricted diet. The participants attended a screening visit before the intervention period, two clinical investigation days; one at baseline (week 0) and one at the end of the intervention (week 12), and five consultations with a dietician during the intervention with evaluation of body weight and diet. A total of 259 potential study participants underwent an online pre-screening assessment, and a total of 153 study participants were invited to a formal study information meeting. A total of 118 subjects completed the formal screening examination, and a total of 116 study participants were considered eligible for study participation and were randomized to one of the two treatment groups. Within the treatment group receiving fibre supplement, a total of 42 (29 % men) participants completed the study, and for treatment group receiving placebo supplement, a total of 44 (43 % men).

Analyses show no between-group treatment difference for change in body weight, which was considered the primary study outcome. Similarly, no between-group differences were found in analyses of the remaining anthropometric outcomes including waist and hip circumference, sagittal height, fat mass, lean body mass, visceral adipose tissue, and fat percent. Furthermore, no between-group treatment difference was observed concerning lipid profile (total, HDL- and LDL-cholesterol and triglycerides) or inflammatory markers (haemoglobin, white blood cells, C-reactive protein, ALAT, and ASAT).

With regards to blood pressure, the group receiving fibre supplement experienced a significantly larger decrease in both systolic and diastolic blood pressure after 12 weeks compared to the group receiving placebo supplement. By contrast, serum insulin levels decreased more in the group receiving placebo compared to the group receiving fibre supplement. This result was further reflected in the analysis of HOMA-IR (homeostatic model assessment of insulin resistance), showing a significant decrease in the group receiving the placebo for 12 weeks compared to the group receiving the fibre supplement. Concerning glucose metabolism, the fibre supplement was not able to decrease the levels of serum insulin to the same extent as the placebo supplement.
Subgroup analyses revealed important sex-related differences concerning the above-described results. Statistical analyses including only the women revealed that the group receiving the fibre experienced a significantly larger decrease in both systolic and diastolic blood pressure, while those receiving placebo showed a larger decrease in insulin and HOMA-IR. In contrast, when analysing only the men, no significant differences were detected.

The analyses showed a difference in the level of physical activity between groups. The placebo group had an increased level of physical activity compared to the fibre group after the 12 weeks of intervention. UCPH did not detect significant differences between the groups when stratifying for sex regarding physical activity.

CSIC has completed the microbiota analysis of the subjects involved in this intervention by the 16S rDNA amplicon sequencing (metagenomics analysis is ongoing). The alpha diversity analysis of the microbiota revealed that the intervention tended to reduce the uniformity (Simpson's evenness) of the microbial species abundance and the diversity (Reciprocal Simpson's index) in the two groups (fibre and placebo). The intervention reduced the evenness of the microbial communities in both groups, indicating that the caloric restriction alters the intestinal microbiota allowing for some species become dominant. The results also suggest that the fibre administration contributed to reducing the gut microbiota evenness caused by the energy restriction.

A principal coordinate analysis was carried out to visualize the effects of different variables (diet, time, sex/gender, etc.) on the microbial communities. This analysis suggested that some microbiota components were responding to fibre intake in a specific manner and that the response to the diet differed in females and males. Therefore, sex-related effects were considered in further analysis to establish relationship between the different variables and the taxonomic groups detected.

Interestingly, the largest microbiota variation associated with sex/gender was observed for the Paraprevotella genus, which was significantly more abundant in men than women (p = 0.004). The genera Bilophila, Hungatella, Acetanaerobacterium and Faecalibacterium showed the largest significant positive associations with the BMI, indicating that the higher the BMI, the higher the abundance of these species (p < 0.050). These results are in agreement with previous observations in animal models of diet-induced obesity (DIO), where Bilophila wadsworthia abundance correlated positively with high fat intake and body weight (Schneeberger et al. Sci Rep. 2015;5:16643), and in human studies where this particular species was positively associated with BMI, insulin resistance, and inflammation (Brahe et al. Nutr Diabetes. 2015;5:e159).

The fibre intervention exerted a bifidogenic effect, a feature also recognized for other dietary fibres (Benítez-Páez et al. Trends in Food Science & Technology, 2016). Furthermore, the fibre tested increased the abundance of the Parabacteroides genus, a bacterial group known to be positively modulated by starch-based dietary supplements (Holscher. Gut Microbes. 2017;8(2):172-184). Additionally, we observed that potentially harmful bacteria such as Bilophila species were reduced in the group receiving fibre. This observation is of particular relevance given the strong association of these microbes with obesity, insulin resistant, and inflammation as previously stated (Schneeberger et al. 2015).

Metagenomics, metabolomics and lipidomic analyses are ongoing and data integration with clinical readouts will be done by the beginning of 2019.
Data Analyses and Integrative Studies (WP9)

To be able to understand the contribution of the microbiome to human health and thereby go from correlation to causation, microbiome data of many health outcomes in different people (with a different physiological status) are needed. An example on such analysis was performed for the relation between age and microbiome (O'Toole and Jeffery. Science 2015, 350(6265):1214-5) and between diet and health and microbiome (Claesson et al., Nature. 2012;488(7410):178-84). These studies show that many factors impact the microbiome and that many of them interact, e.g., if people living in a community will exchange bacteria, but often also eat similar products. To use these factors to steer the microbiome to individually health levels we should find the causal relations between environmental factors, microbiome composition, and health. It is not feasible to perform a human study that incorporates enough data and variability of meta-data. Therefore, it is essential to integrate data from a multitude of studies, which was the aim of WP9 (led by J. Bouwman at TNO).

For the efficient integration of data, standardization is essential. For this purpose, the FAIR (findable, accessible, interoperable and reusable) data principles are developed (Wilkinson et al. Sci Data. 2016 Mar 15;3:160018). Those principles define that (meta)data should be assigned to a globally unique and persistent identifier, contain enough metadata to be able to fully interpret the data and indexed in a searchable source. Data are retrievable and include their authentication and authorization details. The (meta)data use vocabularies that make it possible to link to other (meta)data and (meta)data are released with a clear and accessible data usage license. Nowadays many human studies also analyse the microbiome. Integrating these data will improve our understanding of the interactions between host and microbiome. However, these data are regularly not fully shared upon publication (as is the standard in transcriptomics analysis). If they are shared, they often lack the physiological individual data of the host and details on the study design are not well annotated or even lack. Therefore, in MyNewGut we used the Phenotype database (www.dbnp.org) to make our data FAIR. In addition, the precise detail of the accessibility of these studies was included. The data and meta-data were made interoperable in high detail so that it was ready for integrated analysis. For this, a mapper was developed that made it easier to map the terms onto ontologies (standardized terminologies with relations).

All analysis was done in close collaboration with the data owners; this is essential since the corresponding partners are the only one that fully know the details about the studies and thereby understand the outcomes of the data integration. For this reason, we had several digital and face-to-face meetings with other partners.

We first started with data integration on old datasets of TNO. This preparatory work helped to resolve the well-known issues of data integration of microbiome data. We showed the technical variances over different runs using a sample that was produced by mixing several samples from a large study. From this analysis we recommend to always include a mixed sample in the run of microbiome data as a test of technical variation, an analysis of the quality of the run and a solid basis of defining a sequence level cut-off. Unfortunately, this is not anymore possible for the studies that are performed within MyNewGut as the analyses had been performed already before this work was ended. We then performed and integrated analysis on the baseline of different intervention studies of WP3 (HPD led by INRA, WBE-AXOS/PUFA led by UCPH, and FMT/butyrate led by AMC). It shows the importance of...
careful processing of the data. Clearly, it is necessary to harmonize data by using uniform analytical pipelines to align the raw, unprocessed data. To enable this, the research community should make codes available through interactive portals. Still many issues were encountered during the analysis process. However, we have shown that it is possible to integrate data from different clinical trials, while keeping its individual value.

At last, we analysed the complete WP3 studies along three axes: the microbiome, the metabolome and the dietary fibre intake. We showed that although there are limitations it is possible to integrate data from different clinical trials and it is also possible to integrate data from different analysis platforms. This systemic view led to new insights on the intervention effects and additive value from existing data. We have made progress in the development of the prepossessing, analysis pipelines and standardization of data to facilitate this in further projects. This work will be published in two peer-reviewed papers and will be used in the following projects.

Production of Innovative Food Prototypes to Control Brain-and Diet-Related Disorders (WP10)

Production of bakery, cereal-based and dairy food prototypes containing prebiotics fibres

Cargill and UCPH agreed on the use of a WBE rich in AXOS in the high fibre diet arm of the intervention trial in WP3 (varying content of fibres and fat composition). The aim was to provide a dose of 15 g WBE/day in the form of bakery products (5 g/d) and sachets each containing the pure product (10 g/d). Recipes were optimised to obtain a daily dose of 5g WBE/day by consuming 2 biscuits and 2 crackers. To obtain these levels, 13% WBE (dough weight) was used in both the recipes for biscuits as crackers resulting in final levels of 1.25 g WBE per baked piece. The average serving weight size of one biscuits and crackers were 8.7 ± 0.5 g and 8.2 ± 0.5 g, respectively. Additionally, the caloric content and nutritional information of the biscuits and crackers were also analysed and reported. The AXOS content of the WBE was shown to be stable during the baking process, meeting the objectives as scheduled.

CAPSA assumed the tasks related to the production of fibre enriched food product to be used for the intervention of WP8 to test its efficacy on obesity and metabolic syndrome components in human adults. This fibre-enriched product consists of semi skimmed milk and a special mix of two fibres, a digestion-resistant maltodextrin and inulin-type fructans [oligofructose], combined in a 50:50 proportion in a final percentage in the milk of 5%. CAPSA produced both products (fibre and placebo) at industrial scale and filled them in aseptic bottles of plastic (HDPE) of 200 mL. For both products, the corresponding quality and safety assessment was done before starting the shelf life study and sending products to the clinical intervention. Different analysis on physicochemical and nutritional composition of both products were also done. Complementarily, CAPSA characterized both products from a sensorial point of view, with its internal tasting panel. CAPSA evaluated the products through their shelf-life and shipped the products to the UCPH for the clinical intervention.

In addition, CAPSA has worked in the development of alternative products enriched with fibre in other dairy categories, like milk powder, milkshakes and supplements. During the last months of the project, it has designed and produced at industrial scale a powder product (that once reconstituted with water, acquires a consistence of pudding), a shake that fulfil the characteristics of “dietetic food for special medical purposes” and some food supplements, all of them enriched in fibre.

"Microbiome Influence on Energy balance and Brain Development - Function Put into Action to tackle Diet-related Diseases and behavior" – MyNewGut has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement nº 613979
**Production of probiotics to test their effects on stress-related outcomes in human trial**

The goals of Alimentary Health’s (AH) were (i) to optimize the industrial production of probiotics with good preclinical data in stress and cognition, (ii) manufacture products according to good manufacturing practice (GMP) standards for use in interventions in humans in WP8 and, (iii) to monitor the stability of the probiotic product during the intervention period. The probiotic chosen in the first instance was a *Lactobacillus rhamnosus* (JB-1™ strain). This strain was identified as having superior effects on stress and behaviour in validated models (*Bravo et al., PNAS 2011*). Subsequently a *Bifidobacterium longum*, the 1714™ strain, was identified as second candidate. The 1714™ strain was shown to reduce stress-related behaviour, improve cognition in animal models (*Savignac et al., Behav. Brain Res. 2014; Savignac et al., Neurogastroenterol. Motil. 2015*), and more recently to reduce stress in a preliminary human trial (*Allen et al., 2016; Transl. Psychiatry*). For each strain optimum growth conditions were established at laboratory scale to assess growth characteristics using industrial based growth media. Target yields required to ensure commercial feasibility and ability to survive through a freeze-drying process were determined. The strains were produced at larger scale in a commercial food grade fermentation facility to assess robustness for scale-up, and freeze-dried single strain products were produced under GMP conditions. In parallel, the counting methods and quality control (QC) procedures for the strains were optimised in-house. The two products were subjected to independent QC and placed on stability trials. Required minimum shelf life was established prior to commencing each clinical study and quality assurance tests.

A clinical product, active and placebo, were formulated for each strain. QC was performed according to European Pharmacopeia standards to confirm count and quality both in-house and at an independent laboratory. Sufficient product to conduct the interventions was delivered to our MyNewGut partner UCC-APC and stored in controlled conditions for use in the planned human interventions. Stability trials on the formulated products were carried out and each product stayed within specification for the duration of the trial.

**Recommendations and Guidelines for European Public Health and Scientific Requirements for Health Claims (WP11)**

**Recommendations based on integrated overview of the scientific project results**

Based on the integrated overview of the scientific project results given in the Project Final Report and the six Position Papers (to be) published in Clinical Nutrition (see for details below) NutriClaim, supported by CSIC and TNO, provided a concise summary of microbiome-informed conclusions and recommendations for dietary guidelines and public health policies in the D11.1 Deliverable Report *Recommendations for public health interventions and policies based on integrated overview of results*. In addition to the dietary and some life-style recommendations published for scientists in the six Position Papers and for non-specialists in the D11.2 Report and some widely distributed semi-scientific publications, the D11.1 report includes also as regulatory recommendation the proposal for the EU and EFSA to recognise that not only dietary fibres as naturally present in foods but also most fibres added to foods have “beneficial physiological effect as demonstrated by generally accepted scientific evidence” as was recently concluded and reported in detail both by the FDA and by Health Canada. As is observed in the Position Papers and a range of other authoritative scientific publications, a substantial increase in fibre intake will increase a gut microbiome contributing both to ‘gut health’ and to a major risk reduction for colon cancer. Such a substantial increase in intake cannot be realised when, as at present, only fibres naturally present in foods are recommended in dietary guidelines and...
not the added fibres, including also (almost) all prebiotic fibres. A more detailed justification for the increased fibre intake is provided in the D11.2 report.

Stakeholder-targeted semi-scientific summary of the project results

The Position Papers provided the scientific overview of project results focussing on specific messages to the scientific community regarding dietary recommendations, taking into account microbiome-mediated effects. Based on the information provided in general by project results and in particular by these Papers and the draft versions thereof, EUFIC prepared a semi-scientific summary intended for a wide range of stakeholders – with major support provided by NutriClaim and CSIC related to dietary and lifestyle recommendations. Also the many presentations in conferences and workshops by MyNewGut scientists and the questions and feedback received contributed to the optimisation of this semi-scientific summary.

A summary of these recommendations and other project results has been published in the widely distributed multi-language EUFIC FOOD TODAY article: Five years in review of ’MyNewGut’: How ‘gut bugs’ affect our physical and mental health.

Due to the limitations in the EUFIC FOOD TODAY format, some details, regarding specific quantitative information about recommended intake of fibres and proteins, relevant for policy-makers and other stakeholders could not be included in the EUFIC article. NutriClaim included these in the D11.2 Deliverable Report, submitted Month 60.

Implications of project results for public health policy

The MyNewGut subgroup formed by ICC and NutriClaim for developing an optimal approach for including gut microbiome related information and insights in public health policy and dietary guidelines selected as optimal approach the publication of six MyNewGut review-type Position Papers (also called Opinion Papers) in a high impact peer-reviewed journal. Based on the agreement realized with Clinical Nutrition (the official journal of ESPEN, the European Society for Clinical Nutrition and Metabolism), the six papers were submitted in 2018 for publication in consecutive issues of the journal. The over-all theme and aim of these review-type papers is to provide microbiome-informed dietary and public health recommendations for promoting metabolic and mental health. The authors drafted the Opinion Papers in close interaction with the leadership and members of the subgroup, for avoiding overlap and for harmonising the set-up and style of the papers. Authors also presented, before and after submission to Clinical Nutrition, conclusions and (dietary and other) recommendations of their paper in workshops and conferences including meetings organised by MyNewGut partners such as ‘DF18’ the 7th International Dietary Fibre Conference, organised by ICC (Rotterdam, 4-6 June 2018), the MyNewGut Workshop preceding DF18, and the MyNewGut Final Conference, organised by EUFIC (Brussels, October 18th 2018). The feedback obtained in meetings confirm that broad consensus exists regarding the views and recommendations expressed in the Position Papers.

Titles and status of the papers, all submitted before November 2018 are as follows.


5. Cerdó et al. Microbiome and early nutrition programming of neurodevelopment: recommendations from the MyNewGut project [under review].


By sharing draft versions of all papers with EUFIC and EFFoST and co-editing the articles the main conclusions of the six position papers are included in key dissemination articles:
- The EUFIC FOOD TODAY article “Five years in review of ‘MyNewGut’: How ‘gut bugs’ affect our physical and mental health”
- The EFFoST on-line magazine Taste of Science in the forthcoming article Linking diet and health – what is the gut’s microbiome role?

The public Deliverable 11.2 “semi-scientific report Summary of project results and recommendations for public health policy” is a somewhat extended version of the EUFIC FOOD TODAY article.

Very shortly summarised, the position papers indicate that diet may have gut microbiome mediated health implications, with high protein, high saturated fat diets showing negative effects on microbiota diversity, metabolites and inflammatory and metabolic adverse effects, whereas, in contrast, increasing fibre intake and choosing poly-unsaturated over saturated fatty acids is likely to have a beneficial effect on the gut microbiome diversity as well as on metabolic and mental health.

**Recommendations on the scientific and regulatory requirements for health claims related to the gut microbiome**

Since it was considered it as essential that, where possible, MNG studies should fit in later health claim dossiers the WP11 leader informed all participants and distributed a document on *Essential requirements for studies aimed for health claim substantiation* in the 1st MyNewGut Project meeting, Valencia, 19-20 February 2014.

One key message already indicated in this document, recommending to select insulin resistance as a key factor in studies for relating modulation of the gut microbiome to health claims, was published: *Stoffer Loman and Jan-Willem van der Kamp (2016) Insulin resistance as key factor for linking modulation of gut microbiome to health claims and dietary recommendations to tackle obesity, Trends in Food Science & Technology 57 (2016) 306-310.*

This message was considered as of major importance, since of all the physiological effects resulting from changes in the microbiome, insulin resistance is the most direct diet-modifiable parameter related to obesity.
NutriClaim assessed which innovative pre- and probiotics studied in MyNewGut have potential for a health claim application. These appeared to be a mix of two fibres (inulin-type fructan and resistant maltodextrin, (see for details WP8) and a *Bifidobacterium longum* strain (see for details WP8 and 10). Guidance documents on the scientific requirements for gut microbiome related health claims and for regulatory requirements for market entry of novel foods were issued in three Deliverable Reports,

- **D11.4a.** (public document) *Guidance document on the generic scientific requirements for gut microbiome related health claims*. This document includes guidance for taking into account EFSA criteria in scientific publications.
- **D11.4b** (confidential document) *Guidance document on the scientific requirements for health claims related to specific potential probiotic bacteria and ingredients, foods and diets for prevention of diet- and brain-related disorders*. This document provides guidance for the pre- and probiotics mentioned above.
- **D11.5.** Guidance document on the regulatory requirements for market entry of the newly developed, innovative food prototypes

By active participation in the health claim related discussions in different meetings (included in the general list of dissemination activities), as regular and invited speakers, partners of WP11 could share their views and learn from discussions. This contributed to the new guidelines documents issued in two project reports.

### MYNEWGUT INVOLVEMENT IN REGULATORY MEETINGS/WORKSHOPS - OVERVIEW

<table>
<thead>
<tr>
<th>Meeting / Workshop</th>
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<tr>
<td><strong>Paula Trumbo</strong> (FDA, USA)</td>
<td><strong>MyNewGut Workshop preceding DF15, 01/06/2015, Paris</strong>&lt;br&gt;<strong>6th Intl Dietary Fibre Conference ‘DF15’, 01-03/06/2015, Paris</strong>&lt;br&gt;Comments on MNG plans and results with focus on options for microbiome related health claims&lt;br&gt;Dietary Fibre- State of the Art and Future Perspectives for Health Claims – Views from the US Food &amp; Drug Administration</td>
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<tr>
<td><strong>Hans Verhagen</strong> (RIVM, NL, EFSA NDA Panel)</td>
<td><strong>MyNewGut Workshop preceding DF15, 01/06/2015, Paris</strong>&lt;br&gt;<strong>6th Intl Dietary Fibre Conference ‘DF15’, 01-03/06/2015, Paris</strong>&lt;br&gt;Comments on MNG plans and results with focus on options for microbiome related health claims</td>
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*"Microbiome Influence on Energy balance and Brain Development - Function Put into Action to tackle Diet-related Diseases and behavior" – MyNewGut has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement nº 613979*
Dissemination and Exploitation (WP12)

Overall, the communication strategy applied to both social media and general news media proved to be very successful in building a community and establishing a relationship with the press. The major opportunity for communication and exploitation of MyNewGut’s research findings was to find a human angle that would make the topic of the gut microbiota translatable and understandable for both experts and a lay audience. The dissemination of the results on social media showed high engagement and interest among a diverse audience. Overall, MyNewGut social media community (Twitter and Facebook) currently counts over 8,500 followers combined. Information and news coming from the project was shared on the project’s public website www.mynewgut.eu. Across the duration of the project, the website has received 114,311 page views, during 42,368 sessions, from 28,403 website users. On the MyNewGut website it is possible to consult with an online press review which lists all media articles where the project was mentioned.

An important role was played by project beneficiaries, who not only provided the necessary content but also network through which communication and dissemination efforts were multiplied. Partners gave over 200 presentations at scientific conferences, and participated in 19 workshops, at high-level events and/or those related to the microbiome topic, and distributed leaflets elsewhere, reaching nearly 10,000 relevant stakeholders.

Dissemination materials included project leaflets (6,400 printed), 2 press releases, 2 lay-language articles, newsletters, podcasts, webinars, video interviews, roll-ups, and scientific posters and papers. All materials made use of a strong project identity to build awareness and visibility of the project.

The major results of MyNewGut were shared on Thursday 18th October 2018, in Brussels. The conference marked the culmination of the project, which over the last five years investigated the gut microbiome to prevent diet-related and behavioural disorders. The event was attended by around 120 participants, including representatives from the European Commission, project partners and interested stakeholders, as a result of a strong promotion via the project website and its social media channels. To further the outreach of the results, the presentations of the MyNewGut final conference were recorded as a webinar. In addition to recordings of the presentations during the event, a number of key partners were interviewed on their role within the MyNewGut project and the project’s findings.

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During the MyNewGut project, a number of results and outcomes have been identified that may be used for further research activities, developing, creating and marketing a product or process, creating and providing a service, or for standardisation activities. The details of this have been provided in an exploitation plan.

**Ethical Issues**

All studies have been conducted complying with the fundamental ethic principles and national and EU legislations. Human study protocols (WP3, WP4, WP6, WP8) were submitted to the local ethics committee and a copy of the ethical approval of the study protocol and of the informed consent form were submitted to the EC prior to the commencement of the relevant part of the research. A Good Clinical Practice (GCP) monitor was hired for each intervention to ensure patient safety. An insurance policy also covered any possible serious adverse events. In case of incidental findings, all participants were informed by the study responsible and received the necessary information on the findings and assistance (if needed). In relation to animal experiments (WP5 and WP7), the partners ensured full compliance with the revised Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes, which came into force on 1 January 2013 and national regulations throughout the lifetime of the project. The applicants also submitted copies of ethical approvals for research on animals to the EC prior to the commencement of the studies.

**The potential impact** (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results (not exceeding 10 pages).

**Human Intervention Trials to Understand the Ability of the Gut Microbiome to Influence the Metabolism of Nutrients and Energy Balance (WP3)**

High-protein diets (HPDs) seem apt for weight loss in as much as energy intake is reduced; however, the effects of HPDs on the microbiota-derived metabolites and gene expression in the gut raise new questions on the impact of these diets on large-intestine mucosal homeostasis, and on individuals at risk of kidney disease; leading MyNewGut to recommend some caution regarding the use of HPDs, notably recurrent and/or long-term. Also, the role of the source of protein should be considered for future research and dietary recommendations.

From the dietary intervention study conducted with varying fibres and fat composition, we have obtained valuable knowledge about the effect of WBE-AXOS intake on gut microbiota, its functions and metabolic health markers. The results suggest that this dietary fibre could exert effects on glucose metabolism via microbiota-mediated mechanisms although long-term intervention trials should be conducted to confirm this hypothesis since only trends could be detected in the intervention. Although we did not observe an effect of PUFA intake on the microbiota, results indicate the gut microbiome does not respond quickly, and future study design should be changed to assess the microbial effects related to dietary fat composition. These results are therefore important for future research in dietary modulation of the gut microbiome and in the development of microbiome-based dietary recommendations. 

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The intervention based on faecal microbiota transplant proves the causal role of the gut microbiota in regulating the gut-brain axis, impacting the dopaminergic system and food intake in humans. Further research could enable researchers to specifically target this gut brain axis (by either enriching diet, providing the identified microbial strains and metabolites as novel therapeutic or a combination of all) to beneficially change food intake, mood and behaviour in obese human subjects with metabolic syndrome.

**Epidemiologic Studies to Identify the Influence of Lifestyle and Other Factors on the Gut Microbiome and Its Role in the Development of Obesity and in Eating Behaviour (WP4)**

Dietary recommendations to reduce obesity risk have to consider the macronutrient and total daily energy intake, diet diversity (based on internationally accepted indexes) and the individual microbiome structure with the purpose of avoiding combinations of diet and microbiome configurations that are likely to favour obesity onset in children.

This information could also be the basis for developing new disease risk biomarkers as well as new tailored probiotics, simulating microbial communities that contain the keystone taxa and functions associated with a lean phenotype. In other words, the new products will contain not only a single- or multi-strain combination, but a proper indigenous bacterial community.

**Preclinical Trials to Understand the Mechanism of the Relationship between Diet, Behaviour and Gut Microbiome in the Control of Obesity and Related Disorders (WP5)**

The identification of new bacterial strains isolated from humans with proven pre-clinical efficacy on obesity and metabolic dysfunction in diet-induced obesity, via different mechanisms, could give rise to a new generation of probiotics able to tackle obesity and its metabolic complications more effectively, with an important economical and societal impact. The biobank of human indigenous gut bacteria generated by the CSIC harbours valuable biological material, which can be exploited for future screening in the search of other functional traits and strains.

Despite their use in clinical practice and regular prescription to diabetic patients, the effect of the DPP-4 inhibitors at the intestinal level remains poorly studied. UCL has described for the first time that the DPP-4 inhibitor vildagliptin influences gut microbiota and improves intestinal homeostasis in animal models. This might represent a novel mechanism whereby vildagliptin improves human health beyond its standard use as an antidiabetic drug.

Gluten might contribute to the impairment of metabolic health, which could be ameliorated if gluten is consumed accompanied by specific prebiotics according to the evidence from obesity models. Indeed, the prebiotics tested in MyNewGut are naturally found in gluten-containing ingredients, which would reinforce the interest of ingesting whole foods to promote metabolic health in humans.

TUM demonstrated that obesity and insulin resistance seen in human donors cannot be initialized in C57BL/6 mice by transferring patient-derived faecal microbiota under their experimental conditions. The changes in weight, glucose tolerance and insulin resistance were only triggered by high-fat feeding, but not by different microbial environments. The transfer of human microbiota into mice...
resulted in a substantial change in the bacterial community structure, richness and diversity compared to those of the donor, suggesting that human obesogenic taxa remain within the group of non-transferable bacteria. Diet-induced obesity, impairment of glucose tolerance and liver steatosis were independent of patient donor microbiota. These observations provide data of interest for future studies.

The data obtained by MUG (in collaboration with UCL, UREAD and CSIC) disclose a novel gut microbiota-leptin-brain network that is of relevance to diet-dependent mental health. If these preclinical findings could be translated to humans, they would indicate that specific dietary interventions represent, on the one hand, an important approach to preventing mental disorders associated with obesogenic nutrition and, on the other hand, would attribute specific dietary interventions an important place in the therapy of mental disorders.

**Epidemiologic Studies to Identify the Effects of Environmental Factors on Gut Microbiome and its Influence on Brain, Immune, and Metabolic Programming, Development and Function (WP6)**

Caesarean section rates in the E.U. exceed 30% while levels of around 12% are recommended by the WHO. The results outlined in WP6 have clear potential to influence policy with regard to prophylactic antibiotic use in the first 4 days of life in conjunction with the escalating numbers of unnecessary C-sections being performed across the developed world, and also occurring more and more frequently in developing countries. A clear negative impact on the neurodevelopment of young children caused by antibiotic use in the first few days of life provides further evidence that early seeding of the gut microbiota is vital in neurodevelopmental processes. Furthermore, the impact of being born by C-section is evident throughout young adulthood, whereby young adults born by C-section are more vulnerable to psychological stressors. Minimising the rate of unnecessary C-section, and reducing the use of prophylactic antibiotics in the first days of life, may help decrease stress susceptibility in adulthood.

The impact of the maternal metabolic condition on the establishment and functionality of the offspring gut microbiota and the persistency of the effects during childhood until 6 years of age, linked to infant body composition, neurodevelopment and brain structure and function, should be considered in further research and lifestyle recommendations intended to improve metabolic and mental health programming.

**Innovative Food Ingredients and Products Targeting the Human Gut Microbiome (WP7)**

Currently there exists a fibre gap (in terms of dietary fibre) in the nutrition of current society and there is a need for high-fibre ingredients, including soluble fibres, to increase fibre intake. The relevant implications of fibre intake for health and disease call for urgently needed identification and implementation of novel approaches. WBE-AXOS offers such an opportunity and hence has been tested for its applicability to crackers, milks etc., by several industrial partners (Cargill, CAPSA). Additionally, appropriate (and consensual) quantitative and qualitative information about dietary fibre is missing in most food composition tables. Given the data obtained by UCL and WP3 partners in MyNewGut, and recent publications in the field, we have progressed in the evaluation of the fibre sources –such as wheat bran (WB)- that beneficially impact specific components of the gut microbiota and metabolism. This has contributed to discovering new players and mechanisms of action that could...
support specific health-outcomes. In the final step of the project, based on the existing state of the art and future development, dietary guidelines related to dietary fibres have been proposed, taking into account their interaction with the gut microbiota (as indicated in WP11).

In terms of innovative food prototypes including probiotics, the new fermented probiotic milk could have an impact on the market because it has better chemical and sensory properties than similar existing commercial products. It is denser, less acidic and has better mouthfeel. Companies and consumers are constantly searching for products with better characteristics and acceptability by end users. The properties of the new fermented product make it attractive for further commercialization. Also, it is planned to apply for registration of this novel probiotic yogurt in the form of a technical solution, one of the ways to protect its formula and IP.

MyNewGut partners have substantially progressed towards the development of a new generation of probiotics isolated from the indigenous microbiota of humans, regarding the evaluation of their safety, the feasibility of scaling-up their production and improving their stability. These could constitute case-examples of future alternatives for tackling diet-related disorders like obesity more effectively. The approach undertaken by MyNewGut could also inspire and guide other innovative actions in this field.

**Human Intervention Trials to Evaluate the Efficacy of Innovative Food Prototypes and Ingredients on Brain and Diet-Related Disorders (WP8)**

The results outlined in WP8 highlight the difficulties in translating promising pre-clinical results from probiotic intervention studies to the human population. The result of the intervention study with the strain JB1™ of *L. rhamnosus* will help inform research questions surrounding design and implementation of future probiotic intervention studies. Positive results from the intervention study with the strain 1714™ of *B. longum* highlight the strain-dependent effects of probiotics in targeting the microbiota-gut-brain axis in the human population.

The results of the intervention in overweight subjects under an energy restricted diet support some beneficial effects of a specific fibre combination on cardio-metabolic disease risk markers, since it reduced both systolic and diastolic blood pressure. Sex-related effects were also detected. The study could guide the design of future intervention trials and help progress towards refined dietary recommendations, particularly for overweight subjects with indexes of metabolic syndrome.

**Data Analyses and Integrative Studies (WP9)**

We showed that although there are limitations it is possible to integrate data from different clinical trials and different analytical platforms. The results of the data integration provide new insights on the intervention effects and add value to the existing data from individual studies. The progress made in the standardization of data and analysis pipelines will facilitate data integration and re-use in further projects.

**Production of Innovative Food Prototypes to Control Brain-and Diet-Related Disorders (WP10)**

The optimization of large-scale production of probiotic strains and milk products supplemented with fibres carried out in MyNewGut made their evaluation possible in clinical trials. These activities, as well as the results of the intervention trials (WP8), will inform future research studies and facilitate their commercialization.
**Recommendations and Guidelines for European Public Health and Scientific Requirements for Health Claims (WP11)**

Despite the growing amount of scientific publications highlighting the impact of diet and food on the gut microbiome and its influence on physical and mental health, these insights are not yet playing any role in current public health policy and related dietary recommendations. Also, no pro- and prebiotics related health claims have been authorised by EFSA.

MyNewGut convincingly communicated the importance of the impact of gut microbiome for dietary recommendations and other public health issues related to physical and mental health in:
- Peer reviewed review-type Position Papers in the high impact journal Clinical Nutrition
- Semi-scientific messages for dietitians, nutritionists, doctors, public health policy makers and related stakeholders for public health, dietary recommendations and related regulations, such as the widely distributed multi-language EUFIC FOOD TODAY article *Five years in review of 'MyNewGut': How 'gut bugs' affect our physical and mental health.*

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**PLEASE PROVIDE THE PUBLIC WEBSITE ADDRESS (IF APPLICABLE), AS WELL AS RELEVANT CONTACT DETAILS.**

- Project public website: [www.mynewgut.eu](http://www.mynewgut.eu)
- Facebook: [https://www.facebook.com/mynewgut](https://www.facebook.com/mynewgut)
- Twitter: [https://twitter.com/mynewgut](https://twitter.com/mynewgut)
- MyNewGut Final Conference: Presentations (webinar) [https://www.youtube.com/playlist?list=PLiQer0r4t_JwnTcW_3Tp3sJkJR3owrqx](https://www.youtube.com/playlist?list=PLiQer0r4t_JwnTcW_3Tp3sJkJR3owrqx)
- MyNewGut Final Conference: Interviews (video interviews) [https://www.youtube.com/playlist?list=PLiQer0r4t_Jzy4kWA51nZa6Dsfi0_4904](https://www.youtube.com/playlist?list=PLiQer0r4t_Jzy4kWA51nZa6Dsfi0_4904)

**Contacts**
Microbiome Influence on Energy balance and Brain Development – Function Put into Action to tackle Diet-related Diseases and behavior

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2. Use and dissemination of foreground

- **Section A**

MyNewGut has delivered impact through engaging with the scientific community, clinicians, nutritionists, industry including SMEs, regulatory bodies and the general public through a wide range of activities that aimed to disseminate project outcomes and communicate on the overall context of the project. Partners have organised 19 workshops and presented at 110 conferences on the project to engage with the scientific community, policy makers, health professionals and other health and research networks, throughout the duration of the project. Further, 2 lay-language, 2 technical articles, 115 scientific publications, 5 newsletters and 6 press releases coinciding with events or outcomes of interest were published, in addition to podcasts, webinars and video interviews on the final outcomes. All events, newsworthy information and project outcomes were disseminated via MyNewGut’s Facebook and Twitter channels and are hosted on the website (www.mynewgut.eu) until November 2021. The website will continue to be updated with relevant information on project outcomes as it becomes available (e.g. media mentions from the last press release, scientific publications).

Impact will be sustained by partners of the MyNewGut consortium by taking learnings into account in upcoming H2020 projects which are starting by the end of 2018 and early 2019. The Coordination and Support Action MicrobiomeSupport (www.microbiomesupport.eu) and the Innovation Action CIRCLES being examples thereof. Further, the MyNewGut consortium will await the announcement and start of other EU projects funded under, for instance, SC1-BHC-03-2018 (exploiting research outcomes and application potential of the human microbiome for personalised prediction, prevention and treatment of disease) and LC-SFS-03-2018 (microbiome applications for sustainable food systems).

The MyNewGut final newsletter, a news item on the website, as well as a tweet to the MyNewGut Twitter community will alert readers and followers of these new projects which will take up the MyNewGut legacy ensuring that the MyNewGut followership is provided an opportunity to continue engaging in the evolving science around the gut microbiome.

- **Section B**

During the MyNewGut project, a number of results and outcomes have been identified that may be used for further research activities, developing, creating and marketing a product or process, creating and providing a service, or for standardisation activities.

The outcomes that have been identified as being exploitable include the following:

- **New products and prototypes:** The innovations from MyNewGut have led to three new food ingredients and product prototypes, including next generation probiotics and live bio-therapeutics as well as new healthy food products containing probiotics and/or prebiotics. Such products may be brought to the market in the future.
- **Database:** The analyses from MyNewGut and other projects have been integrated into a combined database, which is expected to be a valuable resource for comparing data and generating new results. This database will be further enriched with the results from future research projects and data from other labs.

- **Dietary recommendations:** The knowledge gained from the MyNewGut studies may be a foundation for health claims and dietary recommendations.

- **Spin-off:** The company WellMicro is a spin-off from the Microbial Ecology Laboratory at the University of Bologna, Italy, who were a partner in the MyNewGut project. They will make use of innovative sequencing techniques to show how the microbiota impacts human health.

As mentioned above, the results from MyNewGut will form the basis for new R&D projects, such as the EU-funded projects MicrobiomeSupport and CIRCLES. A number of other proposals have also been submitted by the MyNewGut partners.