Project 3

Name/title of the PhD course	**Clinical and Experimental Medicine**
Name of the PhD coordinator	Professor Francesco Beguinot
Name/Title of the PhD project	***Postprandial glucose response in patients with type 1 diabetes: possible role of gut microbiota composition in moving toward a personalized approach***
Department of reference	The Department of Clinical Medicine and Surgery (http://dmcc.dip.unina.it)
Working conditions, research team, infrastructures, equipment	The Department of Clinical Medicine and Surgery, afferent to Federico II University of Naples, is basically oriented towards the study of prevention and treatment of chronic degenerative diseases. The Department is the seat of the PhD course in Biomedical and Advanced Surgical Therapies and Research Centers. The Department has 193 employees, of which 106 as scientific staff (47 professors and 59 researchers) and 87 as technical staff. Within the department, the Diabetes, Nutrition, and Metabolic Disease Unit has specific experience in the field of nutrition and metabolic diseases. Epidemiological and controlled intervention studies in healthy people, high risk individuals, diabetic and hyperlipidemic subjects have investigated the effects of dietary fibre, different types of carbohydrates, different types of dietary fat and other dietary components, such as polyphenols, on glucose metabolism, insulin resistance, and lipid metabolism, oxidation, subclinical inflammation, and liver fat. The Unit is part of the European Clinical Research Infrastructure Network (ECRIN). The most relevant achievements are the demonstration of 1) beneficial effects of dietary fibre on glucose and lipid fasting and postprandial metabolism (Lancet 1980; Diabetologia 1984; Diabetes Care 2001, AJCN 2008) (Diabetes Care 2009). 2) the effects of the different types of dietary fat on insulin resistance, postprandial lipid metabolism, lipase activities, liver fat (Diabetologia 2001, Atherosclerosis 2003, Clinical Nutrition 2008, NMCD 2008, Metabolism 2010, EJCI 2012, Diabetes Care 2012). 3) the effects of supplementation with long-chain n3-fatty acids on insulin resistance (Diabetes Care 1996, Atherosclerosis 1991). 4) the effects of diets naturally rich in long chain n3-fatty acids and/or different types of polyphenols on lipid metabolism and oxidative stress (AJCN 2014) and glucose metabolism (Diabetologia 2015). 5) the effects of extra-virgin olive oil on postprandial glycaemia in patients with type 1 diabetes (Diabetes Care 2016, Clin Nutr 2019). The Diabetes and Metabolic Disease Unit is operating within the University Hospital and has clinical responsibility of in- and outpatients. The Unit disposes of 1) a metabolic kitchen to prepare and store foods, 2) a metabolic ward. 2) an investigation room fully equipped with 2 beds and all the appropriate instruments to perform postprandial studies, indirect calorimetry, and clinical physiology investigations, 3) a research laboratory (low speed centrifuges, refrigerated centrifuges, ultracentrifuges, a cold-room, −20°C and −80°C freezers, an auto-analyzer for clinical chemistry, an auto-analyzer for ELISA, a gas chromatography system.
Scientific context	Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by insulin deficiency and resultant hyperglycemia. Patients with T1D need insulin administration to maintain blood glucose within acceptable levels. However, mainly due to high inter-individual variability in insulin needs, this goal is hard to achieve, especially during the postprandial phase. Managing postprandial glucose response (PGR) –a main determinant of metabolic control in type 1 diabetes (T1D)– is challenging also with advanced technologies. In the hybrid closed-loop systems (CLS) approved for clinical use, basal insulin is automatically infused according to algorithms relying on continuous glucose monitoring (CGM), still meal insulin doses are calculated by the patients. So, while glycemic control is generally improved by CLS, the majority of time spent out of optimal blood glucose range with this system is due to algorithm failures in blunting steep blood glucose changes after meals. In addition to the lag time of insulin absorption, this is mainly due to poor knowledge of determinants of postprandial glycemia. The carbohydrate content of the meal is the main dietary factor influencing PGR. However, many other nutritional issues matter, firstly fiber content and/or glycemic index (GI) of foods. High GI foods induce an early and high PGR, while low GI foods induce a blunted early and late rise of blood glucose. Dietary fat and protein also influence PGR, not only in terms of amounts but also of quality, as indicated by the reduced early PGR to a high GI meal with extra-virgin olive oil but not with butter. Therefore, alternate methods to carbohydrate counting could be considered to enhance CLS functioning. A feasible approach could be adjusting meal boluses according to glycemic load –grams of carbohydrate multiplied by GI. With glycemic load counting, compared to carbohydrate counting, for meals identical for carbohydrate content, a higher insulin dose is injected before high GI meals and a lower one before low GI meals. This improved postprandial glucose variability in a real-life setting in T1D patients on insulin pump. With CSII, a dual-wave bolus may improve PGR to a low GI meal, while a standard bolus may still be unable to blunt the early PGR after a high GI meal. As the hybrid CLS only needs calculating a standard bolus eventually adjusted through its algorithm, the differences between the doses calculated by glycemic load counting or carbohydrate counting could have an even greater impact on PGR. Among non-nutritional determinants of PGR, gastric emptying and gut microbiota composition may also play a role. Gastric emptying rate influences the time of glucose peak, and gut microbiota may individualize PGR. With the hybrid CLS, the insulin infusion rates could help individualize PGRs related to different combinations of meals/ microbiota composition/ gastric emptying features.Personalized medicine has the potential to approach the different inter-individual responses and, in this context, gut microbiome may play an important role. Recent research in healthy individuals has shown that subject-specific signatures in the gut microbiome may help explaining differences in post-prandial glucose response to a meal. This could have clinical relevance in individuals with diabetes.Against this background, we hypothesize that taking into account carbohydrate quality in addition to its quantity would improve postprandial performances of a hybrid CLS. Moreover, we will investigate factors that could explain the interindividual variability in PGR, including gastric emptying and gut microbiota, being a potential target for a personalized approach.

Project Research plan	Two-hundred individuals with T1D will be recruited at the Diabetes Unit of Federico II University Hospital. All patients wearing a continuous glucose monitoring system, consecutively admitted to the clinic for routinary assessment of diabetes complications will be enrolled, unless being pregnant, having celiac disease or taking antibiotics in the 3 months prior to the study. Participants will be asked to fill-in a 7-day food records and collect a fecal sample during the week preceding the hospital admission. On the day of the admission, venous blood will be drawn for determining biochemical parameters and metabolomics, and the EPIC questionnaire completed for assessing dietary habits during the last year. Over the following 2 weeks, at home, participants will eat the same meal on three occasions for the evaluation of intra-individual variability of postprandial glucose response by continuous glucose monitoring.Possible determinants of personalized PGR will be evaluated: 1) the habitual dietary pattern by means of a 7-day food record and measurement of plasma and urine markers of dietary intake 2) gut microbiome composition, 3) plasma gastrointestinal hormones, 4) intestinal permeability by measurement of plasma zonulin, 5) plasma and urine metabolomics. Glyco-metabolic control will be also assessed by measuring HbA1c and plasma lipids.Gut microbiome will be analysed by shotgun metagenomics of fecal samples. The analysis will provide the taxonomic composition and the functional profiling, identifying microbial taxa or genetic potential (gene, metabolic pathways) possibly associated with T1D or the metabolic response upon meal consumption. Random-forest regression models will be developed using all informative features (meal composition, habitual diet, anthropometry, microbiome composition and functional profiles, clinical and biochemical parameters) predicting post-prandial glucose response.
Research and Training Innovative aspects	Individuating the impact of different macronutrients and individual determinants of postprandial glucose response in patients with type 1 diabetes would allow improving pre-prandial insulin dosing and therefore limiting blood glucose fluctuations with a beneficial impact on quality of life of the patients and prevention of chronic and acute diabetes complications. The results of this project will contribute to identifying individual patterns of postprandial glucose response and accordingly an adequate functioning of the modern technologies available for insulin infusion. These results will contribute to maximize the individualization of insulin treatment making closer the realization of a full closed-loop system for insulin infusion
Inter-Multidisciplinary aspects	The project covers different fields and involves research groups with different expertise. The Diabetes, Nutrition, and Metabolic Disease Unit at Federico II University has specific experience in clinical nutrition and research nutritional studies. In this project, individuals with type 1 Diabetes Mellitus will be studied. In these patients, nutrition is critical in relation to insulin therapy and blood glucose control. To this regard, Diabetes care is the main area of interest and research for Roche Diabetes Care Italy S.p.A that will host the PhD student for 6 months. Essential is the role of the strict collaboration with the Department of Agricultural Sciences, Federico II University, that will analyse gut microbiome composition. Consistent with the overall field of microbiome research, the Dvision of Food and Nutrition Science at Chalmers University of Technology will provide its expertise and computational infrastructure for OMICs analysis, in exposure biomarkers, nutritional metabolomics and personalized nutrition
Secondment opportunities	**Chalmers Technical University,** Department of Biology and Biological Engineering, Division of Food and Nutrition Science., Sweden (www.chalmers.se/sv/institutioner/bio) , a research unit with 45 staff members conducting research and education in the area of food and health. The division will contribute to the project by hosting one PhD student for a secondment of four-six months during which he/she will come in contact with its research and training and will get hands on training in methodologies capturing gut microbiota activities and relate such to outcomes in human intervention studies. The unit has access to the absolute fore-front mass spectrometric equipment for metabolomics experiments and computational infrastructure to carry out large scale OMICs analysis**. Professor Rikard Landberg** will be the person in charge acting as co-supervisor. His expertise is in exposure biomarkers, nutritional metabolomics and personalized nutrition. He is the PI of large molecular phenotype projects and of several RCTs to evaluate novel personalised nutrition concepts and health effects of plant-based diets. **Roche Diabetes Care Italy S.p.A**.. Roche Diabetes Care has been pioneering innovative diabetes technologies and services for more than 40 years. They will contribute to the project by hosting one PhD student for a secondment of six months during which he/she will be exposed to their Business being part of the team and collaborating across the functions gaining expertise in Diabetes Management with a business perspective. More in details the person identified will collaborate with various department keeping a special focus on Medical Affairs is **Dr. Maria Grazia Bellotti**,

Main Supervisor: Dr Lutgarda Bozzetto (https://www.docenti.unina.it/lutgarda.bozzetto)	
Brief CV	Present role: Assistant Professor in Food Sciences and Dietetics at the Department of Clinical Medicine and Surgery, Federico II University of Naples. Previously: Research fellowship at the Department of Clinical Medicine and Surgery (2012-2015). Visiting scientist at the Obesity Research Unit, University of Helsinki, Helsinki, Finland (2018).Research assistant at the Department of Clinical Medicine and Surgery (2015-2020). Membership of scientific associations. - Società Italiana di Diabetologia (SID) - European Association for the Study of Diabetes (EASD) - European Atherosclerosis Society (EAS) - Società Italiana di Aterosclerosi (SISA) **Clinical activity:** Mostly for patients with diabetes mellitus and metabolic diseases, since 2004 at the Diabetes Unit of the Federico II University Hospital, at the Center for insulin pump therapy, and the Internal Medicine ward.
Publications	**The 5 most significant/recent publications in the microbiome field are:** -Costabile G, Vetrani C, **Bozzetto L**, Giacco R, Bresciani L, Del Rio D, Vitale M, Della Pepa G, Brighenti F, Riccardi G, Rivellese AA, Annuzzi G. Plasma TMAO increase after healthy diets: results from 2 randomized controlled trials with dietary fish, polyphenols, and whole-grain cereals. Am J Clin Nutr. 2021 Oct 4;114(4):1342-1350. doi: 10.1093/ajcn/nqab188. PMID: 34091663.

	- Vetrani C, Calabrese I, Cavagnuolo L, Pacella D, Napolano E, Di Rienzo S, Riccardi G, Rivellese AA, Annuzzi G, **Bozzetto L.** Dietary determinants of postprandial blood glucose control in adults with type 1 diabetes on a hybrid closed-loop system. Diabetologia. 2022 Jan;65(1):79-87. doi: 10.1007/s00125-021-05587-0. Epub 2021 Oct 23. PMID: 34689215; PMCID: PMC8660714. - **Bozzetto L**, Della Pepa G, Vetrani C, Rivellese AA. Dietary Impact on Postprandial Lipemia. Front Endocrinol (Lausanne). 2020 Jul 7; 11:337. doi: 10.3389/fendo.2020.00337. PMID: 32733374; PMCID: PMC7358426. - Vetrani C, Maukonen J, **Bozzetto L**, Della Pepa G, Vitale M, Costabile G, Riccardi G, Rivellese AA, Saarela M, Annuzzi G. Diets naturally rich in polyphenols and/or long-chain n-3 polyunsaturated fatty acids differently affect microbiota composition in high-cardiometabolic-risk individuals. Acta Diabetol. 2020 Jul;57(7):853-860. doi: 10.1007/s00592-020-01494-9. Epub 2020 Feb 29. PMID: 32114641. **- Bozzetto L**, Costabile G, Della Pepa G, Ciciola P, Vetrani C, Vitale M, Rivellese AA, Annuzzi G. Dietary Fibre as a Unifying Remedy for the Whole Spectrum of Obesity-Associated Cardiovascular Risk. Nutrients. 2018 Jul 21;10(7):943. doi: 10.3390/nu10070943. PMID: 30037123; PMCID: PMC6073249.
Projects participation	**EU funded projects (past and/or current) in the microbiome field:** -INDIGUMET Project- Effects of a diet rich in fermentable fiber from cereals on NAFLD indices and the role of gastrointestinal hormones: ancillary DIGUMET study. Granted in the context of the European project "HDHL INTIMIC-Knowledge Platform (KP) on food diet, intestinal microbiomics and human health" - ETHERPATHS project "Characterization and modelling of dietary effect mediated by gut microbiota on lipid metabolism". Work Package 5: Food products and nutritional intervention trials. *Experience in supervising PhD students*: 4 students supervised for PhD courses on “Advanced Biomedical and Surgical Therapies” and “Clinical and Experimental Medicine”.

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