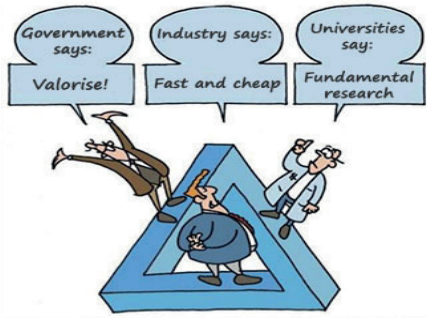
Mapping amino acid and short-chain fatty acid metabolism in man

Valorization

Knowledge valorization refers to "the process of creating value from knowledge by making knowledge suitable and/or available for economic and/or societal use and translating that knowledge into competitive products, services, processes and entrepreneurial activity."

(National Valorization Commission 2013)



In other words: the creation of societal and economic value from scientific knowledge. Examples include developing a product or a medicine, or applying scientific knowledge to a system or process. In fact, public investments into science should generate returns that benefit the economy. It is a strategy that brings science closer to society. The term 'knowledge valorization' is akin to the term 'innovation'. Innovation means converting inventions to reality, thereby applying them to a product or process. An invention alone is not considered an innovation; innovation only applies if the invention is used in practice. At Maastricht University, the Maastricht Valorization Center (MVC) provides support to researchers, students and businesses in promoting knowledge valorization.

For knowledge utilization, it is necessary that knowledge can be used outside the laboratory; that there is market potential and interest from investors. The studies presented in this thesis were funded by Top Institute Food and Nutrition (TIFN), a public-private partnership on pre-competitive research in food and nutrition. As such, TIFN provided the linking pin between the university and the marketplace. It brought together the supply of knowledge from the university and the demand for knowledge from the marketplace. Experts of international food industries, clinicians, and health care professionals collaborated with the researchers in this project, referred to as the Gastrointestinal Health project 'microbiota, metabolism and energy harvesting'. During frequent meetings, these partners helped the researchers continue to develop their knowledge through direct interaction and exchange of relevant knowledge from their side. This collaboration led to demand-driven research that, in turn, gave the nutritional industry insight into the major role the gut microbiota may play in tackling worldwide obesity. A major global societal objective is after all the reduction of obesity and science is expected to have a crucial role in achieving this goal.

Obesity is a major epidemiologic challenge facing today's doctors, with an ever increasing annual allocation of healthcare resources for the disease and related comorbidities. Worldwide, obesity has nearly tripled since 1975. In 2016, 39% of adults aged 18 years and over were overweight, and 13% were obese. More concerning than the rise in obesity among adults is the increased prevalence of obesity among children. Over 40 million children under the age of 5 were overweight or obese in 2016. While obesity is clearly a major public health issue, obesity and being overweight turned out to be among the top 10 of leading causes of global mortality and burden of disease. The overall morbidity seen in this growing patient population remains a key issue contributing to decreased quality of life. Impairment in activities of daily living such as eating, dressing, and transferring to and from a bed or wheelchair occur at a younger age in obese patients compared to non-obese controls. If overall mortality decreases but the diagnosis and treatment of obesity-related conditions continue to increase, the cost of managing the obese patient population could be overwhelming. As such, obesity inflicts a tremendous economic burden on health care systems.

The leading cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Importantly, several studies over the last decades have recognized the gut microbiota as an additional factor contributing to this obesity crisis. However, the understanding regarding which microbes are 'beneficial' and how these microbes interact with host metabolism, especially in humans, have remained unclear and require thorough investigation. Therefore, the overall aim of this research thesis was to elucidate the role of the gut microbiota in human substrate and energy metabolism by combining a detailed characterization of gut microbiota composition and functionality with measurements of interorgan SCFA trafficking. SCFA were identified as important metabolites in the crosstalk between the gut microbiota and host.

A highly promising intervention for preventing or treating metabolic diseases including obesity and type 2 diabetes would therefore be a change in the gut microbiota composition and function. As a proof-of-concept, in this thesis antibiotics were used to temporarily modulate the gut microbiota composition in a robust way, and the effects on insulin sensitivity, energy and substrate metabolism were examined. 57 obese men with impaired insulin resistance were orally treated with 1500mg/day amoxicillin, vancomycin, or placebo for 7 days. Although vancomycin treatment decreased microbial diversity, no significant impact of antibiotic treatment on insulin sensitivity, energy and substrate metabolism was found. At 8 weeks follow-up, gut microbial composition was still considerably altered as compared to baseline. This finding once again indicates the importance of research into the effects antibiotics have on gut microbiota composition and function considering that antibiotics are widely used in clinical settings. Important here is to take into account that the extent to which antibiotics may affect the gut microbiota composition is highly variable between persons. As diet is an important factor shaping the gut microbiome, the impact of antibiotics might be related to a person's dietary habits as well. A patient-specific diet-induced manipulation of the gut microbiota targeting specific microbial responses seems an important aspect for future

research to tackle obesity and related quality of life and healthcare costs. Research into prebiotics, for example, might be an effective way to alter host metabolism in obese insulin resistant subjects when applied at the individual rather than on a population level.

Prebiotics already received considerable attention because of their potential to beneficially affect gut health. Given the growing interest in exploring the best location of administration of prebiotics, the interorgan exchange of the microbial fermentation products of prebiotics was determined in men. These studies revealed that the exchange of SCFA and amino acids differs in the proximal colon versus the distal colon. Whereas amino acids were predominantly released by the proximal colon, SCFA were mainly released by the distal colon. This may reflect the fact that a larger part of absorbed SCFA are metabolized by the intestinal mucosa of the proximal colon. Indeed, the SCFA receptors GPR41 and 43 are mainly expressed in the ileum and the proximal colon. With respect to the minor amino acid exchange in the distal colon, it may be possible that the remaining amino acids have been utilized by the gut microbiota to produce SCFA contributing to the high SCFA exchange in the distal colon. Finally, the liver showed a significant uptake of all SCFA. Hence, no intestinally produced SCFA escaped the splanchnic area indicating efficient hepatic metabolic clearance of SCFA. The clinical relevance of these findings needs to be further investigated as this unique human model may make it possible to study the influence of manipulating amino acid and/or SCFA availability in the intestines (using pre- and/or pro-biotics) in order to elicit beneficial effects on human substrate and energy metabolism and glucose homeostasis. Larger patient groups and additional metabolic diseases have to be investigated, i.e. insulin sensitive versus insulin resistant patients. However, the differences in microbial composition at baseline could have influenced fermentation rates and the production of amino acids and/or SCFA. Therefore, it is advisable to take feces samples in future studies to determine fecal amino acids and/or SCFA concentrations next to plasma amino acid and SCFA concentrations. In this way, clinicians and dieticians in the field of obesity can contribute to personalized care by screening the microbiota profile in combination with the metabolic phenotype before designing a weight-loss program or dietary intervention.

Overall, this research thesis may have benefits for the food industry as well. The findings have implications for the development of slowly fermentable fibers that increase SCFA specifically in the distal intestines given the higher SCFA release by the distal intestines. Using this information, food industry may want to optimize their existing product formulations. Several studies increasingly demonstrated that targeted SCFA delivery in the human colon exerts beneficial effects on energy and substrate metabolism. In addition, replacing digestible fibers with fermentable fibers attenuates the rise in blood glucose after a meal. However, various categories of prebiotic dietary fibers show different health benefits. The traditional prebiotics of fructo-oligosaccharides (FOS), inulin, and galacto-oligosaccharides (GOS) still provide the most evidence of beneficial health effects due to their fermentation, but many other categories of compounds may

be as effective as, or more effective than, the traditional prebiotics. Moreover, it may be of interest to investigate what potential effects these prebiotics have on microbial amino acid metabolism. In fact, the end-products of protein fermentation in the distal colon have well-established detrimental effects on the colonic microenvironment and epithelial health. Studies revealed that some of the metabolites from amino acid catabolism by bacteria might increase the risk of developing colon cancer. Gaseous compounds (hydrogen sulfide and nitric oxide), ammonia and phenols for example exert structural damage to the colonic mucus layer stimulating the synthesis of pro-inflammatory cytokines. As such, increasing the delivery of fermentable fibers to the colon to shift bacterial activity away from protein fermentation on top of stimulating the SCFA production, should become an additional target in the design of any intervention. Since different fermentable fibers differentially influence fermentation activity, modifying total protein intake and the type of amino acids consumed should be taken into account as well. With respect to the minor amino acid exchange that we observed in the distal colon, it may be possible that part of the remaining amino acids have been utilized by the gut microbiota to produce SCFA, indirectly contributing to the beneficial high SCFA exchange in the distal colon. Thus, stimulating SCFA production by shaping the diversity of amino acid fermenting bacterial communities in the distal colon would mean double profit. Hence, these results are also very important to food manufacturers that target the sports nutrition and active adult markets that feature protein as a key ingredient. Collectively, this work will lead to development of new concepts for the formulation and management of foods to improve the well-being of humans and bacteria.