Modification of white adipose tissue biology and metabolic profiles in humans by nutritional bio-actives

Valorization

What is valorization / knowledge valorization?

The term valorization (surplus value, "Verwertung" in German) describes according to Marx's theory "the productive use of a resource, and more specifically the use or application of something (an object, process or activity) so that it makes money, or generates value, with the connotation that the thing validates itself and proves its worth when it results in earnings, a yield" [1]. Nowadays, the term valorization / knowledge valorization is also applied in academia and project management. According to the Dutch Ministry of Education, knowledge valorization is defined as "the transformation of knowledge into economic and social value". Its main purpose is the increase of the likelihood that scientific knowledge is more easily accessible and effectively used for public benefits [2]. Hence, here we discuss the social and economic relevance of the investigated aspects and the potential of the scientific findings with respect to their best use, and how specific population groups, health professionals and industries could benefit from applying the scientific knowledge described in this thesis.

Social and economic relevance

Multifactorial health conditions such as obesity and obesity-related cardiometabolic disorders are associated with complex interactions between the genome and various environmental factors. Diet composition, the intake of total calories, a reduced physical activity and a sedentary lifestyle are the major drivers of obesity. Hence, obesity has become an epidemic and is one of the major metabolic risk factors leading to non-communicable diseases (NCDs) like cardiovascular disease, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). According to the most recent study led by the Imperial College London and the WHO the number of obese adults increased 7-fold from 100 million in 1975 to 671 million in 2016 with another 1.3 billion adults being overweight. Shockingly, childhood and adolescent obesity increased 10-fold from 11 million in 1975 to 124 million in 2016 and additional 213 million overweight children and adolescent, which are likely to become obese [3, 4]. Evidence suggests that adipose tissue (AT) dysfunction rather than an increased total fat mass *per se* links obesity to its accompanying metabolic impairments such as hyperlipidemia and –glycaemia, chronic low-grade inflammation, endothelial impairment, and ectopic lipid accumulation that subsequently increase the prevalence of cardiovascular disease, T2DM and NAFLD. It is estimated that NCDs are the main cause of almost 86% of deaths in Europe, and 77% of the disease burden; and therefore, the WHO considers obesity to be the 5th leading death risk worldwide [5]. Due to the growing prevalence of obesity and related NCDs, also in young individuals, the health care costs for obesity combatting strategies (like gastric banding, liposuction, gastroduodenal bypass, in addition to lifestyle changes) are increasing dramatically as well. The European Association for the Study of Obesity (EASO) points out that policymakers should consider

that currently direct obesity-related costs range from 1.5 to 7% of health care expenditure in the EU [6] and diabetes accounts for approximately 10% of the total health care spending [7, 8]. Furthermore, these direct costs are accompanied by indirect costs (sick leaves, early retirement, use of social security funds, premature mortality, reduced productivity) summing up to ~ €390 billion in 2010 for obesity and diabetes. In addition, to these direct and indirect costs, the continued consumption of highly processed, sugary, and fatty foods poses not only a threat to the global health care systems but also to the future economic growth. Morgan Stanley, a large scale commercial bank, estimates an 0.3% OECD[1] annual GDP[2] growth in a high sugar scenario compared to a 2.2% with a low sugar scenario in 2035 ("The Bitter Aftertaste of Sugar" [9]). This awareness of the financial sector should further stimulate public sector education and private sector innovation, and foster academic investigations to tackle the "diabesity" (a term introduced for "coexistence of obesity and diabetes" due to the strong association between both conditions) effects on health costs and productivity [9], because currently government actions including food taxes and stricter regulations for food industry are not greatly effective in reversing the epidemic.

While surgical interventions provide immediate effect (the long-term effectiveness of surgeries remains still unclear [10]), they are associated with high costs, mortality, post-operative complications, and nutrient malabsorption, and therefore are not suitable as public health solution. Hence safe, effective, and easy-to-comply-with treatments and prevention modalities are needed to mitigate the development of obesity, paralleled by aiming to improve AT dysfunction and thereby metabolic impairments. Improving the unhealthy diets and physical activity levels will eliminate the main causes of obesity-related NCDs, and there are lifestyle interventions e.g., diabetes prevention studies (DPS, SLIM and EDIPS[3]) that have proven to be effective by obtaining a ~50% reduction of the diabetes incidence rate. However, long-term compliance and weight maintenance is difficult to achieve and requires thus much more efforts by governments, health care services and food industries to implement these healthy lifestyles everywhere. One attempt to make these lifestyle interventions more effective is to supplement our diets with nutritional bio-actives. Nutritional bio-actives, also called dietary bioactive components, can be defined as substances that are non-essential constituents in foods and dietary supplements, which improve cell, organ and overall body function and health. Unlike the essential nutrients, they (e.g. polyphenols, carotenoids, and polyunsaturated fatty acids (PUFA)) are not needed to maintain basic body functions, but they can have an impact on the health status [11]. Therefore, the identification and scientific substantiation of nutritional bio-

[1] <OECD> Organization for Economic Cooperation and Development
[2] <GDP> Gross Domestic Product
[3] <DPS> Finnish Diabetes Prevention Study, <SLIM> Study on Lifestyle intervention and Impaired glucose tolerance Maastricht, <EDIPS> European Diabetes Prevention Study

actives that enhance AT function and mitigate metabolic risk factors may improve the general health of the metabolically impaired population. The consumption of bio-actives represents an easily achievable preventive measure with moderate effects and the potential to reach most segments of the population, thereby having a high health impact. It seems also a promising business for the food and supplement industry (value of dietary supplements market in EU was €7.2 billion in 2015 [12]). However, although the scientific knowledge is growing and an increasing number of studies indicate beneficial effects of bio-actives on obesity-related metabolic diseases, inconsistent data are reported regarding the effects of individual nutritional bio-actives on AT biology, metabolic profiles, and energy metabolism. Moreover, well-controlled human intervention studies are scarce. It is therefore of interest to investigate how single and combinations of dietary bioactive components (with partly distinct mechanisms of action) impact adipocytes and AT function and whether such combinations could show favorable (additional / synergistic) effects on the metabolism of obese humans beside weight loss promotion.

The goal of this PhD project was to fill knowledge gaps in the field of nutritional bio-actives for the prevention and treatment of metabolic disorders by investigating the combinatory effects of the well-studied polyphenols resveratrol (Res) and epigallocatechin-3-gallate (EGCG), the carotenoid lycopene and the PUFA eicosapentaenoic acid (EPA) in a unique human cellular model and in a placebo-controlled human intervention study with overweight-obese subjects. Therefore, the translational research described in this thesis was structured going from method development and validation to *in-vitro* experiments and finally *in-vivo* intervention studies.

Relevance of studies

The studies conducted in this thesis expand the current knowledge regarding the potential role of nutritional bio-actives on AT substrate and energy metabolism, thereby contributing to the fundamental and applied research ambitions according to the strategic program 2017-2021 of Maastricht University (UM, section Research, Action: “Maintain a healthy balance between fundamental and theme-driven research”). The thesis itself is part of the intended high output of PhD theses, which fosters education, attracts researchers / students, and keeps the university competitive on the national and international level [13]. On the one hand, *in-vitro* models are useful and can be effectively applied for high-throughput screening of the metabolic health effects of nutritional bio-actives. However, considering nutrient-nutrient interactions, as well as nutrient-gut-microbiome interactions and systemic bioavailability of bio-actives, human intervention studies are not only necessary but also remain the gold standard in studying the effects of nutritional bio-actives on human health and well-being.

On the other hand, nutritional human studies are challenging and require further standardization, refinement, and careful execution to high standards like the conduct in accordance with GCP, rigorous monitoring, and well-controlled and documented dietary intake. In more detail, cellular models allow for the investigation of several components, their derivatives / metabolites [14], and combinations in a fast, easy, and well-controlled manner and thus are valuable tools for high-throughput screening and mechanistic understanding. Because evidence is accumulating that primary human multipotent adipose-derived (hMADS) cells retain their donor characteristics and can be differentiated *in-vitro* into adipocytes, they represent a convenient AT model to investigate *in-vitro* responses in metabolically distinct groups. The presented research has corroborated that this process is responsive to individual nutritional bio-actives and their combinations. The hypothesis for the conducted studies was that combinations were more effective than individual compounds regarding their influence on lipid accumulation, various AT functions and metabolic parameters such as insulin sensitivity. This is suggested by a reduced lipid accumulation and pro-inflammatory secretion in *in-vitro* differentiated adipocytes (Lyc/Res), a prevented decrease of fat oxidation *in-vivo* and increased skeletal muscle mitochondrial capacity (EGCG/Res) and modified transcriptional patterns in adipocytes, AT, and muscle after treatment with combinations. However, these findings were not reflected by increased energy expenditure, changed food intake, improved insulin sensitivity or white AT loss after 12 weeks supplementation. Therefore, such results should not be advertised as long-term weight loss solutions to consumers, but they may indicate that the supplementation of "multi-bio-actives" combined with a healthy lifestyle could help combat metabolic dysfunction accompanied by excessive weight. In addition, to the putative health benefits of correcting metabolic parameters this is an attractive business opportunity comparable to established multi-vitamin / mineral products. Furthermore, attention must be paid to high and overdosing of nutritional bio-actives and their possible interactions with other nutrients, semiluxury foods and drugs. The impact of high doses and such interactions may be detrimental for human health and well-being and needs to be further investigated and communicated to healthcare professionals advising on lifestyle as well as directly to consumers. Evidence is accumulating that *in-vivo* effects of dietary bioactive components seem dependent on the metabolic status, gut microbiota composition, and gender of the subjects and may be organ-specific. Thus, a generalization that supplementation with such bio-actives is beneficial for human health first requires a better characterization of subject subgroups such as responders / non-responders and reliable biomarkers.

Target Groups, Activities, and Products

In-vitro and human intervention studies provide detailed knowledge about toxicity, effectiveness, and safety of different dosages of bio-actives during short and longer-term treatment / supplementation. This contributes to the establishment of recommended intake values [11] and scientifically sound health effects of these constituents. Defining recommended intakes of non-essential nutritional bio-actives is yet difficult due to contradictory results of human intervention studies. Several reasons may account for this e.g., differences in i) the extent of obesity and body composition, ii) habitual dietary intake, iii) physical activity levels, iv) the compliance of the subjects, v) the genetic background, and vi) gut microbiota diversity. Consequently, for future investigations larger, and longer studies are necessary, carefully adjusted for confounding or interacting factors e.g., enrolling well-characterized subjects with different metabolic risk factors, and including adequate controls, to identify beneficial effects for sub-populations and to establish recommended intake levels. Such studies will provide valuable information for industries that commercialize dietary supplements and functional foods and will help raise the awareness of a healthy lifestyle including the consumption of nutritional bio-actives containing fruits and vegetables [11]. A critical evaluation of beneficial and non-effective nutritional research outcomes by (inter-)national organizations and independent institutions e.g., EFSA[4] should be mandatory to position results in the right context and protect consumers from health claims without sufficient scientific evidence. In addition, such overarching evaluations include inconsistencies in data from human nutrition studies e.g., as reported for Res [15] and addressed in a recent study by Kjaer *et al.* [16] and shows that drawing conclusions from a limited number of studies in different sub-groups are misleading. Therefore, defining new biomarkers for the intake of bio-actives and their impact on metabolic health with the help of -omics technologies [17] can give indications regarding sub-populations which benefit from the supplementation with nutritional bio-actives and subsequently will promote more directed prevention / intervention strategies. Another option to identify possible target groups is to make all generated data sets accessible to institutes and companies that integrate the knowledge into the evolving “big data” approach. This is in accordance with another objective of knowledge valorization: to rapidly share results with the scientific community and health professionals through publication in international peer-reviewed journals and presentation at international scientific conferences. Furthermore, sharing of research outcomes in layman’s terms, in accessible university media channels (www.uctv.tv; https://www.mumc.nl/en/research) can be a suitable way to translate and spread scientific knowledge for the society.

[4] <EFSA> European Food and Safety Authority

The cell model chapters emphasize that further improvement, standardization, and validation of *in-vitro* models are necessary to i) better mimic *in-vivo* micro-environments (3D structures, co-cultures, nutrient supply (supra-physiological vs. physiological nutrient concentrations)), ii) generate reproducible results (e.g. pro- or anti-adipogenic effects of Res) in independent labs and experiments, and iii) enable the transition from *in-vitro* to *in-vivo* results. These cost-effective *in-vitro* settings could eventually reduce or skip future animal experiments (as done in this thesis). The *in-vitro* assay established herein to investigate lipid droplet accumulation is also a good starting point to develop a high-throughput commercial screening method. This method could facilitate a faster identification of dietary components regarding their effects on adipocyte lipid droplets biology from individual donors. Moreover, it could determine additive or synergistic effects of combinations and unravel underlying mechanisms, before investigating selected combinations in more sophisticated and expensive human *in-vitro* models, or clinical trials.

The *in-vitro* anti-inflammatory and anti-adipogenic effects of the bio-actives described in this thesis could however not be translated *in-vivo* into beneficial longer-term health effects (including improved insulin sensitivity and AT morphology). This could be due to the huge complexity of the potentially involved mechanisms (interactions of numerous cell types and organs) or due to the enrollment of healthy overweight-obese subjects, which could partly explain some discrepancies described in the literature. Nevertheless, the multi-organ impact and cross-talk of nutritional bio-actives *in-vivo* will hamper that implications from even improved *in-vitro* cell-based studies (human cell lines, organoids, 3D-/co-culture models) add significantly to our understanding of how nutritional bio-actives contribute to human metabolic health.

However, *in-vitro* data are valuable for the generation of intellectual property (IP). Hence the inhibitory effects of the combinations on the lipid accumulation in differentiating adipocytes were already filed in a patent application before publication of our results. In contrast, human intervention studies offer only small possibilities to create IP because they need a primary objective and an ethical justification to conduct the investigation, which is mostly based on previous research findings. Unfortunately, the supplementation with the polyphenol combination EGCG/Res did not show additive or synergistic (more likely to be patentable) effects when comparing results with other human invention studies investigating individual polyphenols.

The studies, experiments, and analyses described in this thesis were conducted at the department of Human Nutrition and Health at DSM Nutritional Products (Basel, Switzerland), the research department of Human Biology (NUTRIM, Maastricht University, Netherlands), and the Nutrition, Metabolism, and Genomics Group (Division of Human Nutrition, Wageningen University, Netherlands). Moreover, the human study was supported by a grant of the Alpro Foundation (Gent,

Belgium). This sets a good example for a fruitful collaboration and networking between universities and nutrition industry partners, which promotes applied research and joint utilization of resources for a faster valorization of results. Taken together, the results of this thesis were only achieved due to these collaborations and importantly, they give novel impulses for further investigations in the field of nutrition.

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