LUDC-IRC Postdoctoral Program 2020 – Project 2

Polyphenol-rich food for prevention and treatment of obesity and type 2 diabetes in humans – Physiological effects, molecular mechanisms and long-term associations

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Purpose and aims

The overall purpose is to understand and define the role of dietary polyphenols in prevention of metabolic diseases and maintenance of health. The specific goals are to evaluate the metabolic effects and to identify cellular and molecular targets of berry polyphenols as well as the long-term associations with a high habitual polyphenol intake. Previous promising results from mice studies will thus be taken to the next step to be tested and proved in human intervention studies as well as studying the role of berry polyphenols in long-term associations studies using the available cohorts.

State-of-the-art/background

Plant-derived food has been shown to delay or even inhibit the development of obesity and type 2 diabetes (T2D) (Jannasch 2017). One hypothesis is that food components rich in phytochemicals, e.g. polyphenols, exert anti-diabetic effect (Bahadoran 2013). Polyphenols are present in plants for natural defence but they also provide taste and colour to e.g. fruits, berries, vegetables, cereals, tea, coffee and wine. Previously, polyphenols health properties were attributed to the direct antioxidative effect by acting as free radical scavengers, however, recent data reveal that polyphenols can influence host health by regulation of gene expression, especially genes related to inflammation and modify the gut microbiota composition in a positive direction (Fraga 2019). Previous papers have reported beneficial effects on postprandial glucose and insulin responses after acute lingonberry, blackcurrant or bilberry intake in human subjects (Linderborg 2012, Bell 2017). However, the identity of the active substances and the mechanisms are not understood.

The gut microbiota has been identified as an important factor in the interplay between processing of food and physiological processes of the host. The metabolic potential of the gastrointestinal tract and its microbiota are increasingly recognised as promising targets to improve glycaemic control and treat type 2 diabetes (Delzenne 2011, Brunkwall 2017). The dietary polyphenol profile is suggested to be important for gut microbiota composition and production of beneficial bioactive metabolites for prevention and treatment of obesity and type 2 diabetes (Moco 2012). In fact, a human intervention study by Cotillard et al. has shown that increased consumption of healthy foods such as fruit and vegetables lead to an increase in bacterial richness and improved clinical symptoms associated with obesity and type 2 diabetes.

Significance and scientific novelty

According to WHO, beyond the appropriate medical treatment for those already affected, the approach of prevention is considered to be the most cost-effective, affordable and sustainable course of action to cope with the chronic disease epidemic worldwide (WHO 2003). Improving nutrition is the single most important contributor to reduce the burden of diabetes in Europe, yet we spend less than 1% of the total health care costs for prevention of diseases. Our previous results regarding different berries, especially lingonberries, are very promising in the anti-obesity, anti-diabetic and anti-inflammatory point of view. Very limited number of studies has previously been reported regarding polyphenols in the relation to obesity, diabetes and inflammation, so this has a very high news value. In particular, there is a need for high quality human studies of metabolic effects of berries, and especially Nordic wild berries, since they have a unique composition of phytochemicals.

Preliminary and previous results

Polyphenol-rich berries in high fat-fed C57BL/6J mice. We have recently reported about effects and mechanisms of polyphenol-rich berries in high-fat fed mice. The first paper revealed for the first time that

lingonberries, but also blackcurrant and bilberries, efficiently prevented the development of obesity and associated metabolic disturbances while the Brazilian açai berry had the opposite effect (Heyman 2014). Genome-wide hepatic gene expression profiling indicated that the protective effects of lingonberries and bilberries are accounted for by several-fold downregulation of genes involved in acute-phase and inflammatory pathways (e.g. Saa1, Cxcl1, Lcn2). In contrast, açai-fed mice exhibited marked upregulation of genes associated with steatosis (e.g. Cfd, Cidea, Crat) and lipid and cholesterol biosynthesis, which is in line with the exacerbation of HF-induced hepatic steatosis in these mice. In silico transcription factor analysis together with immunoblot analysis identified NF-κB, STAT3 and mTOR as upstream regulators involved in mediating the observed transcriptional effects (Heyman-Lindén 2016(1)). In a follow-up study, mice were fed HF diet with or without a supplement of lingonberries. Lingonberries reduced plasma levels of markers of inflammation (SAA) and endotoxemia (LBP) and positively altered gut microbiota composition and functionality compared to the HF control. Two genera associated with healthy gut mucosa and anti-inflammation, Akkermansia and Faecaliebacterium, were identified as biomarkers, (Heyman-Lindén 2016(2)).

Metabolite profiling in plasma collected from mice fed HFD supplemented with two different batches of lingonberries. The impact of two different batches of lingonberries was investigated regarding the phenotype and plasma metabolome in mice (Hamimi 2017). Multivariate statistical analysis of phenotype and metabolite profiling data were used to link alterations in metabolism to phenotypic features being shared between both diets or unique to a particular diet. Both improved liver function and reduced inflammation, which associated with alterations in sphingomyelin metabolism. Moreover, a batch dependent reduction in circulating glucose levels was found to be associated with elevated FFA levels, suggesting a normal metabolic control with a different set point.

Short-term effect of lingonberries in high-fat fed mice. High-fat diet has recently been shown to increase body weight and impair glucose control in mice already after four days of HFD (Hansson 2018). Since lingonberries reduce adiposity in mice, we specifically have examined the molecular effects of lingonberries or isolated lingonberry polyphenols, on glucose uptake and lipid metabolism in visceral and subcutaneous white adipose tissue. In collaboration with Ass. prof Karin Stenkula, adipose tissue and primary adipocytes from mice fed HFD with or without berry supplementation for only 4 days have been isolated. The effect on lipogenesis, lipolysis, glucose uptake and insulin resistance have been examined in primary adipocytes and transcriptome microarray have been performed in adipose tissue . Key players in insulin signalling and insulin resistance, e.g. GLUT4, PKB, AMPK and PPARg have been analysed and adipocytes have been sorted according to size distribution.

Research plan and project organization

After the very convincing results from animal studies, lingonberries (and possibly also other polyphenol-rich berries) will be tested for effects in humans. Studies will be accomplished both in healthy individuals and subjects with obesity and insulin resistance (IR). Healthy individuals will be recruited by advertisement in daily press and online platforms while obese/IR subjects preliminary will be recruited among patients attending the obesity clinic at the Department of Endocrinology, University Hospital SUS. The studies in humans will be performed as acute and short-term studies. (Long-term studies will follow later according to financial possibilities and achieved results.) In addition to the intervention studies, the association between polyphenol-rich food and type 2 diabetes will be investigated in epidemiological studies using Malmö Diet and Cancer Study (MDCS).

The ethical permission is not yet submitted since all details regarding the studies are not completed. Probably, there will be enough time for these preparations while finding the right postdoc candidate for the project.

Year 1:

Acute studies will investigate the impact of different doses of lingonberries on postprandial glucose and insulin response. The study will be designed as a double-blind, counterbalanced, crossover in sugar-matched conditions. Three different doses of lingonberries (mixed from different batches) will be tested at different time points with at least one week between the occasions. The lingonberries will be served as a smoothie-breakfast. Participants will be asked to follow a low polyphenol diet for 24 hours prior to each visit and fast over night before attending. On arrival, baseline blood glucose will be recorded. Participants will consume the smoothie in an opaque flask to maintain double blinding. Blood samples will be taken at pre-

consumption, 15, 30, 45, 60, 90, 120 and 150 minutes postprandially. From the blood samples, Area Under the Curve (AUC) for glucose, insulin and incretins will be analysed. This acute study will be performed in healthy individuals and the plan is to recruit 25 persons and each person will be tested for 3 doses of lingonberries and 1 matched control product without lingonberries.

End of year 1 and year 2:

The short-term studies will focus on effects on postprandial glucose and insulin secretion, inflammatory markers and changes in gut microbiota before and after daily ingestion of lingonberries for 7 days. The included subjects should add lingonberries, corresponding to 200-300 g berries per day (the dose will be decided according to the result from the acute studies), to their ordinary diet. The berries will be given both as frozen berries, freeze dried berry powder and lingonberry juice. The participants will also achieve recipes and serving suggestions. They will also be told to minimize the intake of fruits and berries 3-4 days prior to the start of the study and fill in a food diary during the study period. The study will be designed as a counterbalanced crossover study in which all participants supplement the diet with lingonberries for 7 days and eat a control diet without ingestion of any berries and a restricted amount of fruits for 7 days with a wash-out period of four weeks in between. The food pattern should in all other matters be equal between the test periods.

In the start and in the end of each test period the participants will be told to have an overnight fast and the next morning blood samples will be taken before and 15, 30, 45, 60, 90, 120 and 150 minutes after a standardized carbohydrate-rich breakfast for analysis of AUC for glucose, insulin and incretins. In addition, in the start and in the end of each test period a blood sample will be taken for analysis of inflammatory markers (LBP, SAA, TNF-alpha, PAI-I and CRP) and metabolite profiling (e.g. sphingomyelin). In addition, effects on satiety will be evaluated using the plasma hunger/satiation/satiety markers (ghrelin, PYY, GLP-1) but also by estimation of hunger/satiation using a VAS scale. Fecal samples will be collected and frozen in -80°C before and after the test periods. For global analysis of gut microbiota, the V1-V3 region of the 16S rRNA gene will be amplified from bacterial DNA and sequenced using next-generation sequencing.

This short-term study will in the first place be performed in healthy individuals but thereafter also in obese/insulin resistant individuals. The plan is to recruit 20 persons and each person will be its own control according to the cross over design.

Year 1 and 2 in parallel with intervention studies:

Epidemiology studies using the well-characterized and large Malmö Diet and Cancer Study (MDCS) of 28,098 individuals (Manjer 2002) with >20 years of follow-up will be used to correlate the intake of polyphenol-rich food to the new subgroup classification of diabetes patients (Ahlqvist 2018). Type 2 diabetes cases (4284 incident cases until 31 dec 2016) are identified through regional and national registers, including Swedish National diabetes register, Diabetes 2000 register, hospital discharge register, and prescribed drug register. The baseline examination included assessment of dietary habits (using 7-day food diary, 168-item dietary questionnaire and a 1-hour dietary interview), lifestyle factors, anthropometric and blood pressure measurements, and collection of blood (non-fasting samples). Associations between polyphenol intake and the different diabetes classification will allow better prevention strategies.

Competences and roles of the participating researchers in the project

Assoc. Prof. Karin Berger will together with the postdoc be responsible for the intervention studies, although the study design and study outcome will be performed in close collaboration with Assoc. Prof. Emily Sonestedt and a medical doctor (to be determined). Karin Berger has previously been involved in a double-blind cross-over human intervention study in which overweight individuals were tested for metabolic changes after intake of rosehip (Andersson 2012). The project is also dependent on a nurse for blood sampling. Analysis of microbiota from fecal samples can either be in collaboration with a research group in the field of microbiota analysis at Lund University or analysed by a company having this service. Emily Sonestedt will together with the postdoc be responsible for the epidemiological studies. Emily Sonestedt is a member of the steering committee of the Malmö Diet and Cancer cohort with specific responsibility for the dietary database and have extensive experience in processing data from the cohort.

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