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Berry intake changes hepatic gene expression and DNA methylation patterns associated with high-fat diet☆

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Abstract

The liver is a critical organ for regulation of energy homeostasis and fatty liver disease is closely associated with obesity and insulin resistance. We have previously found that lingonberries, blackcurrants and bilberries prevent, whereas açai berries exacerbate, the development of hepatic steatosis and obesity in the high-fat (HF)-fed C57BL/6J mouse model. In this follow-up study, we investigated the mechanisms behind these effects. Genome-wide hepatic gene expression profiling indicates 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 exhibit 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. To gain further insight into mechanisms involved in the gene expression changes, the HELP-tagging assay was used to identify differentially methylated CpG sites. Compared to the HF control group, lingonberries induced genome-wide hypermethylation and specific hypermethylation of *Ncor2*, encoding the corepressor NCoR/SMRT implicated in the regulation of pathways of metabolic homeostasis and inflammation. We conclude that the beneficial metabolic effects of lingonberries and bilberries are associated with downregulation of inflammatory pathways, whereas for blackcurrants, exerting similar metabolic effects, different mechanisms of action appear to dominate. NF-κB, STAT3 and mTOR are potential targets of the health-promoting effects of berries.

Keywords: Berries; Inflammation; Liver steatosis; Gene expression; Methylation; High-fat diet

1. Introduction

Overweight and obesity are health issues that continue to increase and are now affecting almost 40% of the global adult population [1]. Obesity is associated with metabolic changes including insulin resistance, nonalcoholic fatty liver disease (NAFLD), low-grade inflammation and dyslipidemia, which may lead to development of type 2 diabetes (T2DM) [2,3]. The liver is a critical organ for the regulation of whole body energy homeostasis due to its central role in lipid and glucose metabolism, as well as its close connection via the portal vein to nutrient uptake in the intestine. The prevalence of NAFLD is increasing in parallel with the epidemic of obesity and insulin resistance that is coupled to the diet of the Western lifestyle [3,4]. High-fat (HF) diets have been shown to cause dysregulated hepatic gene expression with perturbations in lipid, cholesterol, inflammatory and oxidative pathways [5]. NAFLD is characterized by accumulation of lipids and lipid derivates sensitizing the liver for further damage by inflammation and fibrosis, potentially by a second hit involving oxidative stress [6,7]. The hepatic fat accumulation may derive from increased dietary lipid intake, increased lipid synthesis (*de novo* lipogenesis) and/or decreased oxidation. In addition, obesity and T2DM states may associate with increased uptake of free fatty acids from adipose tissues into nonadipose tissues such as the liver [7,8].

The types of foods we eat are important for maintenance of a healthy body weight. It is possible that increased consumption of vegetables and fruit protects against body weight gain [9], thereby

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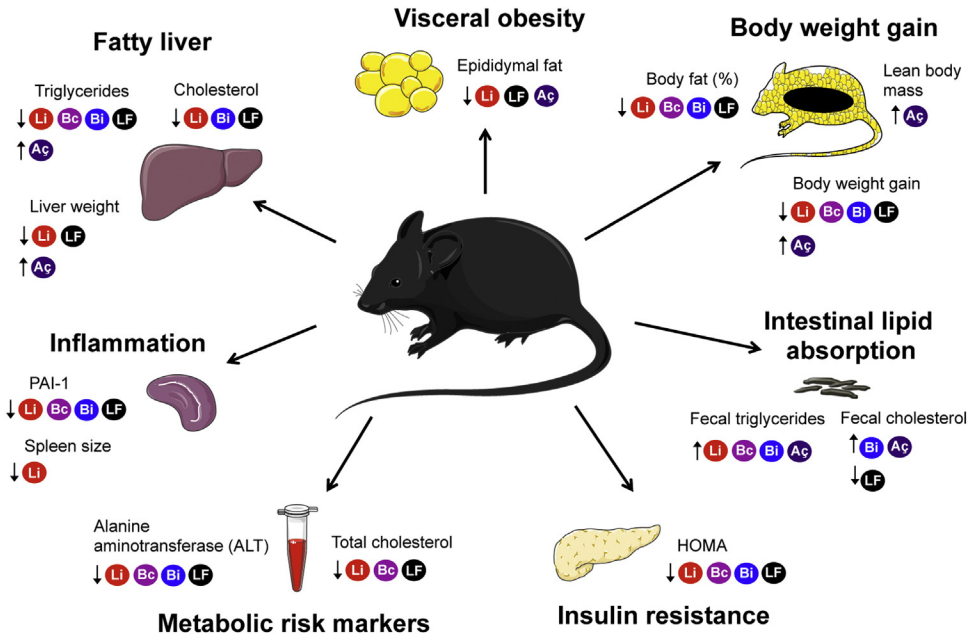

Fig. 1. **Overview of metabolic effects of supplementing HF diet with different berries.** Mice received HF diet without supplementation or HF diet supplemented with 20% lingonberry (Li), blackcurrant (Bc), bilberry (Bi) or açai (Aç) for 13 weeks [10]. One group received a low-fat (LF) diet. Arrows indicate the statistically significant effects ($P<.05$ or less) compared to the control mice receiving HF diet. PAI-1 and ALT are markers for inflammation and liver dysfunction, respectively. Homeostatic model assessment (HOMA) index reflects the level of insulin resistance. Illustrations were obtained and modified from Servier Medical Art by Servier, http://www.servier.com/Powerpoint-image-bank, licensed under Creative Commons Attribution 3.0 Unported License, http://creativecommons.org/licenses/by/3.0/.

preventing obesity and NAFLD. In a previous study [10], we found that addition of different berries to HF diets can prevent development of obesity and fatty liver in C57BL/6 mice, a mouse model used to study diet-induced obesity and prediabetes [11]. The previous findings are schematically described in Fig. 1. In brief, mice were protected against HF-induced weight gain when the diet was supplemented with lingonberries (−21%), blackcurrants (−14%) or bilberries (−10%), whereas açai promoted weight gain (+14%) [10]. Furthermore, mice fed lingonberries, blackcurrants and bilberries showed a drastic reduction of fat accumulation in liver (−77%, −57% and −43% mg/g liver), whereas the açai berry had the opposite effect (+73%) compared to mice receiving a HF diet without berries. In addition, plasma levels of the liver injury marker alanine-aminotransferase (ALT) were reduced by around 30% by lingonberries and blackcurrants. Characteristic of colorful berries is that they are rich in polyphenolic compounds, which are proposed to have a range of health properties [12]. Polyphenols are generally metabolized in the intestine and liver, they recirculate in the enterohepatic circulation and certain metabolites have been suggested to accumulate in association with hepatic fat droplets and immune cells [12,13]. Diets rich in natural antioxidants [14] as well as Nordic berries [15] have been shown to improve liver function in humans. However, very little is known about what pathways in the liver are affected by berries *in vivo*. Both the abovementioned human studies and our recent mouse study suggest that the liver is a major site of action for the beneficial health effects of berries. In order to get insight into the mechanisms underlying these effects, we sought to follow up the findings by in-depth characterization of livers from the same cohort of animals as in our previous study [10].

Here, expression microarrays were employed to analyze the hepatic transcriptome in mice that were protected against HF-induced obesity and liver steatosis (i.e. HF diets supplemented with lingonberries, blackcurrants or bilberries) and compared to mice that were not protected (HF control) or with even increased obesity and fatty liver (HF diet with açai). Furthermore, changes in DNA methylation of CpG sites, which represent a potential mechanism by which nutrients and natural compounds may regulate gene expression [16,17], were assessed as genome-wide DNA methylation of individual CpG sites in livers from mice receiving lingonberry — the berry with the most pronounced health-promoting effects according to our previous study [10].

2. Methods and materials

2.1. Animals and study design

The liver tissue used for gene expression analysis was taken from male C57BL/6JBomTac mice receiving HF diets (45 kcal% fat) supplemented with 20% (w/w) of different freeze-dried berries for 13 weeks [10]. The general composition (kcal%) of the diets was as follows: for the HF diets, 45% fat, 35% carbohydrate and 20% protein; for the low-fat (LF) diet, 10% fat, 70% carbohydrates and 20% protein. In addition, the diets were formulated to have the same glucose, fructose and sucrose content. The study was approved by the Animal Ethics Committee in Lund, Sweden, (Permit Number: M185-11) and was in accordance with the Council of Europe Convention (ETS 123). Dietary composition as well as phenotypical and metabolic characteristics of the mouse study is described in Ref. [10]. In the current study, liver tissue and plasma (n=6–12) were used from 4-h-fasted mice receiving the following diets: HF diet (control group), LF diet (10 kcal% fat) or HF diet supplemented with lingonberries, blackcurrants, bilberries or açai.

2.2. DNA and RNA isolation from liver

DNA and RNA were extracted with the AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) and purity and concentration were determined using spectrophotometry (NanoDrop). RNA integrity was evaluated by an Agilent 2100 Bioanalyzer (Agilent Technologies, Loveland, CO, USA).

2.3. Microarray processing

Global gene expression profiles in livers were determined by the Swegene Center for Integrative Biology Genomics DNA Microarray Resource Center (SCIBLU, Lund, Sweden) using MouseWG-6 v2.0 Whole-Genome Expression Beadchips (Illumina, San Diego, CA, USA). Expression analysis was conducted on 6 randomly selected mice (from a total of 12) per diet group, giving 36 microarrays in total. Images and raw signal intensities were acquired using the Illumina BeadArray Reader scanner. Data preprocessing and quantile normalization were performed using Illumina GenomeStudio software. The data have been deposited in NCBI's Gene Expression Omnibus (GEO) database (accession number GSE66711).