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Connections with gut microbiota composition and cardiometabolic risk markers

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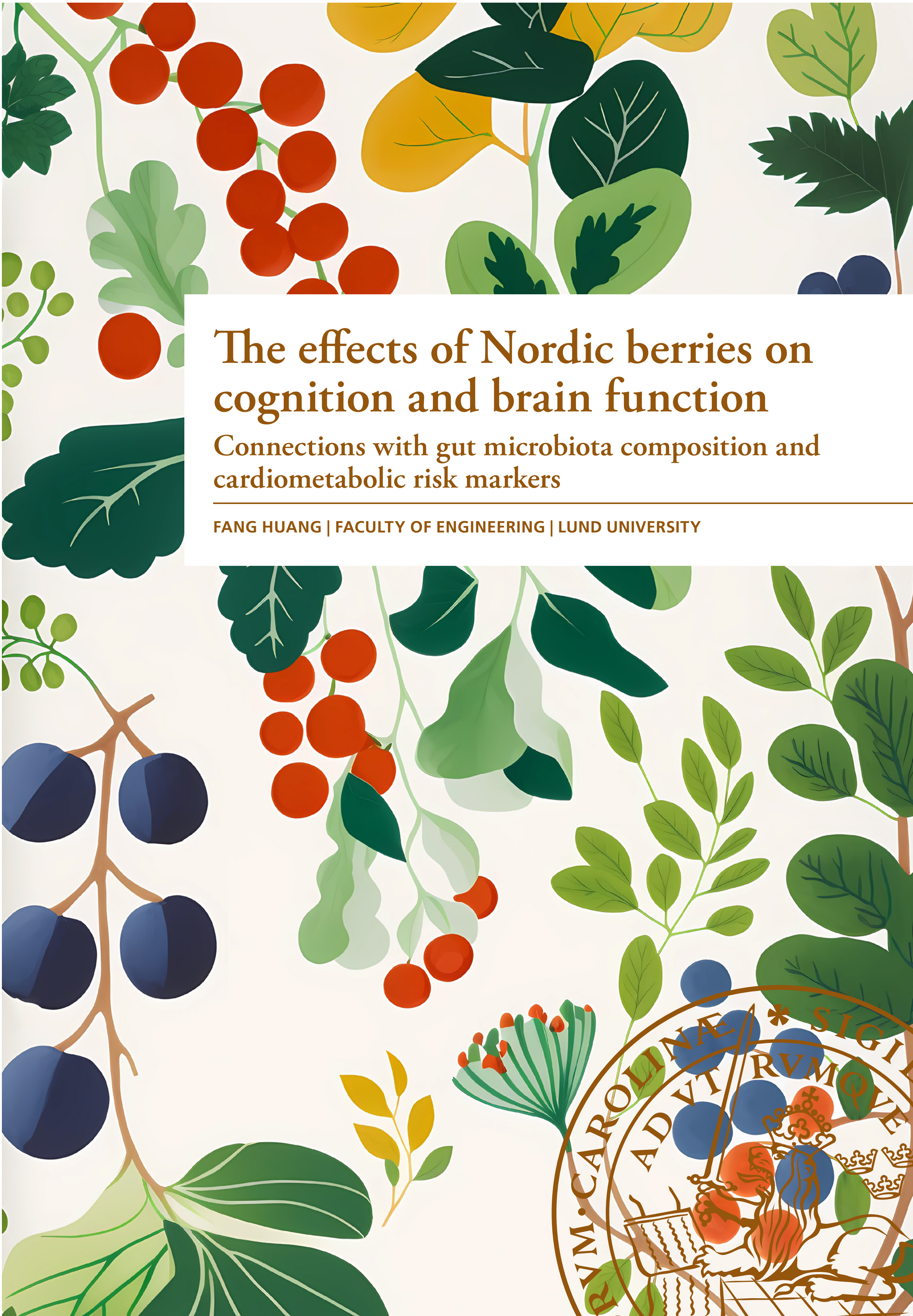

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FANG HUANG | FACULTY OF ENGINEERING | LUND UNIVERSITY

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Abstract

An aging population is associated with an increased prevalence of cognitive decline and related diseases. Gradual cognitive decline is a natural feature of aging, but may also develop into a more severe loss of cognitive function, manifesting as mild cognitive impairment or severe cognitive disease. Currently, no medical treatments can fully prevent these conditions or diseases. One approach is to identify foods that have cognitive benefits to promote healthy aging in the population. Emerging evidence suggests that consuming fruits high in polyphenols and fiber, such as berries, may help improve memory and cognitive function in humans and rodents. Nordic countries are abundant with wild berries, but the cognitive health benefits of these berries remain largely unexplored.

This thesis investigates the effects of Nordic berries on cognitive function in mice fed a high-fat (HF) diet, assessed by a series of behavior tests. The studies also aim to explore other health effects of berry supplementation, such as the impact on levels of cardiometabolic risk markers and gut microbiota composition. Additionally, this thesis also examines a potential probiotic – *Prevotella copri* DSM18205^{T} – and assesses its ability to utilize various carbon sources including the carbohydrate constituents in the berries investigated in the thesis.

The results suggest that lingonberry, bilberry, blackcurrant, cloudberry, and blueberry positively affect brain function in middle-aged HF-fed C57BL/6J mice, as demonstrated by improved performance in the T-maze alternation test. Additionally, a berry mixture containing lingonberries and bilberries significantly improved spatial and learning memory in C57BL/6J mice, as seen in their enhanced performance in both the T-maze and the Barnes maze tests.

Supplementation of the berry mixture to a HF diet may aid in reducing body weight, fasting insulin as well as neuroinflammation levels in C57BL/6J mice. In addition, in apolipoprotein E knockout mice (ApoE–/–) a HF diet including lingonberries significantly reduced body weight and improved glucose response.

An additional finding is that berries strongly influence the composition of the gut microbiota, promoting the relative abundance of the bacterium *Akkermansia muciniphila*, which has previously been linked to protective effects on cognition. The work in this thesis also revealed the growth characteristics and cultivation techniques of *P.copri* DSM18205^{T}.

The significance of these findings provides recommendations for dietary strategies that incorporate berries, these strategies may aid in mitigating the growing prevalence of cognitive decline and diseases associated with the aging global population. Lastly, the knowledge gained on the growth and metabolite characteristics of *P.copri* DSM18205^{T} will provide a basis for future research on its potential uses.

Key words: Nordic berries, cognition, brain function, gut microbiota, cardiometabolic function

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To my family

致我的家人

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This thesis investigates the effects of Nordic berries on cognitive function in mice fed a high-fat (HF) diet, assessed by a series of behavior tests. The studies also aim to explore other health effects of berry supplementation, such as the impact on levels of cardiometabolic risk markers and gut microbiota composition. Additionally, this thesis also examines a potential probiotic – *Prevotella copri* DSM18205^{T} – and assesses its ability to utilize various carbon sources including the carbohydrate constituents in the berries investigated in the thesis.

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The significance of these findings provides recommendations for dietary strategies that incorporate berries, these strategies may aid in mitigating the growing prevalence of cognitive decline and diseases associated with the aging global population. Lastly, the knowledge gained on the growth and metabolite characteristics of *P.copri* DSM18205^{T} will provide a basis for future research on its potential uses.

Popular scientific summary

As we age, our memory may not function as well as it did when we were younger. It may take longer to learn new things, and it may be more challenging to recall previously learned information. This is a normal part of the aging process and is not necessarily a sign of a medical condition. However, memory loss can also be a symptom of certain medical conditions, such as Alzheimer's disease.

When we are overweight or obese, it is not just our heart and waistline that could be at risk. Studies have shown that being obese or having metabolic disorders like diabetes can also increase the risk of developing cognitive impairment. While there seems to be a connection between aging, obesity, and cognitive decline, these are not the only factors that matter. Genetics and lifestyle also play a significant role in the development of cognitive impairment. Consequently, staying physically active and eating a diet rich in fruits, vegetables, and healthy fats can support brain health and cognitive function.

Berries are a colorful group of fruits that can make up a part of a healthy diet. They are packed with vitamins, antioxidants, and fiber. Some berries, like lingonberries and bilberries (European blueberries), have been shown to reduce body weight, improve metabolic function, reduce gut inflammation, and improve cognitive function. But what is the connection?

Recent research suggests that the gut microbiota – the ecosystem of microorganisms living in our bowels – is crucial in many aspects of our overall health. Scientists believe that our gut and brain are closely connected and can communicate. Studies have found that individuals with cognitive impairment and brain plaque (a protein that can build up in the brain and cause damage) tend to have abnormal gut microbiota.

When we eat berries, the antioxidants and fibers in the berries make their way to our gut, where they are consumed by the microorganisms living there. These food compounds can change the makeup of our gut microbiota and promote the growth of beneficial bacteria. This can positively impact our well-being and protect us from certain illnesses. One specific example is a type of bacteria called *Prevotella copri*. This bacterium was promoted in healthy individuals when consuming fiber-rich diets. It can be considered a good bacterium because the presence of *P. copri* in high amounts is associated with improved blood sugar management.

This thesis explores several Nordic berries: lingonberry, bilberry, blackcurrant, cloudberry, and sea buckthorn, also including American blueberry. It does so in studies on mice. Some of the Nordic berries have not been studied before in terms of their potential effects on cognitive function, metabolic function, and the effect on gut microbiota composition.

Our findings suggest that bilberry, blackcurrant, blueberry, lingonberry, and cloudberry positively affect brain function in mice. This is demonstrated by improved cognitive function when evaluated in a series of memory tests. In addition, a berry mixture containing lingonberries and bilberries may also help to reduce body weight gain caused by a high fat diet and improve metabolic function.

We also found that the intake of lingonberries, blackcurrants, cloudberries, sea buckthorn, or blueberries contributed to a more diverse and robust gut microbiota compared to a diet without berries, suggesting that a more balanced bacterial community can be achieved by eating these berries. Additionally, the supplementation of lingonberry, bilberry or sea buckthorn could promote the presence of a beneficial bacterium called *Akkermansia muciniphila*. This bacterium has previously been linked to a protective effect against cognitive deficits.

We also investigated the growth behavior and cultivating technique of another potentially beneficial bacterium mentioned above, *P. copri*. Our results will help to explain and evaluate the role played by *P. copri* in our bodies.

Taken together, these findings suggest that eating certain Nordic berries could be a valuable strategy for restoring balance to the gut microbiota and improving brain function. So next time you reach for a handful of bilberries in the Nordic wilderness, remember that they may be doing more than just satisfying your sweet tooth!

Populärvetenskaplig sammanfattning

När vi blir äldre kan minnet bli mindre tillförlitligt. Det kan ta längre tid att lära sig nya saker och det kan bli svårare att komma ihåg. Detta är en del av livet och är inte nödvändigtvis ett tecken på sjukdom. Minnesförlust kan dock i vissa fall vara ett symptom på vissa medicinska tillstånd, till exempel Alzheimers sjukdom.

Vid övervikt och fetma är det inte bara vårt hjärta och vår midja som kan vara i fara. Studier har visat att övervikt eller ämnesomsättningssjukdomar som diabetes också kan öka risken för att utveckla försämrad kognitiv förmåga.

Även om det finns ett samband mellan åldrande, fetma och försämrad kognitiv förmåga så är dessa inte de enda faktorerna. Genetik och livsstil spelar också en viktig roll för utvecklingen av försämrad kognitiv förmåga, och därför kan fysisk aktivitet och en kost som är rik på frukt, grönsaker och hälsosamma fetter hjälpa till att bevara hjärnhälsa och kognitiv funktion.

Bär är en färgglad grupp frukter som kan utgöra en del av en hälsosam kost. De är fulla av vitaminer, antioxidanter och fibrer. Konsumtion av vissa bär, som lingon, hallon och blåbär, har tidigare visat sig kunna minska kroppsvikten, förbättra ämnesomsättningen, minska tarminflammation och främja den kognitiva funktionen. Men vad finns det för samband?

Det är här tarmfloran kommer in: det ekosystem av mikroorganismer som lever i dina tarmar. Ny forskning tyder på att tarmfloran spelar en avgörande roll för många aspekter av vår hälsa. Forskare tror att vår tarm och hjärna är nära sammankopplade och kan kommunicera. Studier har visat att personer med kognitiv nedsättning och hjärnplack (ett protein som kan ackumuleras i hjärnan och orsaka skador) tenderar att ha en ohälsosam tarmflora.

När vi äter bär når antioxidanter och fibrer våra tarmar, och kan konsumeras av de mikroorganismer som lever där. Dessa kostkomponenter kan förändra sammansättningen av vår tarmflora och främja tillväxten av nyttiga bakterier. Detta kan påverka vårt allmänna välbefinnande positivt och till och med skydda oss från vissa sjukdomstillstånd.

Ett specifikt exempel är en bakterie som kallas *Prevotella copri.* Denna bakterie främjas hos friska individer när de konsumerar fiberrik kost. Den anses vara en bra bakterie eftersom riklig förekomst av *P. copri* är förknippad med förbättrad blodsockerhantering.

I denna avhandling undersöks flera nordiska bär som inte tidigare har studerats med avseende på deras potentiella effekter på kognitiv funktion, ämnesomsättning samt inverkan på tarmfloran. Detta görs i prekliniska studier på möss.

Vårt resultat tyder på att europeiska och amerikanska blåbär, svarta vinbär, lingon och hjortron påverkar hjärnfunktionen positivt hos möss, som uppvisade förbättrad kognitiv förmåga i en serie minnestester. Vissa bärsorter visas också kunna minska kroppsvikten. Vi upptäckte också att vissa av dessa bär har en betydande inverkan på tarmfloran, särskilt genom att främja förekomsten av den nyttiga bakterien *Akkermansia muciniphila*. Denna bakterie har tidigare kopplats till en skyddande effekt mot försämrad kognitiv förmåga.

Vi undersökte också tillväxtprofilen och odlingstekniker för en annan potentiellt nyttig bakterie, *P. copri.* Den kunskap vi bidragit med kommer att hjälpa till att förklara och utvärdera den roll som *P. copri* spelar i vår kropp.

Sammantaget tyder dessa resultat på att det kan vara bra att äta vissa nordiska bär för att återställa balansen i tarmfloran och förbättra hjärnans hälsa. Så nästa gång du sträcker dig efter en handfull blåbär i den nordiska vildmarken, kom ihåg att de kan göra mer än att bara tillfredsställa ditt sötsug!

中文科普简介

人们的记忆力可能会随着年龄渐长而逐渐减退。学习新事物可能需要更长的时间，回忆以前学过的东西更具挑战性。这是衰老过程中的一个正常情况，并不一定是严重疾病的标志。然而，记忆力减退也会有可能是某些认知性疾病的征兆，如阿尔茨海默氏症。

在超重和肥胖的情况下，受到威胁的不仅仅是我们的心脏和腰围。研究表明，肥胖或患有糖尿病等代谢性疾病也会增加患认知障碍的风险。

虽然衰老、肥胖和认知能力下降之间存在一定联系，但它们并不是唯一重要的因素。遗传和生活方式在认知障碍的发展中也起着重要作用。生活方式的选择具体来说，保持适当体育锻炼和健康的饮食。比如吃水果、蔬菜和健康脂肪的饮食等等。这些做法都可以在一定程度上维护大脑的健康，影响认知功能。

莓果是一组五颜六色的水果，它们可以构成健康饮食的一部分。莓果富含维生素、抗氧化剂和膳食纤维。一些可食用莓果，比如越橘，美国蓝莓，先前已被科学研究证明有利于控制体重，改善代谢功能，减少肠道炎症，并且影响改善认知功能。

但吃莓果和对健康有益之间具体的联系是什么呢？部分答案也许可以在我们的肠道微生物群里找到。

最近的研究表明，肠道微生物(或者又叫肠道菌群)在人体健康的许多方面都起着关键作用。科学家们认为，我们的肠道和大脑是紧密相连的，简而言之，它们可以通过直接或者间接的方式进行交流，互相影响。研究证实，有认知障碍的人往往也伴有异常的肠道菌群组成。

当我们吃莓果时，莓果中的抗氧化剂和膳食纤维会进入我们的肠道，被生活在那里的微生物摄入。这些食物成分可以改变我们肠道微生物菌群的构成，促进有益细菌的生长。这可以对我们的整体健康产生积极影响，甚至可能保护我们免受某些疾病的侵害。

一个具体的例子是一种叫作 *Prevotella copri* 的普雷沃氏细菌。当人们食用富含膳食纤维的饮食时，这种细菌在健康人的肠道中得到促进。同时也因为当有更多 *P. copri* 菌存在的时候，这类人群似乎能够更好地控制和管理自身血糖。因此，*P. copri* 被建议为也许是一种有益人体健康的细菌。

这篇论文研究了五种来自北欧本地野生可食用的莓果，包括越橘(lingonberry)，欧洲野生蓝莓(bilberry)， 黑加仑(blackcurrant)，云莓(cloudberry)，沙棘(sea buckthorn)，以及来自美国的种植蓝莓

(blueberry)。重点研究目的是针对这些莓果对认知功能，代谢功能以及对肠道微生物群组成的潜在影响。在此之前，人们对几种北欧莓果于认知功能的影响知之甚少。本论文采用的主要研究方法是在小鼠实验中完成的。

我们的研究结果表明，通过在实验小鼠的食物里加入越橘，野生蓝莓、黑加仑、云莓 或者美国蓝莓，小鼠在一系列记忆力测试中表现得更好，由此证明这些莓果对认知功能有益处。此外，我们的研究结果还证明了富含莓果的饮食，特别是越橘和野生蓝莓，也有助于提高大脑健康，体重控制以及血糖管理。

但益处还不止于此。研究结果还发现，越橘，黑加仑，云莓，沙棘以及美国蓝莓对肠道微生物群的组成也有很大的影响，摄入这些莓果可以潜在地提高肠道健康。另外，越橘，野生蓝莓和沙棘被证实能够特别促进一种名为 *Akkermansia muciniphila* 的有益细菌的存在。这种细菌被认为有助于人体健康。

这篇论文还深入研究了上面提到的另一种潜在的有益细菌-*Prevotella copri* 的生长繁殖行为和培养技术。通过了解这种菌的生长习性，代谢产物的成分，研究人员能够更好地解释或者评估这种菌在我们身体里扮演的角色和作用。

综合来看，这些发现表明，食用北欧莓果可能有助于肠道微生物群的发展组成以及改善大脑健康。因此，当你在北欧的森林发现并收获了一把野生蓝莓的时候，请记住它们可能不仅仅只是美味而已!

List of Papers

Paper I

Marungruang, N., Kovalenko, T., Osadchenko, I., Voss U., **Huang, F**., Burleigh, S., Ushakova, G., Skibo, G., Nyman, M., Prykhodko, O., & Hållenius, F. F. Lingonberries and their two separated fractions differently alter the gut microbiota, improve metabolic functions, reduce gut inflammatory properties, and improve brain function in ApoE−/− mice fed high-fat diet. Nutritional Neuroscience, 23:8, 600-612, DOI: 10.1080/1028415X.2018.1536423

Paper II

Huang, F.,* Marungruang, N.,* Kostiuchenko, O., Kravchenko, N., Burleigh, S., Prykhodko, O., Hållenius, F. F., & Heyman-Lindén, L. Identification of Nordic Berries with Beneficial Effects on Cognitive Outcomes and Gut Microbiota in High-Fat-Fed Middle-Aged C57BL/6J Mice. Nutrients, 14(13). DOI: 10.3390/nu14132734 (*=equal contribution)

Paper III

Huang, F., Marungruang, N., Martinsson, I., Nguyen, T, D., Karlsson, N, E., Deierborg, T., Öste, R., & Heyman-Lindén, L. A Mixture of Nordic Berries improves Cognitive Function, Metabolic Function and Alters the Gut Microbiota in C57BL/6JMice. (Manuscript)

Paper IV

Huang, F., Sardari, R., Jasilionis, A., Böök, O., Öste, R., Rascón, A., Heyman-Lindén, L., Holst, O., & Karlsson, E. N. (2021). Cultivation of the gut bacterium *Prevotella copri* DSM 18205^{T} using Glucose and Xylose as Carbon Sources. MicrobiologyOpen, 10(3), e1213. DOI: 10.1002/mbo3.1213

Author’s contribution to the papers

Paper I

The author prepared the fiber fractions for the research diets. The author took part in performing the animal experiment including the oral glucose tolerance test and the behavior tests. The author participated in the collection of the samples, and contributed to the analysis and the data acquisition of the SCFAs result.

Paper II

The author took part in the animal experiment, collected the samples, and analyzed the behavior data. The author took part in the biochemical analysis of plasma and brain samples. The author evaluated and visualized the results. The author wrote the bulk of the manuscript and submitted it as the corresponding author.

Paper III

The author took part in the planning of the experiment and the berry mixture production. The author took part in the experimental diets’ preparation and conducted the behavior tests. The author analyzed the behavior test data and conducted the majority of plasma metabolic markers analysis. The author visualized the data and wrote the bulk of the manuscript.

Paper IV

The author planned the experiments with the help of Roya R. R. Sardari. The author performed the experiments. The author collected, evaluated, interpreted, and visualized the data. The author wrote the manuscript and submitted it as the corresponding author.

Papers not included in the thesis

Paper I

Huang, F., * Nilholm, C., * Roth, B., Linninge, C., Höglund, P., Nyman, M., & Ohlsson, B. (2018). Anthropometric and metabolic improvements in human type 2 diabetes after the introduction of an Okinawan-based Nordic diet are not associated with changes in microbial diversity or SCFA concentrations. International Journal of Food Sciences and Nutrition, 69:6, 729-740, DOI: 10.1080/09637486.2017.1408059 (*=equal contribution)

Paper II

Gondo, T. F., **Huang, F**., Maruangruang, N., Heyman-Lindén, L., & Turner, C. Extractability and degradation of antioxidant compounds in berry smoothie beverage. (Manuscript)

Abbreviations

ACNs	Anthocyanins
AD	Alzheimer's disease
ApoE–/–	Apolipoprotein E
AUC	Area under the curve
AX	Arabinoxylan
BDNF	Brain-derived neurotrophic factor
CVD	Cardiovascular disease
DCX	Doublecortin
DG	Dentate gyrus
g	General traditional intelligence
G*c*	Crystallized intelligence
GCL	Granule cell layer
G*f*	Fluid intelligence
GI	Gastrointestinal tract
GLP-1	Glucagon-like peptide-1
dwb	Dry weight basis
HDL	High-density lipoprotein
HF	High fat
HF+Berry	High fat diet supplemented with berry mixture
IL	Interleukin
insLB	Insoluble fraction of lingonberries
LBP	Lipopolysaccharide binding protein
LF	Low fat
MAPK	Mitogen-activated protein kinase
MCI	Mild cognitive function impairment
MetS	Metabolic syndrome
NF-κB	Nuclear factor-kappa B
NGF	Nerve growth factor

NLR	Novel location recognition
NOR	Novel object recognition
OD	Optical density
OGTT	Oral glucose tolerance test
OH	Hydroxyl radical
OTUs	Operational taxonomic units
OxLDL	Oxidized low-density lipoprotein
PYY	Peptide YY
PYX	Peptone yeast xylose
PYG	Peptone yeast glucose
ROS	Reactive oxygen species
SAA	Serum amyloid A
SCFAs	Short chain fatty acids
solLB	Soluble fiber fraction of lingonberry
STR	Stirred tank bioreactor
TNF-α	Tumor necrosis factor-alpha
TGF- β	Transforming growth factor-β
wLB	Whole lingonberries
w/w	Weight for weight
16S rRNA	16S ribosomal RNA

1 Introduction

The aging population is a growing concern in many parts of the world. While it represents a remarkable feat for society, it also poses a challenge as the elderly face an increasing risk of developing health problems and conditions. In addition, modern dietary habits, resulting from the globalization and industrialization of food supply, are linked to increasing consumption of high-fat (HF) foods [1], which have been associated with various health issues, including metabolic syndromes and cognitive impairment. As a result, it is important to explore dietary approaches that facilitate a healthy lifestyle, with a particular emphasis on promoting healthy aging and brain function.

An estimated 15% to 20% of people aged 65 or older have mild cognitive impairment (MCI) [2]. The causes of MCI are not yet completely understood; however, it is believed that MCI precedes the dementia stage. To date, there is no effective medical cure for cognitive function decline, and it is therefore important to apply prevention strategies such as dietary guidance and physical activities for patients with MCI before the onset of neurodegenerative diseases.

There is growing evidence that polyphenols, a group of compounds found in fruits and berries, may improve cognition and reverse age-related declines in memory and learning in mammals [3]. Berries are particularly rich in polyphenols, especially anthocyanins, and have been shown to promote health benefits, including improved cardiovascular, neuroprotective, and brain function in humans and animals [4-12]. In addition to polyphenols, berries are also a good source of dietary fiber, both soluble and insoluble, which has been linked to improved metabolic function, colonic health, and the prevention of cardiovascular disease, as well as delayed cognitive decline [13-16].

Dietary fiber represents the macromolecules in the diet that resist digestion by human endogenous enzymes. Gut microbiota plays a specific mediator role in metabolizing dietary fiber through anaerobic fermentation, and exerts potentially beneficial effects [17]. For example, a higher prevalence of *Prevotella* has been reported in non-Western populations that typically exhibit diets rich in fruits and vegetables [18]. Among several *Prevotella* species that inhabit the human gut, *Prevotella copri* is the most abundant species and is associated with improved glucose and insulin tolerance in subjects who have received fiber-rich diets [19], as well as exerting antidiabetic effects [20]. Studies on *P. copri* have attracted increased attention in recent years; however, since the bacteria strain was first isolated from human feces in Japan [21], knowledge remains limited regarding cultivation techniques, growth, and bacterial phenotypic characterization. A deeper understanding of certain bacteria in the context of interacting with both the host and other microbiomes in the environment requires a fundamental understanding and increased knowledge of the characteristics of the bacteria of interest.

Bearing in mind the current situation of an aging population and the increasing prevalence of cognitive decline in the global context, it is important to discuss efficient dietary supplementation, including berries, to help protect against the brain aging, and to lower the risk of MCI development and other metabolic disorders. Such measures require further evaluation of different berry species regarding their potential to influence cognitive function and modulate gut microbiota composition in relation to certain types of beneficial bacterial strains.

Nordic environments are abundant in understudied berries. Some berries, such as bilberries, have been shown to have much higher total anthocyanin content than cultivated American blueberries [22]. Nordic wild berries remain largely unexplored, and currently, there is a lack of preclinical studies charactering these berries, particularly their effect on memory and cognitive function.

This work explores the effects of Nordic berries on cognitive function, metabolic syndrome and gut microbiota composition in HF diet fed mice. Furthermore, a potential probiotic bacteria strain, *Prevotella copri* DSM 18205^{T}, is characterized by its growth profile. The generated knowledge can contribute to the development of potential applications in future research.

2 Background

This chapter introduces the main topics and concepts of the thesis. This collection of topics revolves around the basic concept of cognitive function, its assessment in animal models, and numerous factors that can impact cognitive function. Specifically, the topics cover the general biological changes in the aging brain, hippocampal function in spatial memory, the role of neuroinflammation, obesity, and metabolic syndrome in cognitive function, potential cognitive benefits of Nordic berries, and the influence of gut microbiota and the blood-brain barrier. The role of specific bacteria, such as *Prevotella copri*, and the potential benefits of symbiotic relationships are also discussed. By exploring these topics, we can better understand the complex factors that impact cognitive function and potential interventions that can improve it.

2.1 Cognition overview

Human cognitive function encompasses various mental abilities, including perception, memory, learning, attention, decision-making, and language [23]. These processes allow us to acquire and manipulate knowledge, reason, and make decisions.

Psychometric and neurocognitive methods are commonly used to define and measure cognitive function. The psychometric approach uses cognitive tests to identify constructs such as general intelligence (g), fluid intelligence (Gf), and crystallized intelligence (Gc) (Figure 1) [24, 25]. The neurocognitive approach focuses on brain-behavioral relationships, which allows researchers to investigate how different brain regions are involved in cognitive processes such as attention, memory, language, and decision-making [26]. This type of evaluation is commonly seen in cognitive and behavioral assessment in both humans and rodents [27-29].

In addition to the two assessment methods (the psychometric approach and the neurocognitive method) mentioned above, Park has described four mechanisms of cognitive aging [30], and these mechanisms include: 1) the rate of perceptual speed, which affects the speed of cognitive processing; 2) working memory function; 3) inhibitory function; and 4) sensory function.

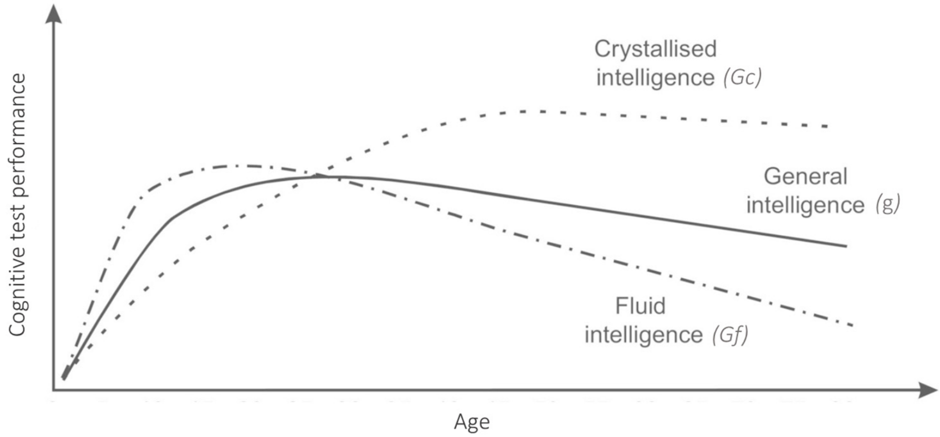


Figure 1 Development of fluid and crystallised intelligence according to the model of Cattell. General intelligence (g) is the foundation of all cognitive abilities. Fluid cognitive abilities (*Gf*) refers to reasoning or thinking, processing speeds, and one's ability to solve problems in novel situations, independent of acquired knowledge. Crystallized cognitive abilities (*Gc*) refers to "acquired knowledge," which includes accumulating lifetime intellectual knowledge and achievements. Source for figure: Modified from Elsevier Books, chapter 2 of Theories of Cognitive Aging and Work, Fisher, G.G., M. Chacon, and D.S. Chaffee, p. 17-45. Copyright (2019), with permission from Elsevier.

Perceptual speed is one of the main elements of the cognitive process, and is essential for all cognitive tasks. It can be defined as the time it takes a subject to complete a task or a previously learned task, and is usually related to the speed at which a subject can understand and react to the information they receive. Working memory is a cognitive system responsible for processing and temporarily storing information during complex cognitive tasks, such as comprehension, learning, and reasoning [30]. The inhibition function refers to age-related differences in how well individuals filter out irrelevant information that can distract from focused attention on relevant information [31], which can be crucial for an effective memory system. The last of these four mechanisms are sensory function, including visual and auditory acuity [24].

This thesis provides a general concept of cognitive functions, various methods for evaluating the cognitive function in rodents, and the underlying mechanisms of age-related cognitive decline.

2.2 Aging brain

As people age, various changes occur in the brain that can affect its structure and function. These changes can contribute to a decline in cognitive abilities and an increased risk of certain neurological and psychiatric conditions.

It has been widely found that the volume of the brain and/or its weight declines with age at a rate of around 5% per decade after the age of 40 [32]. The rate of decline may increase, especially after the age of 70 [33]. There are multiple factors believed to cause this phenomenon, including the loss of neurons and reduced neurogenesis. Additionally, in healthy aging, reduced synaptic plasticity and synaptic loss precede neuronal death is associated with cognitive decline and can lead to memory loss. These changes involve a decrease in postsynaptic response to neurotransmitters and a reduction in neuronal excitability [34].

During nonpathological aging, the chemical makeup of the brain may also change. Neurotransmitter levels, which are essential for facilitating communication between brain cells, may be reduced in older adults. For example, dopamine and serotonin, which play vital roles in mood and motivation, may decline in quantity [35]. Reduced blood flow to the brain is another common occurrence in aging individuals, which may interfere with the delivery of oxygen and nutrients to brain cells and can result in cognitive decline and an elevated risk of dementia [36].

According to previous studies, brain aging may also show hallmarks in other structure such as mitochondrial; it has been found that chronic positive energy balance can accelerate brain aging and contribute to the development of neurodegenerative diseases through mitochondrial dysfunction, the accumulation of neurotoxic proteins, and inflammation in the nervous system [37].

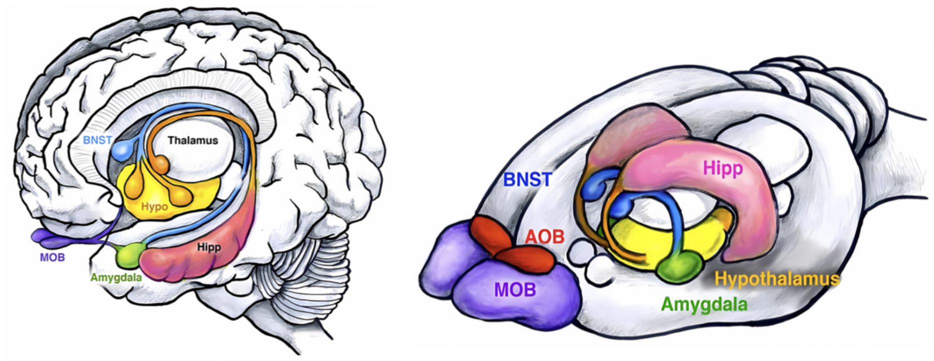
'Mild cognitive function' is a term that was introduced in the 1980s to describe individuals who showed memory loss or other cognitive impairments, but did not meet the criteria for dementia [38]. Mild cognitive impairment (MCI) is considered a cognitive stage between the normal decline associated with aging and the more severe impairment seen in dementia. People with MCI have a greater degree of cognitive impairment than expected for their age and education, but can still perform daily activities [39]. During the normal aging process, it is common to experience a decline in the ability to learn new information, slower mental processing, slower speed of performance, and an increased tendency to be distracted. However, MCI is distinguished from normal aging by its impact on recognition, intelligence, and long-term memory, which can be assessed through clinical tests and a review of the person's medical history [40]. Some people with MCI may experience only minor memory decline that does not worsen over time, while others may develop neurodegenerative conditions such as Alzheimer's disease (AD).

The causes of the development of MCI and AD remain unclear, but increasing age is one of the greatest risk factors for MCI prevalence and the onset of AD. An estimated 40% to 60% of individuals aged 58 years and older with MCI have underlying AD pathology [41]. Most of the individuals with AD are 65 or older, and the incidence of the disease at the age of 80 is twenty-fold that at the age of 60 [42]. Meanwhile, global life expectancy has increased by more than six years between 2000 and 2019, from 66.8 years in 2000 to 73.4 years in 2019 [43].

Thus, it is vital to investigate which preventative or treatment strategies might slow down cognitive decline in a growing population of the elderly.

2.3 Hippocampus and spatial memory

The hippocampus, a small, seahorse-shaped structure located in the medial temporal lobe of the brain, is considered one of the most important structures in the brain for learning and spatial memory [44] (Figure 2).



(a) (b)

Figure 2. Main structures of the human and rodent limbic system.
(a) Human brain showing the hippocampus (pink), bed nucleus of stria terminalis (BNST, blue), hypothalamus (yellow), amygdala (green), and olfactory bulbs (MOB, purple). (b) Similar structures are found in rodents. Rodents have enlarged olfactory bulbs compared to humans, and additional accessory olfactory bulbs (AOB, red) that are not present in humans. Source for illustration: Modified from Sokolowski K and Corbin JG (2012). Wired for behaviors: from development to function of innate limbic system circuitry. Front. Mol. Neurosci. 5:55. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://www.creativecommons.org/licenses/by-nc/3.0/).

Neurons in the hippocampus, known as place cells, fire in response to specific locations within an environment. As an individual moves through the environment, different cells fire, creating a unique pattern of activity that represents the individual's current location. This activity is thought to be critical for forming spatial memories, as the unique pattern of place cell firing can be replayed during subsequent recall, allowing individuals to remember the location of objects and landmarks within their environment [45]. The hippocampus also receives input from other brain regions involved in sensory processing, such as the visual and auditory cortex, which allows it to integrate spatial information with other sensory cues [46].

The hippocampus is made up of several subregions, including the dentate gyrus [47], CA1, CA2, and CA3, each of which plays a role in memory formation and retrieval. Previous research has demonstrated that the DG can acts as preprocessor of incoming information, preparing it for subsequent processing in CA3 [48], whereas the CA1 subregions provide the dominant outflow of the hippocampal circuit [49, 50]. In addition, doublecortin (DCX) is a microtubule-associated protein expressed by neuronal cells and immature neurons in the brain and it is thought to be involved in forming new neurons in the brain. Specifically, the level of DCX in the DG region of the hippocampus can be used as a neurogenesis marker associated with spatial memory.

However, as we age, the hippocampus may undergo structural and functional changes that can impair its ability to form and retrieve spatial memories.

2.4 Behavioral testing in animals

Despite being different species, rodents are shown to have similar patterns in disease pathogenesis and organ and systemic physiology compared to humans [51]. The use of rodent models, specifically mice, for evaluating behavior has developed over decades [52-54]. The behavioral characteristics of mice are complex and can differ significantly based on factors such as genetics, gender, and interaction with the environment, adding to the complexity of the study [54-56].

Assessing behavior and cognition in animal models is important when investigating the underlying mechanisms of brain aging and neurodegenerative diseases. While in vitro models can provide some insights, the complex interactions of neural systems that contribute to cognitive function are better studied in more complex animal models.

Cognitive tests are a widely used method for evaluating brain function in rodents, including the Y-maze, the T-maze, the Water Morris test, the Barnes maze, the fear conditioning test, the novel object recognition (NOR) test, and the novel localization recognition (NLR) test [57-61]. These tests assess cognitive abilities such as learning, memory, attention, problem-solving, and decision-making. By utilizing specific cognitive tests, researchers can gain valuable insights into the function of various brain regions and systems involved in cognitive processes and the impact of genetic, environmental, and pathological factors. For instance, the Barnes maze is a commonly employed test to evaluate spatial learning and memory in mice [27].

Wild-type mice models such as C57BL strains have been proven to help age research [62, 63] and studying nutritional strategies to combat cognitive impairment [64-66]. In addition, genetically manipulated models such as atherosclerosis-prone apolipoprotein E knock-out (ApoE−/−) mice are increasingly used in developing models for aging-related complication research [66].

The Methods and strategies section will further explain the animal model selection.

2.5 Neuroinflammation

Neuroinflammation refers to the inflammation of the nervous system, which includes the brain, the spinal cord, and nerves [67]. It is a complex process involving the activation of immune cells and the release of inflammatory mediators in response to various stimuli, including infection, injury, and neurodegenerative disease [68, 69]. While acute neuroinflammation can be beneficial, as it helps to remove damaged tissue and stimulate repair processes, chronic neuroinflammation has been linked to a range of neurological disorders, including mild cognitive decline and various neurological diseases such as AD and Parkinson's disease [68, 69]. There is growing evidence that neuroinflammation has played a key role in developing and progressing these conditions or diseases, and that targeting neuroinflammatory pathways may be a potential therapeutic strategy for treating these conditions or diseases.

The exact mechanisms by which neuroinflammation contributes to cognitive decline are not fully understood, but several theories have been proposed.

One theory is that neuroinflammation is associated with producing proinflammatory cytokines, including tumor necrosis factor α (TNFα) and interleukin (IL)-1β [70, 71]. Excessive production of these proinflammatory cytokines has been linked to the development of neurological disorders and cognitive decline. These cytokines may also contribute to neuron cell death, which can lead to a further decline in cognitive function [68].

Neuroinflammation may also contribute to the development of amyloid plaques and tau tangles, which are hallmark features of AD and other neurodegenerative disorders. These plaques and tangles are believed to disrupt communication between brain cells and contribute to the decline in cognitive function [72-74].

It is believed that inflammation plays a role in the development and progression of various neurological disorders and the decline in cognitive function that can occur with aging. More research is needed to understand the complex relationship between neuroinflammation and neurological disorders, and to determine the most effective methods for targeting neuroinflammatory pathways.

2.6 Obesity and metabolic syndrome

Obesity is a major global health issue that affects people of all ages, and is characterized by excess body fat. According to the World Health Organization, obesity has more than doubled since 1980, with over 1.9 billion adults aged 18 years and older being overweight in 2019 [47]. The prevalence of obesity varies widely among countries and regions, with higher rates generally seen in high-income countries and urban settings; however, obesity is also a significant problem in many low- and middle-income countries, where it is often accompanied by undernutrition [75].

Obesity is a complex condition influenced by several factors, including genetics, environment, and lifestyle [76]. It occurs when a person consumes more calories than they burn, and the excess energy is stored as fat. Over time, this excess fat can accumulate to unhealthy levels, leading to obesity. Obesity in humans is defined as a body mass index (BMI) greater than 30 kg/m^2 or a waist circumference over 102 cm for men and over 88 cm for women. These measures are strong predictors of insulin resistance and metabolic dysfunction [77].

Obesity is a major risk factor for several chronic diseases, including type 2 diabetes (T2D) [78], heart disease [78], and stroke [79]. These diseases are associated with a higher risk of premature death. In addition, obesity can be associated with other health problems such as dementia [80].

Metabolic syndrome is a cluster of metabolic risk factors that increase the risk of developing cardiovascular disease (CVD) and T2D. These risk factors include hypertension, hyperglycemia, dyslipidemia, and obesity. Hyperglycemia, or elevated blood sugar levels, is a characteristic of insulin resistance or glucose intolerance. Dyslipidemia can be characterized by low levels of high-density lipoprotein (HDL) cholesterol, the ‘good’ cholesterol, or high triglycerides.

Obesity and metabolic syndrome can be associated with cognitive decline for several reasons. One of the main mechanisms by which obesity and metabolic syndrome may affect cognitive function is inflammation and oxidative stress, which can contribute to developing brain inflammation (neuroinflammation) [81, 82], accumulation of fat deposits in the brain, and the alteration of neurotransmitter systems [82-84]. Obesity and metabolic syndrome are associated with an increased risk of cardiovascular disease [84], which can lead to reduced blood flow to the brain and potentially impair cognitive function.

Previous studies have used HF feeding to induce cognitive deficits in rodents to model human cognitive impairment [64, 65, 85]. A polyphenol-rich diet may counter the HF intake associated with memory decline; for example, raspberries, blueberries, and Indian gooseberries have been studied in this context, and have been shown to alleviate HF-induced cognitive impairment [64, 85-87].

2.7 Potential beneficial components in berries

Dietary fiber is a type of carbohydrate that is found in plant-based foods and is not digested by the body. Dietary fiber can be classified based on its chemical composition, source, solubility, fermentability, and physiological effects. A common classification method is to divide it into two groups: soluble and insoluble, based on their ability to dissolve in water. Soluble fibers, often broken down by gut bacteria, include pectin, fructan, mixed linkage glucans (gums), and some types of resistant starch [88]. Insoluble fibers, which are less fermentable, include cellulose, some other types of beta-glycans, and lignin [89]. Soluble fiber can be further divided into viscous (such as pectin and galactomannan) and non-viscous (such as fructo-oligosaccharides and inulin), based on viscosity. Fiber intake has been associated with various health benefits. Soluble and viscous fiber can generally decrease transit time in the digestive tract, slow glucose absorption, and lower cholesterol levels [90, 91]. Additionally, gut microbiota can ferment soluble fiber to produce short-chain fatty acids (SCFAs) that promote the growth and activity of beneficial bacteria. Insoluble fiber can help prevent constipation and promote regular bowel movements [89, 92]. Both types of fiber are essential for good health, and it is recommended to include a variety of high-fiber foods in the diet.

While dietary fiber has been shown to have potential benefits for health, it is just one component of a healthy and balanced diet. To maintain good health, it is crucial to incorporate a variety of food and nutrients, including other important compounds such as polyphenols.

Polyphenols are a group of plant compounds found in various foods, including berries, vegetables, nuts, and tea [93-95]. There are many different classes of polyphenols, including phenolics, flavonoids and non-flavonoids [96]. Flavonoids can be further categorized into subclasses such as anthoxantins and anthocyanins (ACNs) (Figure 3).

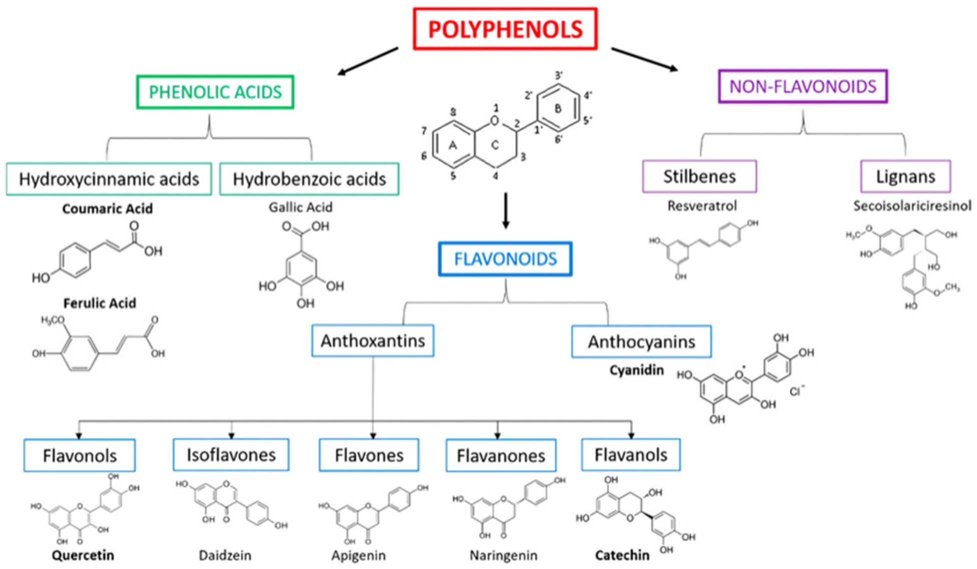
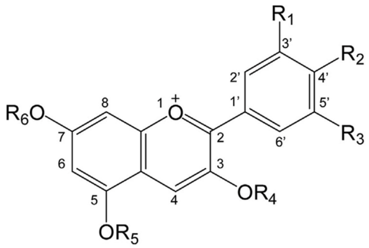


Figure 3. Schematic classification of polyphenols and examples of chemical structures.
Source for illustration: Beconcini, D., et al (2020) Antioxidant and Anti-Inflammatory Properties of Cherry Extract: Nanosystems-Based Strategies to Improve Endothelial Function and Intestinal Absorption. Foods, 9, 207. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by/4.0/)

ACNs are one of the compounds specially enriched in many berries. The most predominant ACNs compounds include pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin, found mainly in the external layer of the pericarp [97, 98]. ACNs are comprised of anthocyanidins and their glycosides, which differ in their position and number of hydroxyl groups, degree of methylation, type, and the number of sugar molecules (mono-, di- or tri-glycosides), type of sugars (such as glucose, galactose, and arabinose), and the type and number of aliphatic or aromatic acids (e.g., p-coumaric, caffeic, and ferulic acid) (Figure 4).



	Anthocyanins	R_1	R_2	R_3	R_4	R_5	R_6
1	Pelargonidin 3-*O*-glucoside	H	OH	H	Glc	H	H
2	Cyanidin 3-*O*-glucoside	OH	OH	H	Glc	H	H
3	Peonidin 3-*O*-glucoside	OMe	OH	H	Glc	H	H
4	Malvidin 3-*O*-glucosdie	OMe	OH	OMe	Glc	H	H
5	Cyanidin 3-*O*-galactoside	OH	OH	H	Gal	H	H
6	Malvidin-3-*O*-(6-*O*-acetyl-glucoside)	OMe	OH	OMe	A-Glc	H	H

Glc: glucosyl unit; Me: methyl; Gal: galactosyl unit; A-Glc: 6-*O*-acetyl-glucosyl unit.

Figure 4 The structure of ACNs.
Structural modifications, including glycosylation, hydroxylation, methylation, and acylation, can occur at the C3' (R1), C4' (R2), C5' (R3), C3 (R4), C5 (R5), and C7 (R6) positions. R1-R5 are functional groups derived from glycosyl, hydroxyl, methyl, and acyl units. Representative ACNs and their structures are listed. Source for illustration: Jian Zha and Mattheos A.G. Koffas (2017) Production of ACNs in Metabolically Engineered Microorganisms: Current status and perspectives. Synthetic and systems biotechnology, Vol 2(4). This is an open-access article distributed under the terms and conditions of CC BY-NC-ND 4.0 license (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Among the various types of berries, bilberries, elderberries, blueberries, açai berries, and blackcurrants stand out as particularly rich sources of ACNs [99-103], which are responsible for the dark blue-purple color. ACNs are shown to be associated with several potential health benefits, including anti-neuroinflammatory properties, and attenuate certain disorders, such as diabetes, and cardiovascular and neurological pathologies [92, 104, 105]. Flavonols are also found in berries and are known for their ability to help improve blood flow and lower blood pressure [106].

Emerging evidence in human clinical studies has also shown that diets rich in polyphenols may have beneficial effects on cognition, decrease the rate of cognitive decline seen with aging, and help delay the onset of cognitive symptoms [107].

2.8 Berries

Forests cover up to 69% of Sweden's surface area [108]. The rich forest resource and unique geographic characteristics make Sweden one of the largest producers of wild berries in Europe, with a total biological yield estimated at more than 550 000 tons, mainly bilberries, lingonberries, and cloudberries. Despite this abundant yield, only a small percentage of these berries are currently utilized, suggesting potential for the wild berry industry to grow [109].

Bilberries (*Vaccinium myrtillus L*) – sometimes called European blueberries – are one of the most abundant berry species to be found in the wild. The Swedish word for bilberry is *blåbär*. This literally translates as 'blueberry' in English, and can sometimes be confused with the North American blueberry (*Vaccinium sect. Cyanococcus*) which can be cultivated and is relatively more common and commercialized in global markets. The bilberry is a species that is botanically closely related to the North American blueberry, and the two are superficially similar. However, there is a noticeable difference that distinguishes the two: bilberries have much higher anthocyanin content and have burgundy-red flesh and dark purple juice, whereas the American blueberries have translucent flesh.

Lingonberry (*Vaccinium vitis-idaea,* or *lingon* in Swedish) is another common wild berry species found throughout Northern Europe; it is also known as mountain cranberry or cowberry [110]. These vibrant red berries, commonly used in traditional Swedish cuisine, offer a combination of bitterness and sweetness.

Nordic countries are also abundant with several other less-studied berries. Cloudberry (*Rubus chamaemorus)* is a small, perennial plant that belongs to the Rubus family. It is native to the cooler regions of the Northern hemisphere, including parts of Europe, Asia, and North America. Cloudberry is a soft, orange-yellow fruit with antioxidants and other bioactive compounds, including polyphenols, flavonoids, and carotenoids [111]. Sea buckthorn (*Hippophae rhamnoides*) is known for its orange berries, which are high in flavonoids (quercetin, isorhamnetin, catechin) [112]. Blackcurrant (*Ribes nigrum)* is a flowering plant in the Grossulariaceae family, and is native to Europe and parts of Asia. It is a shrub that produces small, edible berries, typically black or deep purple.

The health properties of berries have attracted Increased attention and research In recent decades.

Previous research has demonstrated that berries can positively impact metabolic function. Several preclinical studies have shown that the aqueous extract of Brazilian Jaboticaba berry (*Myrciaria jaboticaba*) can reduce obesity, improve insulin resistance, and lower plasma glucose level [113-116]. A more recent study by Fotschki et al. [117] found that raspberry polyphenols reduced the weight of epididymal white adipose tissue and hepatic triglyceride content. In another study on the water extract of Indian gooseberry, different doses of the berry extracts were administered in rats daily for 112 days; the cholesterol level was slightly increased in the HF control group and was significantly reduced by the Indian gooseberry extract [86]. Whole lingonberries (as doses of 20% w/w or more) were shown to prevent HF-induced weight gain, adiposity, and elevated glucose levels in C57BL/6J mice [118, 119]. In addition, lingonberries were also shown to counteract the adverse effects of the HF diet feeding on atherosclerosis in ApoE–/– mice [66, 120]. Other less known berries – such as bilberry, with its high levels of ACNs – were also shown to have beneficial effects associated with reduced inflammatory level, reduced blood pressure, and improvement of brain function in mice models [121-123]. There is also a growing body of clinical research that has suggested the beneficial effect of berries on metabolic health, such as blueberries [11, 124, 125].

Moreover, several different types of berries have been associated with improved cognitive performance, including commonly consumed berries like American highbush blueberries (*Vaccinium ashei, Vaccinium virgatum, or Vaccinium corymbosum*) [11, 85, 126], lowbush wild blueberries (*Vaccinium angustifolium*) [10-12], raspberries [65] strawberries [8, 9] and red grapes [127, 128]. In clinical studies, a dietary interview study has shown that intakes of American blueberries and strawberries were associated with slower rates of cognitive decline in older women [129]. Furthermore, similar positive effects on cognition have been observed in *Vaccinium* species and grape varieties [127, 130-132]. Interestingly, one clinical trial also reported some positive effects on working memory in older healthy adults after five weeks of consuming a beverage containing six fruit and vegetable ingredients, including lingonberries and blueberries [133]. Another recent systematic review covering several acute intervention studies concluded that the results across the studies were inconsistent, which made it difficult to generalize about the neuroprotective effects after berry intake [134].

In this thesis, several edible wild Nordic berry species are studied for the first time in terms of their effects on cognitive function. The mechanisms by which these berries may improve cognitive function are also explored, adding to the current understanding of how different foods can influence brain function.

2.9 Gut microbiota and blood-brain barrier

The human body is home to millions of bacteria, which can be found on various parts of the body, such as the skin and the mucous membranes that extend from the mouth to the rectum and in the lumen of the digestive tract. These resident microbes begin in the mouth, and are continuously transported through the gastrointestinal (GI) channel by the swallowing reflex [135].

The number of bacteria decreases as they pass through the stomach, where the low pH reduces the bacterial concentration to around 10^3 colony-forming units (CFU) per gram of gastric juice. The bacterial count remains low in the duodenum and jejunum due to high intestinal motility and the flow of gastric juices, and the bacterial concentration increases drastically in the ileum and colon. In the ileum, there are approximately 10^7 bacteria per gram; this culminates in 10^{11} to 10^{12} cells per gram in the colon [135]. Such high concentrations of living bacteria are rarely found anywhere in nature outside the distal part of the GI tract of mammalians.

Approximately 10^{14} living organisms reside in the human gut; this ecosystem of bacteria, archaea, and eukarya is termed the 'gut microbiota' [136]. The gut microbiota has coevolved with the host over thousands of years to form a complex and mutually beneficial relationship. It represents more than 9 000 000 bacterial genes [137], while the human host represents about 23 000 genes [138].

Bacteria, as living organisms, are classified based on their physical and genetic characteristics, and this classification follows a hierarchical system of categories that go from general to specific. Taxonomically, bacteria are classified based on phyla, classes, orders, families, genera, and species. One example of the taxonomic classification of a specific bacterium, *Prevotella copri*, can be found in Table 1. This classification shows that *P. copri* belongs to the phylum Bacteroidetes, one of the largest and most diverse bacterial phyla [139]. It also belongs to the class Bacteroidia, a group of bacteria commonly found in the human gut.

Table 1 Taxonomic classification of *Prevotella copri*

Phylum	Bacteroidota
Class	Bacteroidia
Order	Bacteroidales
Family	*Prevotellaceae*
Genus	*Prevotella*
Species	*Prevotella copri*
Full scientific name	*Prevotella copri* (Hayashi et al. 2007)

In humans, the dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with the two phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota [135]. The Firmicutes phylum comprises over 200 genera, such as Lactobacillus, Bacillus, Clostridium, Enterococcus, and Ruminicoccus. *Clostridium genera* represents 95% of the Firmicutes phyla. Bacteroidetes consist of predominant genera such as *Bacteroides* and *Prevotella*. The Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* genus [135]. The gut microbiota is shown to be associated with several aspects of human health, including resistance to pathogens [140], the intestinal epithelium function [141], metabolizing dietary and pharmaceutical compounds [141, 142], and controlling immune function [143], and even influence the host's behavior and mood through the so-called 'gut microbiota–brain axis' [144, 145]. The gut microbiota–brain axis refers to the biological systems allowing bidirectional interaction between gut bacteria and the brain [144]. This interaction involves various pathways within the chemical, neuronal, and immunological systems, as well as the enteric nervous system (ENS) and the vagus nerve, the neuroendocrine system, and the hypothalamic-pituitary-adrenal (HPA) axis (Figure 5).

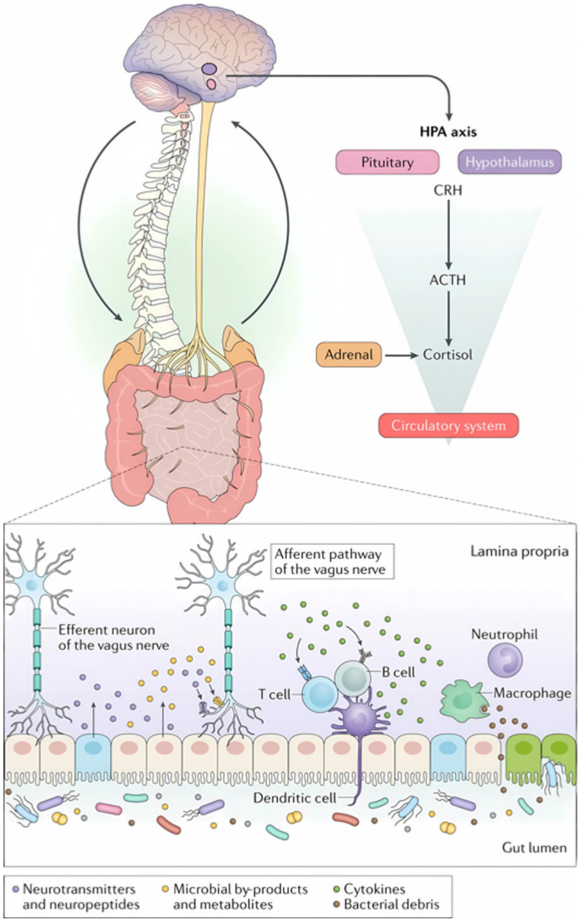


Figure 5 Illustration of gut microbiota-brain axis pathways via chemical, neuronal and immunological systems.
Within the nervous system, stress can activate the HPA axis response that involves neurons of the hypothalamus that secrete hormones such as corticotropin receptor hormone (CRH) into the brain or the portal circulation, triggering the release of adrenocorticotrophic hormone (ACTH), which then initiates the synthesis and release of cortisol. Source for illustration: Morais, L.H., et al., The gut microbiota–brain axis in behaviour and brain disorders, Copyright (2021), with permission from Springer Nature.

The blood-brain barrier (BBB) is a critical component of the gut microbiota–brain axis, as it functions as a physiological barrier that separates the central nervous system (CNS) from the bloodstream [146]. The BBB comprises a specialized layer of brain capillary endothelial cells, connected by tight junctions and supported by an underlying basement membrane, which creates a selective permeability barrier. This barrier restricts the passive diffusion of substances from the bloodstream into the CNS while allowing the transport of essential nutrients and other substances necessary for proper brain function [146]. Disruptions to the BBB have been implicated in various disorders or diseases, such as neuroinflammation, metabolic disorder, cognitive decline, and brain tumor [147-150].

Gut microbes produce a variety of signaling molecules, such as neurotransmitters and hormones, that can impact brain function and behavior via the gut microbiota–brain axis, and the connection between gut microbiota and the BBB highlights the importance of maintaining a healthy gut microbiome for proper brain function and the prevention of neurological disorders. Dysbiosis of the gut microbiota has been shown to be associated with different metabolic dysfunction [151]. In addition, increasing evidence also suggests a role of the gut microbiota in neurodegenerative disorders such as AD [66].

Understanding the gut microbiota's composition is crucial, as it can offer valuable insights into the gut's health and functioning, allowing for the identification of imbalances or abnormalities. This information can help design targeted interventions to restore balance to the gut microbiota, which can significantly enhance health outcomes.

2.10 *Prevotella copri*

The term 'probiotics' refers to a group of beneficial bacteria that interact not only with the host body but also with the complex community of bacteria in the gut microbiota, which occupies a unique ecological niche in the GI tract. A more recent definition of probiotics describes them as living microorganisms that have been scientifically proven to provide health benefits when administered to the host [152].

Maintaining a balanced gut microbiota community and promoting the growth of beneficial bacteria can be achieved through various methods. One approach is to take probiotics, which are living microorganisms that can help to alter the balance between bacteria in the GI tract in a way that supports human health (Figure 6) [153]. Another way to promote beneficial bacteria in the GI tract is through dietary modulation, such as consuming dietary fiber that certain gut microbiota species can ferment to produce SCFAs – a byproduct that linked to several health aspects [154]. The use of both prebiotics (such as dietary fiber) and probiotics to improve gut health and enhance host health is called 'synbiotic' and can contribute to overall well-being and protect against certain metabolic [155-157] and neurodegenerative conditions [158, 159]. This synbiotic effect may modulate gut microbiota-derived metabolites and cellular components, improving brain homeostasis and reducing the risk of neuropsychological problems (Figure 6) [153].

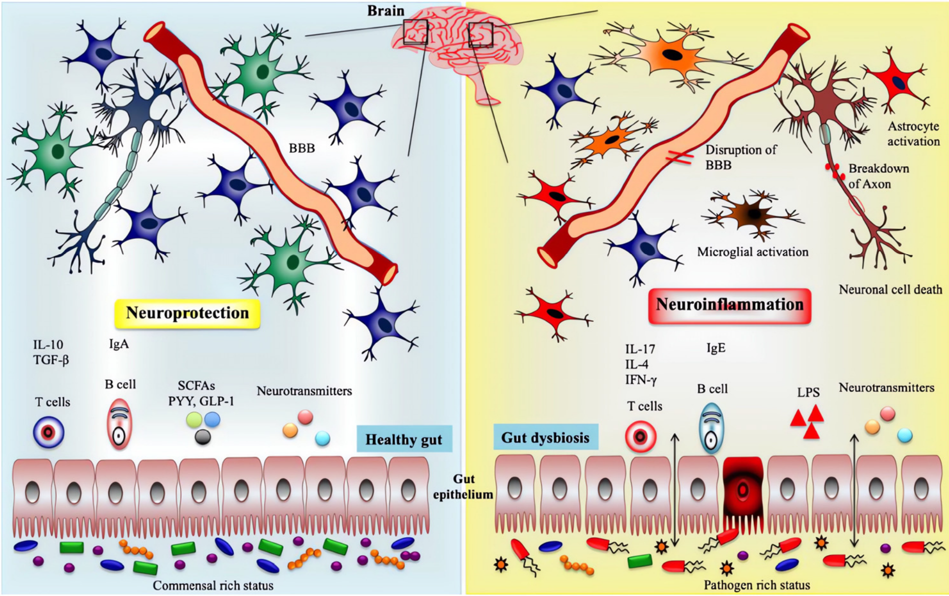


Figure 6 Influence of immune and metabolic regulation by the gut microbiota during the healthy and gut dysbiosis state via gut–brain axis.
Gut microbiota can promote the production of short chain fatty acids (SCFAs), gut-derived peptides, neurotransmitters, and regulatory T and B cells through interactions with intestinal immune cells (left). Gut microbiota may counteract the inflammatory state during the dysbiosis condition (right). Peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and transforming growth factor-β (TGF-β). Source for illustration: modified from Suganya, K., et al. (2020), Gut-Brain Axis: Role of Gut Microbiota on Neurological Disorders and How Probiotics/Prebiotics Beneficially Modulate Microbial and Immune Pathways to Improve Brain Functions, Int J Mol Sci. 21(20):7551. This open-access article is distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Previous studies have shown that *Prevotella is* a common microbial genus in individuals with a plant-based diet (high fibers and low fats and proteins), while an increase in *Bacteroides* is common in individuals with an animal-based diet (low fiber and high fat and proteins). Growth of *Prevotella spp*. Has also been found to be promoted by barley supplementation in healthy individuals and co-occurred with improved glucose metabolism [19, 160]. This finding indicated that some species in the genus *Prevotella* might play a role in the dietary-induced improvement in glucose metabolism in observed individuals Moreover, one bacterial species in the genus, *P. copri*, was particularly associated with dietary fiber intake and considered a potential next-generation probiotic [19, 160-162].

The previous findings Implied the beneficial effect of *P. copri,* given that it is associated with a plant-rich diet; however, other studies have also shown that *P. copri* is linked with inflammatory conditions [163]. According to Fehlner-Peach and colleagues, *P. copri* lineage includes at least four distinct species-level lineages, and the metabolism of polysaccharides among *P. copri* isolates can vary significantly, indicating different metabolic patterns within this group [164]. These inconsistencies between traditional taxonomic classification, species boundaries, and strain-specific differences in metabolism and dietary preferences may explain the conflicting findings regarding the relationship between *P. copri* and human health. These studies emphasize the importance of characterizing each bacteria strain to understand its potential probiotic function.

The bacterial strain studied in this thesis was *Prevotella copri* DSM 18205^{T}. It was initially isolated from the feces of a healthy 52-year-old man in Japan. This strain is an anaerobic, mesophilic bacterium, meaning that it grows best at moderate temperatures, typically between 20°C and 45°C. This bacterium is gram-negative and appears pink or red with a characteristic elongated, cylindrical shape under a microscope.

Exploring the growth conditions and phenotypic characteristics of this bacterium is crucial to gain valuable insights into its behavior and response to various conditions. This information can aid in advancing research and enhancing our understanding of the potential benefits of the bacterium for human health.

3 Aim

The ultimate objective of this industrial PhD project is to investigate the effects of Nordic berries on cognitive function in mice fed a HF diet, assessed by a series of behavior tests. The studies also aim to explore other health effects of berry supplementation, such as the impact on levels of parameters relating to brain neurogenesis, metabolic function, inflammation, as well as changes in gut microbiota composition. In addition, a particular strain isolated from the gut microbiota, *Prevotella copri* DSM18205^{T}, is investigated to reveal the bacterial growth profile and optimal cultivation techniques.

In **Paper I**, the aim is to test how the whole lingonberries (wLB), the soluble fractions of lingonberries (solLB) and the insoluble fractions of lingonberries (insLB) would alter specific group(s) of the gut microbiota responsible for beneficial effects on metabolism and inflammatory status, as well as to examine their possible effects on brain function and behavior in mice. wLB and two fiber fractions (solLB, insLB) are extracted based on the water solubility of the dietary fiber and tested on ApoE−/−mice (8 weeks old) fed HF diets (38%E).

Several varieties of Nordic berries are studied in **Paper II**. This work explores the effects of Nordic berries on cognitive function, including bilberry, lingonberry, cloudberry, sea buckthorn, and blackcurrant. Several of these berries have not previously been investigated in vivo in terms of cognitive function and associated changes in gut microbiota composition. The study uses HF-fed (60E%) middle-aged C57BL/6J mice (24 weeks old) to model age- and lifestyle-induced cognitive impairment.

In **Paper III**, a mixture of two selected Nordic berry species – identified in earlier studies as having the potential to improve memory (**Paper II**) – is supplemented to C57BL/6J mice (8 weeks old) fed HF diet (60E%) to explore further the underlying mechanisms and beneficial effects of these berries on cognition function, as well as correlations with cardiometabolic parameters, brain markers, and gut microbiota composition.

In **Paper IV**, studies were performed to characterize the growth profile of *Prevotella copri* DSM 18205^{T} – isolated from the gut microflora. The aim of the work concerns the growth and propagation of the microorganism, as well as its metabolite production.

4 Study design and overview

Paper I.	
Aim	To investigate the effect of lingonberries on counteracting diet-induced metabolic disorders and gut microbiota composition and neuroinflammation
Model	ApoE–/– mice
Source and treatment	HF (38E%) diet; HF supplemented with lingonberries (26% dwb, wLB); HF supplemented with insoluble fiber fraction of lingonberries (20% dwb, insLB) and HF supplemented with soluble fiber fraction of lingonberries (13.6%, solLB)
Feeding period	8 weeks (wLB, insLB, and the respective HF control) 2 weeks (solLB, the respective HF control)
Cognitive test	T-maze
Analysis parameters	Markers of metabolic function and inflammation, hippocampal synaptic density
Microbiology	Gut microbiota composition, SCFAs
Paper II.	
Aim	To investigate the effect of different Nordic berries on cognitive function and associated changes in gut microbiota composition
Model	Middle-aged C57BL/6J
Source and treatment	HF (60E%) diet; HF diets supplemented with lingonberry, bilberry, cloudberry, blackcurrant, sea buckthorn and blueberry at 6% (dwb), respectively.
Feeding period	16 weeks
Cognitive test	T-maze, NOR test
Analysis parameters	Brain immunohistology, metabolic function, inflammatory makers
Microbiology	Gut microbiota composition
Paper III.	
Aim	To investigate the effects of a mixture of Nordic berries on cognition function as well as correlations with cardiometabolic parameters, brain markers, and gut microbiota composition
Model	Young C57BL/6J mice
Source and treatment	HF (60E%) diet; HF diet supplemented with a berry mixture (20% dwb, HF+Berry)
Feeding period	18 weeks

Cognitive test	T-maze, NOR test, Barnes maze
Analysis parameters	Levels of hippocampal neurogenesis and neuroinflammation, cardiometabolic risk markers
Microbiology	Gut microbiota composition
Paper IV.	
Aim	To investigate and characterize an aerobic bacterium – *Prevotella copri* DSM 18205^T isolated from the gut microflora
Source and treatment	*Prevotella copri* DSM 18205^T
Model	In vitro cultivation
Analysis parameters	Bacterial growth characterization
Microbiology	Fatty acids production

5 Methods and strategies

This section provides an overview of the methods used. For further details, please refer to the respective papers (**I- IV**).

5.1 Experimental diets

5.1.1 HF diet supplemented with berries

In preclinical studies, mouse-fed HF diets are used to study the effects of a dietary intervention on the development of obesity and metabolic syndromes that have a negative impact on cognition [134, 165, 166].

In **paper I**, each group was given a HF diet (38% kcal from fat, 38E%), including either wLB, insLB or solLB, or cellulose with the same level of dietary fiber in the diet. In **paper II**, a HF diet (60% calories from fat, 60%E) and a low-fat diet (10%E) were used for the control groups, and the berry diets were the HF diet supplemented with each of the berry powders at 6% dry weight basis (dwb). In **paper III**, the HF diets (60%E) were supplemented with the berry mixture at 20% dwb, and the control groups included the HF diet (60%E) and the standard chow diet (7.4%E, SDS, RM1).

5.1.2 Preparation of the berry diets

In **Paper I**, a powder milled from freeze-dried lingonberries was used. The insoluble and soluble fractions of lingonberries were separated based on the water solubility of the dietary fiber; both the insoluble fraction (insLB) and the soluble fraction (solLB) were freeze-dried and milled again. In **paper II**, five different Nordic berries – lingonberry (*Vaccinium vitis-idaea*), bilberry (*Vaccinium myrtillus*), cloudberry (*Rubus chamaemorus*), blackcurrant (*Ribes nigrum*), and sea buckthorn (*Hippophae rhamnoides*) – and American blueberry (*Vaccinium ashei*) were used in the study. The Nordic berries (except the blueberries) were freeze-dried, and then ground into fine powders (Figure 7). Blueberries were provided as freeze-dried powder in deoxygenated cans. In **paper III**, a mixture of lingonberry, bilberry, and red grape juice (*Vitis vinifera*) was mixed and prepared as a smoothie; this berry mixture was then freeze-dried. All the berry powders were flushed with nitrogen gas, vacuum-packed in bags with oxygen absorbers, and stored at room temperature until being incorporated into rodent diet pellets to limit the oxidation of potentially bioactive compounds.

In the next step, the berry powders were sent to ALS Scandinavia AB (Danderyd, Sweden) for macro-nutrient analysis to enable the calculation and formulation of the diets. In the last step of the diet preparation, berry powders were shipped to Research Diets, Inc. (New Brunswick, NJ, USA) to be incorporated into rodent pellets with the inclusion of essential macro- and micronutrients.

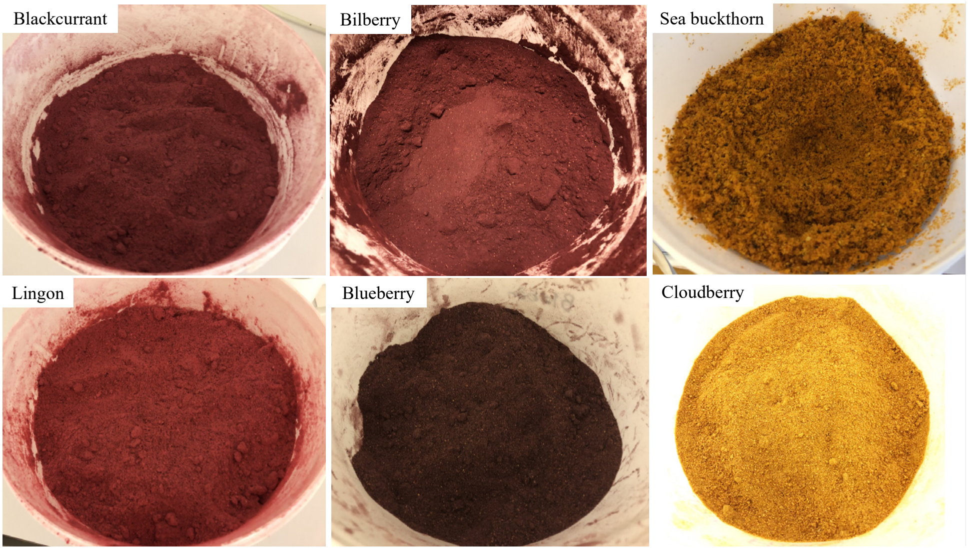


Figure 7 Freeze-dried berry powders used to produce the experimental diets (Paper II).

5.1.3 Quantification of carbohydrate composition of the berry mixture powder

A two-step sequential acid hydrolysis process was carried out to quantify the content of carbohydrates in the various berry fibers, as described previously [167, 168]. The monosaccharide contents of the freeze-dried berry mixture sample were analyzed using high-performance anion exchange chromatography (HPAEC) (ThermoFisher Scientific, Waltham, USA). The separation of the monosaccharides was carried out using a Dionex CarboPac PA-20 analytical column coupled to a Dionex CarboPac PA-20 guard column of the same material and detected using a pulsed amperometric detector. The standards employed in this method included arabinose, xylose, glucose, galactose, mannose, and fructose. The results were based on triplicate measurements and the values were shown as mean and standard deviation (SD).

5.2 Animal models and study design

The study in **paper I** used ApoE−/−mice (Taconic Biosciences Inc., Silkeborg, Denmark). The studies in **papers II and III** used C57BL/6J male mice (Janvier-Labs, Le Genest-Saint-Isle, France). Across **papers I-III**, male mice were consistently utilized.

The C57BL/6J mouse strain is a commonly used inbred mouse strain in research investigating diet-induced T2D and obesity, and impaired cognitive function. In addition, C57BL/6J mice have a relatively long lifespan compared to some other strains of mice, making C57BL/6J a valuable model for studying age-related changes and diseases [169]. Moreover, previous studies have shown that the C57BL/6J mouse strain exhibits an aging phenotype, which increases the relevance of findings to the human research [63]. There are several major sources for C57BL/6J substrains, but there are genetic and phenotypic differences among them. As a result, the same supplier for C57BL/6J has been used in both **paper II and paper III** to eliminate any potential vendor-specific effects.

ApoE−/− mice are a well-established model for studying human atherosclerosis, as the animals lack the gene for the apolipoprotein E protein, which leads to poor lipoprotein clearance and accumulation of cholesterol ester-enriched particles in the blood, ultimately promoting the development of atherosclerotic plaques [170]. ApoE is also an abundant lipid-transport protein in the central nervous system, and its absence in ApoE−/− mice also makes them useful models for neurodegenerative diseases [171, 172]. Additionally, ApoE−/− mice display age-dependent changes in lipid metabolism, brain function, and behavior, and exhibit features of AD, including amyloid deposition, oxidative stress, and neuroinflammation [171, 173].

In **paper I**, ApoE−/−mice were put on the experimental diets at eight weeks, and the mice were 16 weeks old at the end of the experiment, hence two months of the diet intervention. In **paper II**, the diet intervention started when the animals reached the age of 24 weeks, and the entire experimental diet feeding lasted four months. In **paper III**, C57BL/6J male mice aged eight weeks were fed the experimental diets for 18 weeks.

Animals and food were weighed, and the food was replaced every week. Calory intake was calculated and presented as the average consumption per mouse.

In **paper I**, the local ethical committee approved the study for animal experiments in Lund, Sweden (approval number M114-15). In **papers II and III**, the local animal experiment ethical review committee approved the studies in Lund, Sweden (approval number 5.8.18-13,983/2018).

5.3 Assessment of cognitive function

In **papers I, II, and III**, the effects of the berry diets on spatial and recognition memory in mice were assessed through behavior tests such as T-maze, NOR, and Barnes maze.

In **papers I-III**, behavior tests were conducted in a dedicated room designed for the behavior test purpose, visual cues on the walls to enable mice to orient. In **paper III**, an extra measure was taken to simulate night-time conditions − reducing the light density in the room to 45 lux during daytime testing, as this is when the animals are typically more active. This study implemented an assisting handling tool to minimize animal stress. A camera system using the Ethovision XT 14.0 software (Noldus Information Technology b.v., Wageningen, the Netherlands) was used for behavior monitoring and video data analysis.

5.3.1 T-maze

In **papers I and II**, the T-maze protocol was adapted from a previously published work by Deacon and Rawlins 2006 [174]. T-maze is a behavioral test measuring mice's willingness to explore new environments. Mice typically prefer to investigate a new arm of the maze rather than return to one that was previously visited [174]. Many parts of the brain, including the hippocampus, septum, basal forebrain, and prefrontal cortex, are involved in this task [175]. The alternation rate (%) was evaluated as the ratio of correct arm choices to the total number of trials. In **paper III**, a discrete-trial-without-a-reward protocol was adapted from a previous Y-maze protocol [58] (Figure 8). The correct first turn (%) was calculated as the percentage of the animals that have made a correct turn at their first choice of turning; frequency (%) was calculated as the visiting frequency to the correct arm in ratio to the total visits in both arms; and duration (%) was calculated as the time spent (seconds) in the correct arm in proportion to the total time spent (seconds) in both arms.

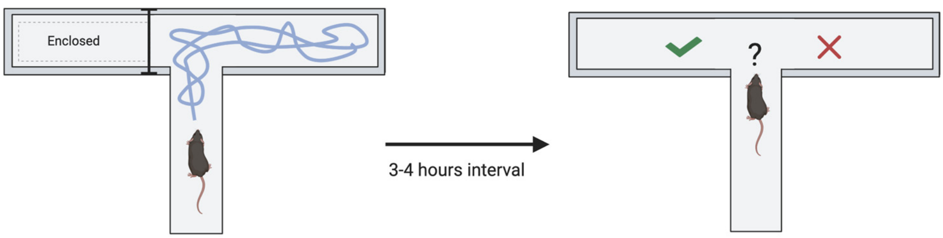


Figure 8 T-maze experimental scheme used in paper III.

5.3.2 NOR test

A NOR test was conducted in **papers II and III**, as previously described by [176], with minor modifications (Figure 9). This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The NOR objects in the **paper III** study were larger than those in **paper II**. In the test session, the visits and the time spent exploring the novel object in ratio to the total visits and the total time spent on both objects were calculated – frequency% and duration% to reveal the animal's preference for the novel object.

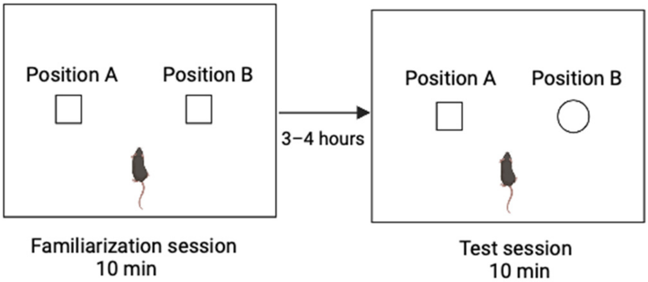


Figure 9 Novel object recognition (NOR) test experimental scheme used in papers II-III.

5.3.3 Barnes maze

The Barnes maze protocol previously described by Attar [177] was adapted to the **paper III** study. Each mouse performed two training trials per day for the following four consecutive days to learn the location of the escape box beneath one of the holes (Figure 10). The latency to find the escape hole or box during the training trials was recorded. A single probe trial was performed two days after the last training trial; the latency and the average distance of the animal to the target hole and the time spent in the target quadrant were measured.

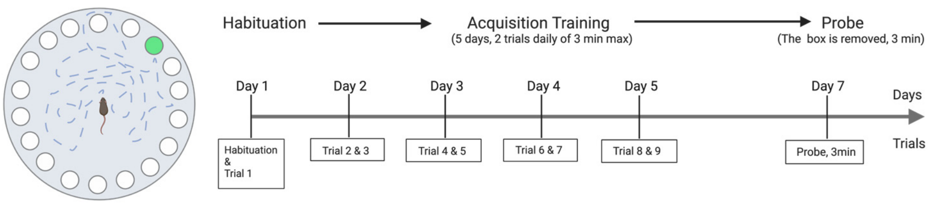


Figure 10 Barnes maze experimental scheme used in paper III.
The apparatus is a 100-cm diameter table with 20 holes lining the perimeter.

5.4 Biochemical analysis

In **papers I-III**, mice were first anesthetized using isoflurane, cardiac puncture was then carried out for blood sampling before termination. The brains were dissected, and the organs were weighed and collected on dry ice. All tissues were snap-frozen and subsequently stored at −80°C until further analyses. Plasma samples were analyzed for various metabolic and inflammatory markers. Brain samples underwent neurogenesis analysis in **papers II** and **III**. Cytokines levels and synaptic markers were measured in hippocampal homogenates in **paper III**.

5.5 Gut microbiota composition analysis

DNA from the cecal tissue and content was extracted. The 16S rRNA genes were amplified using forward and reverse primers containing Illumina overhang adaptors and unique dual indexes. Paired-end sequencing was read on a MiSeq instrument (Illumina Inc., San Diego, CA, USA). In **papers I and II**, sequencing data were analyzed using the open-source bioinformatics pipeline Quantitative Insights into Microbial Ecology (QIIME). Representative sequences (most abundant) from each operational taxonomic unit (OTU) were aligned using Python Nearest Alignment Space Termination (PyNAST); taxonomy was assigned using the Greengenes database (v.13.8). In **paper III**, the default taxonomic assignment of the detected amplicon sequence variant was carried out using a naïve Bayesian classifier algorithm comparing the ASV sequences to the SILVA reference database (v138.1).

The diversity of bacterial species in gut microbiota was measured by Shannon index, which accounts for both the number of different species present (richness) and the evenness of their distribution. OTU richness is another measure of microbial diversity commonly used in gut microbiota studies. An OTU refers a group of closely related sequences that are clustered together based on their similarity. Total OTUs richness is the number of different OTUs present in a sample.

5.6 Anaerobic cultivation of a gut bacteria strain

Paper IV presented cultivation strategies for viable *Prevotella copri* DSM 18205^T strain inoculations and growth in serum bottles and a stirred tank bioreactor (STR). *P. copri* DSM 18205^T (CB7T, Hayashi, et al., 2007) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Braunschweig, Germany). Characterization of the bacterial growth in serum bottles and bioreactors was conducted without using an anaerobic chamber. Cultivations in serum bottles, the inoculum condition, two sampling techniques, the growth pH, and the bacterial growth on peptone yeast glucose (PYG) and peptone yeast xylose (PYX) medium were evaluated (Figure 11). A series of fermentations in PYG and PYX medium in stirred tank cultivation bioreactors was carried out using two types of alkaline compounds (NaOH and NH_4OH) for pH control at two working volumes (0.5 and 1 L), to investigate the need to limit oxygen in the reactor (Figure 11). Cell density, dry cell weight, sugar consumption, and the production of secondary metabolites were determined in both cultivation strategies.

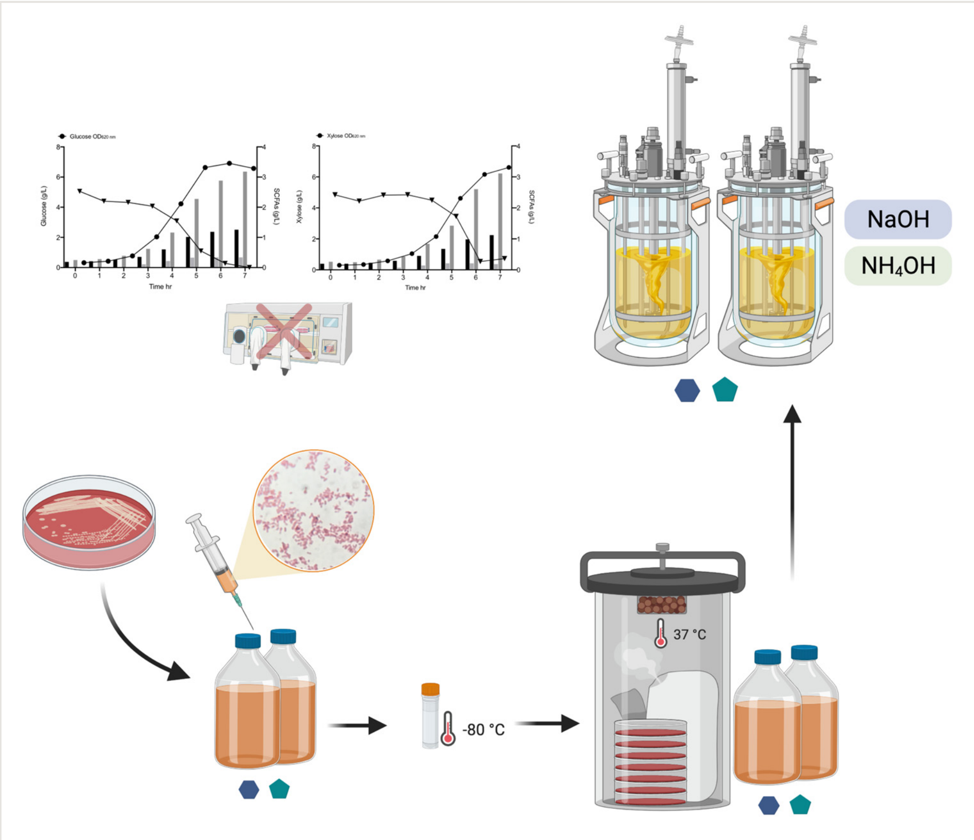


Figure 11 Graphic abstract of the cultivation strategies for *Prevotella copri* DSM 18205^T
Bacterial cells were taken from an agar plate and inoculated into serum bottles for anaerobic cultivation. Once the cells reached the exponential phase, they were transferred to a STR for further cultivation. Growth curves, sugar consumption rates, and metabolite profiles were measured to monitor the bacterial behavior.

6 Results and Discussion

6.1 Overall findings

Paper I showed that the whole lingonberry and its two separated fiber fractions in HF diets induced gut microbiota alternation and improved different levels of diet-induced metabolic disturbance in ApoE–/– mice. Mice fed either the lingonberry or the insoluble fiber fraction exhibited reduced gut inflammation levels and increased hippocampal synaptic density. **Paper II** showed that supplementation with bilberry, blackcurrant, blueberry, lingonberry, and (to some extent) cloudberry has beneficial effects on spatial cognition of middle aged C57BL/6J mice, as seen by the enhanced performance in the T-maze alternation test. The relative abundance of the mucosa-associated symbiotic bacteria *Akkermansia muciniphila* was increased in the cecal microbiota of mice fed diets supplemented with lingonberry, bilberry, sea buckthorn, and blueberry. Based on the previous findings, the work in **paper III** further investigated the effects of berry mixture supplementation containing lingonberries and bilberries. The berry mixture was found to improve spatial memory of young C57BL/6J mice, as evidenced by enhanced performance in T-maze and Barnes maze tests. In addition, the mice receiving the berry mixture tended to have reduced body weight gain and fasting insulin levels. The berry mixture also resulted in a higher level of the inflammation modifying IL-10 cytokine in the hippocampus and a distinct gut microbiome profile, with a significantly higher relative abundance of *Akkermansia muciniphila*. In **paper IV**, a crucial step for reviving *Prevotella copri* DSM 18205^{T} – a potential probiotic isolated from the gut microbiota – without using an anaerobic chamber was discovered. The results include techniques that allow the cultivation of the anaerobic bacterium *P. copri* DSM 18205^{T} in serum bottles and stirred tank bioreactors. The strain was found to be oxygen tolerant during inoculations and culture transfers, thus simplifying handling, but the use of exponential phase cultures to maintain viable cell cultures was crucial for successful inoculations. The specific growth rate of *P. copri* was in the same range when using either glucose or xylose as the carbon source, and yielded a significant production of succinic acid in both cases. In addition, successfully scaling up the cultivation to a stirred tank bioreactor provides a solid base for studying the bacterium for more efficient probiotic production.

6.2 Impact of berry intake on body weight gain

6.2.1 Body weight gain caused by a HF diet can be prevented by consuming Nordic berries

In **paper I**, the mice fed the HF diet supplemented with the wLB, insLB or the solLB showed significantly lower body weight gain compared to the HF (38% kcal from fat, 38E%) control group after 8 weeks (wLB and insLB) or 2 weeks (solLB) of feeding. The wLB group had significantly lower epididymal fat weight and liver weight than the HF control group (Table 2).

In **papers II and III,** the berry supplementation did not have a significant effect on the final body weight gain compared to the HF (60%E) control groups after being fed the experimental diet for 16 or18 weeks, respectively. It is worth pointing out that there was an overall tendency ($p = 0.06$) for the mice fed HF receiving the berry mixture (HF+Berry) to gain less body weight compared to the HF control group (**Paper III**). The animals fed the HF and berry mixture also had significantly lower liver weights but higher epididymal fat weight compared to the HF group.

The results of berry intake on body weight gain are summarized in Table 2.

Table 2 Summary of berry supplements, fat content, dietary fiber composition, as well as body weight gain (papers I-III).

	Berry supplement	Fat content (E%)	Berry dose % (dwb)	Fiber from berry % (w/w)	Added cellulose % (w/w)	Total dietary fiber[&] % (w/w)	Feeding period (weeks)	Body weight gain[€]
Paper I	wLB[1]	38	26.0	6.0	–	6.0	8	↓(*)
	insLB[2]	38	20.0	6.0	–	6.0	8	↓(**)
	solLB[3]	38	13.6	1.5	4.5	6.0	2	↓(*)
Paper II	Lingonberry	60	6.0	1.1	5.0	6.1	16	ns
	Bilberry	60	6.0	1.5	4.9	6.4	16	ns
	Blackcurrant	60	6.0	1.7	4.8	6.5	16	ns
	Cloudberry	60	6.0	2.2	4.8	7.0	16	ns
	Sea buckthorn	60	6.0	1.2	4.9	6.1	16	ns
	Blueberry	60	6.0	1.6	4.9	6.5	16	ns
Paper III	Berry mixture	60	20.0	2.8	3.5	6.3	18	↓(#)

1. wLB: HF supplemented with lingonberries (26% dwb, wLB)
2. insLB: HF supplemented with insoluble fiber fraction (20% dwb, insLB)
3. solLB: HF supplemented with soluble fiber fraction (13.6%, solLB)

Energy (kcal) percentage (E%); dry weight basis (dwb); weight/weight (w/w); no significant difference (ns).

[&]In **paper I**, the dietary fibers in the wLB and the insLB groups were provided by the respective dose of berry supplementation; in the solLB group, dietary fiber coming from 1.5% soluble fiber from the added berry plus the added cellulose. In **paper II-III**, the dietary fiber consisted of the berry fiber and the added cellulose. The HF control diets in each study only contained added cellulose.

[€]Comparison was made between the berry diet groups and their respective HF control group.

6.2.2 Potential factors in berry diets that can affect body weight

There are multiple factors that can affect body weight, such as the length of the intervention, the age of the mice, and any stressors present in the experimental environment. When formulating a diet, it's important to consider dietary factors that can also impact body weight and organ weight. These factors include the amount and form of berries (such as lyophilized, juice, or extracts), the type and percentage of dietary fat, the level of dietary fiber and sugar, and the presence of polyphenol compounds. Below, we will discuss some of these parameters in more detail.

6.2.2.1 Fat content in the berry diet matters

Previous studies have demonstrated that certain types of berries, such as blueberry, bilberry, and lingonberry, may help reduce the body weight gain induced by a HF diet. Additionally, these berries have been associated with decreased epididymal fat and liver weight in mice [118, 119, 178].

Heyman et al. [178] conducted a study in which male C57BL/6J mice, aged 7 weeks, were fed a HF diet (45%E from fat) supplemented with 20% (w/w) freeze-dried lingonberries, blackcurrants, or bilberries for 13 weeks. These mice gained less weight compared to the control group that received a HF diet without berries. In their study, lingonberries, blackcurrants, and bilberries significantly decreased body fat content and hepatic lipid accumulation. These results are consistent with the findings for lingonberries in **paper I**, despite the difference in the animal models between the two studies. Both studies used animals of the same age, with similar fat content in the diets (38%E or 45%E), both lower than those in **papers II and III**. Additionally, the dose of the lingonberry used in **paper I** (26% dry weight basis) and the study by Heyman (20% dry weight basis) was higher than that in **paper II**.

The author suggests that the berry supplements used in **papers II and III** may not have been sufficient to efficiently counteract the detrimental effects of a 60%E fat basis diet on body weight gain. Furthermore, other studies that have used lower doses of berries in a 60%E HF diet also showed similar results to those found in **papers II and III**. A previous study on male C57BL/6I mice showed that adding blueberry powder (1:1 *Vaccinium ashei* and *V. corymbosum* powder) to the HF diet did not protect against HF-induced weight gain after eight weeks of feeding [179]. This finding aligns with the results of Carey et al. [85], who used 9-month-old C57BL/6 mice and fed them HF (60%E) diets with or without 4% freeze-dried blueberry powder for five months. The study found that the low dose of blueberries did not significantly affect body weight.

6.2.2.2 Sugar intake

Different types of sugars may have varying effects on the outcomes of the experiment. For example, there is emerging research indicating that dietary fructose might play a role in the development of obesity [180]. A study by Cox et al. [181] on overweight/obese male and female subjects found that consuming fructose (at 25% of energy requirements) for ten weeks resulted in a significant decrease in resting energy expenditure, thus contributing to the build-up of excess energy substrates, but the equivalent amount of glucose did not have such effect. Other studies have also implicated increased fructose intake in promoting obesity, as rats with access to high fructose corn syrup gained significantly more body weight than rats given equal access to sucrose, even though they consumed the same number of total calories [182, 183].

In **paper II**, the simple sugars present in the berry powders were considered when formulating the experimental diets. All diets were matched on macro- and micronutrients, as well as sucrose, fructose, and glucose, i.e., adjusted for the amounts coming from 6% of the freeze-dried berry powders added.

6.2.2.3 More berry fiber is better for mice receiving a HF diet

In **paper I**, the entire dietary fiber intake (6% w/w) in the wLB and the insLB groups came from their respective addition of berries, and these diet groups also significantly lowered body weight gain compared to the HF control. In **papers II and III**, despite the total dietary fiber being approximately the same amount as in **paper I**, the composition of dietary fiber was a combination of berry fiber and added cellulose. A previous clinical study showed that adding isolated fiber supplements, including fermentable (pectin, β-glucan) or nonfermentable fiber (methylcellulose), did not lead to a reduction in body weight [184]. This may explain the mixture of results in **papers I-III**. Interestingly, when the berry dose was increased to 20% in **paper III** and thus more berry fiber was included, the intake of the berry mixture tended to show an effect on lowering the body weight gain induced by the HF diet (Table 2). These findings suggest that the more berry fiber included, the greater the effect on body weight control.

Does this conclusion contradict the findings in **paper I** where solLB was only 1.5% from the berries? Not necessarily.

6.2.2.4 Soluble fiber fraction of lingonberries may be more effective in terms of body weight control

Previous studies suggest that the energetic value of fiber varies based on its fermentability and antinutritive effects, and the physicochemical properties of fiber can influence nutrient digestion and substrate absorption [185]. Viscous fibers seem to have a particularly pronounced effect on body weight. Human studies have shown that soluble and fermentable fiber from fruits and vegetables can reduce the absorption of fat and protein [186, 187]. This occurs as soluble fiber forms a gel matrix in the stomach, which slows down gastric emptying and traps nutrients, resulting in a slower rate of nutrient absorption [188]. Additionally, soluble fiber can reduce postprandial glycaemic and insulinemic responses, which have been associated with reduced hunger and subsequent energy intake in previous studies [185, 189]. These findings support the results in **paper I,** where 1.5% soluble fiber, comparable to the berry fiber in **paper II**, still reduces body weight gain induced by HF feeding.

There is also evidence that dietary fiber may positively affect cognitive function. For example, studies found that a high dietary fiber intake was associated with better cognitive function and a lower risk of cognitive decline and dementia in the older adults [190, 191]. One explanation for this effect can be found in another study suggesting that dietary fiber may help reduce inflammation in the body, and inflammation has in turn been associated with cognitive decline [192].

The important role of dietary fiber in different health aspects highlights the need for increased attention to fiber composition in raw materials. Thus, the carbohydrate analysis of the freeze-dried berry powder was conducted in **paper III** with a particular focus on the fiber composition of berries.

The analysis of carbohydrate composition in **paper III** suggests that the dietary fiber in the berry mixture powder contained the hemicellulose xyloglucan, as well as components that are most likely neutral side chains from pectic polysaccharides such as arabinan, arabinose and galactose. Acid hydrolysis showed glucose (48.3%, 1.1) to be the main component of the polymeric material, which is in accordance with a high content of xyloglucans (polymers of β-1,4 linked glucose backbone substituted at O-6 with xylose residues) which has been reported as the main type of hemicellulose in dicotyledonous plants, including berries [193]. The xylose likely also originates from bilberry seeds, where it has been found to be a significant component [193, 194]. The arabinose and galactose are thought to originate from neutral side chains of pectic polymers (e.g. rhamnogalacturonan) reported to contribute up to 0.8% of bilberry dry weight [193].

6.2.2.5 Berry polyphenol and dosage effects on body weight gain

Berries contain high levels of polyphenols, including flavonoids (ACNs, flavonols, and flavanols), tannins (proanthocyanins), hydrolysable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids, chlorogenic acid), stilbenoids, and lignans [195, 196]. The concentration of these compounds may vary according to species, genotype, and growing conditions as well as post-harvesting conditions [197, 198]. Thus, the batch variation of the bioactive compounds in raw materials requires consideration in every study.

Additional issues to consider are the ACNs consumed and whether there is a dose-dependent response. Prior et al. [199] investigated 3-week-old male C57BL/6J mice fed a HF diet with or without 10% freeze-dried powders from whole blueberries. The mice fed a HF (45%E) diet plus blueberries had significantly greater body weight gains, body fat (percent of BW), and epididymal fat weights than the HF (45%E) control. In the same study, the mice fed the HF (60%E) diet plus purified

ACNs from blueberries in their drinking water had lower body weight gains and body fat than the HF (60%E) control. Interestingly, the total anthocyanin intake per day per mouse was higher in the whole blueberry supplementation group than that in the purified anthocyanin from blueberries group. The author concluded that the ACNs fed as whole blueberries did not prevent – and may have increased – obesity, whereas feeding purified ACNs from blueberries reduced obesity. Previous research has suggested that ACNs found in berries might help to reduce obesity by decreasing weight and adipose tissue [105].

The possible interaction between the dietary components (e.g. polyphenols and/or sugar) in berries and the fat in the diet can produce unpredictable effects. This may be a contributing factor to the increase in body weight and adipose tissue deposition in HF diet feeding as observed in the results of **paper III.**

Furthermore, the age of the mice and the timing of the dietary intervention are also essential to consider when designing studies and evaluating results. Younger, more metabolically active mice are often used to study metabolic outcomes, whereas more mature adult mice might be more appropriate for cognitive outcomes (Huang et al., unpublished data, manuscript in preparation). Thus, the middle-aged mice in **paper II** may be a less suitable model for obesity-related research questions.

6.3 Impact of berry intake on cognition

Several different types of berries have been associated with improved cognitive performance, including commonly consumed berries like American highbush blueberries (*Vaccinium ashei, Vaccinium virgatum, or Vaccinium corymbosum*)[11, 85, 126], lowbush wild blueberries (*Vaccinium angustifolium*) [10-12], raspberries [65] strawberries [8, 9] and red grapes [127, 128].

The hippocampus is known for its role in memory and spatial navigation, and it is involved in consolidating information from short-term memory to long-term memory [26]. Spatial memory problems, such as getting lost or forgetting where objects have been placed, are a common consequence of hippocampal damage in humans [26]. Damage or dysfunction of the hippocampus can result in memory problems and difficulty with spatial navigation, and is also involved in the pathogenesis of several neurological disorders, including AD [200].

6.3.1 T-maze test: spatial memory is improved by berry intake

T-maze tests are widely used to assess spatial working memory in rodents [57, 174]. Rodents with intact hippocampal functions typically display spontaneous alternation rates of around 75% or higher [57, 174], meaning that their choice to explore the novel arm is based on spatial memory of the previously visited arm, and not due to random chance. The alternation rate normally reflects the motivation of the animals to explore the environment, and rodents have the natural tendency to alternate their choice of goal arm. In **paper I**, no significant differences between the groups were observed in the T-maze alternation rate of ApoE−/− mice receiving different diets. In **paper II**, the supplementation with lingonberries, bilberries, blackcurrants, cloudberries, and blueberries significantly improved the spatial memory in mice on a HF diet. Mice supplemented with these berries had an average alternation rate of 73-83% in the T-maze test (Figure 12).

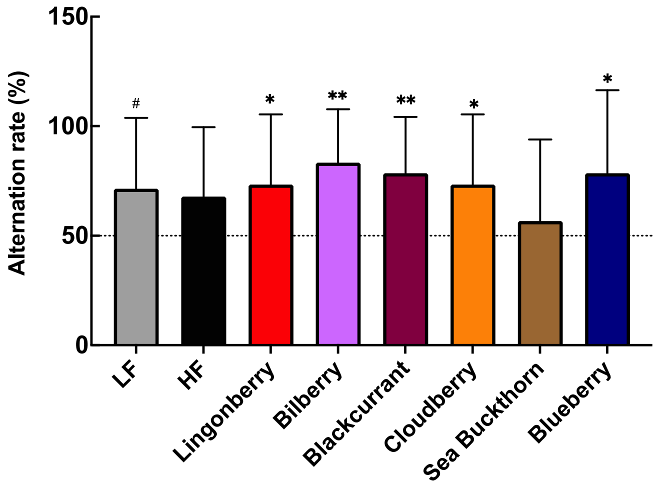


Figure 12 The effects of the HF diet and berry supplementation on the alternation rate in a T-maze test (Paper II).
The alternation rate (%) was evaluated as the ratio of correct arm choices to the total number of trials. Each bar represents the mean alternation rate of the mice in each diet group; low-fat diet (10E%, LF). The Wilcoxon signed-rank test was applied to compare the % alternation of each group against a 50% chance. * $p < 0.05$, ** $p < 0.01$. (# $p < 0.1$ denotes a non-significant trend). Data represent mean SD for n = 14–15 per group.

In **paper III**, a discrete-trial, no-reward T-maze test protocol was adapted from a previous Y-maze protocol [58]. The results showed that the mice fed the HF+Berry performed significantly better than the HF- and chow groups (Figure 13). These findings are consistent with **paper II**, which showed that bilberries and lingonberries, two ingredients in the berry mixture, were associated with improved performance in T-maze tests in mice.

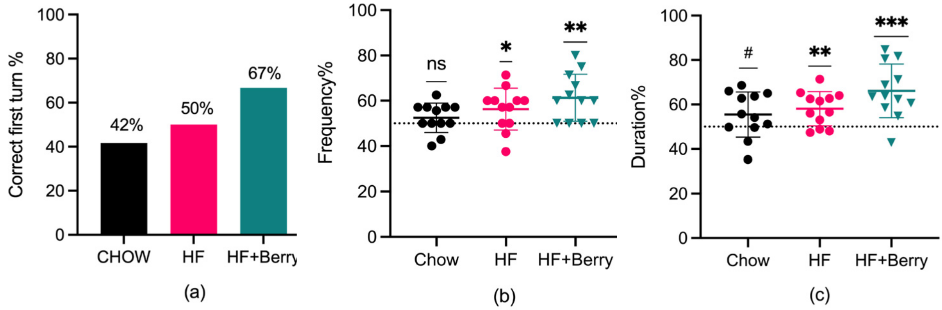


Figure 13 Effects of the chow−, HF−, and HF+Berry diets on the T-maze test (Paper III).
(a) Correct first turn % calculated as the percentage of the animals that have made a correct turn at their first choice of turning; (b) Frequency% calculated as the visiting frequency to the correct arm in ratio to the total visits in both arms; (c) Duration% calculated as the time spent (second) in the correct arm in ratio to the total time spent (second) in both arms. Wilcoxon signed-rank test was applied to compare each group against a 50% chance. Significant differences denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (# $p < 0.1$ denotes a non-significant trend). Values are represented as mean ± SD for n = 12 per diet group.

In these studies, the age of the animals may have played a role in the observed differences in performance. Specifically, the mice used in **papers I and III** were relatively young (16−18 weeks old) at the time of the T-maze experiments, while the mice in **paper II** were considerably older (10 months old). The discrepancy in results could also be attributed to the mouse model used, as a previous study has shown that young ApoE−/− mice do not experience spatial memory decline when fed a HF diet, unlike C57BL/6J mice [201].

Future studies may benefit from using a larger sample size per group and conducting more test repetitions per animal to better elucidate the effects of different diets on behavior. These measures may help to increase the statistical power of the analyses. Additionally, it may be useful to explore other behavioral paradigms that tap into different aspects of cognitive function to gain a more comprehensive understanding of the potential cognitive benefits of dietary interventions.

6.3.2 NOR test: recognition memory is less affected by berry intake

The NOR test is a commonly used method to measure recognition memory, attention, anxiety, and preference for novelty in rodents [202]. In **paper II**, the low-fat (10E%, LF) diet and HF diet supplemented with cloudberries showed a significant increase in the amount of time spent on the novel object during the test session compared to the amount of time spent on the familiar object during the familiarization session (Figure 14). However, no significant difference was observed when supplementing blueberries, which was unexpected in light of previous research by Carey et al. showing that supplementation with 4% blueberry or raspberry reversed the decline in recognition memory caused by a HF diet in C57BL/6 male mice [65, 85]. In **paper III**, there was no significant difference between the diet groups in the NOR test. Previous studies have noted mixed results when investigating the effects of HF diets on NOR in rodents, and these mixed results suggest that diets with different macronutrient compositions could have varying effects on the hippocampal function [190, 203].

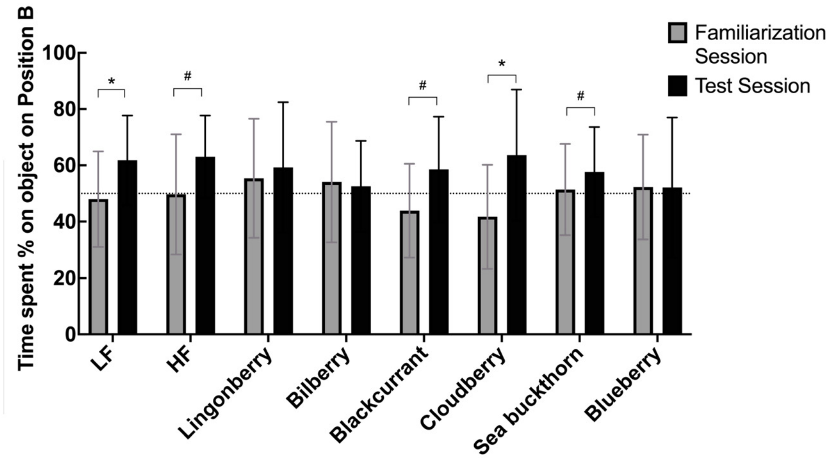


Figure 14 NOR result (Paper II).
The data was presented as the percentage time spent exploring the object on Position B in the familiarization and test sessions. Values are represented as mean and SD (n = 14–15). The results in the familiarization session were compared to the test session using the non-parametric Wilcoxon signed-rank test. * $p < 0.05$. (# $p < 0.1$ denotes a nonsignificant).

The NOR test results can be influenced by several factors, such as the size and the appearance of the objects used, which may not always be clearly reported in other studies. For **papers II and III**, we used objects no larger than 2.5 cm^3 in the work of **papers II and III**. Another factor that can be adjusted in the NOR protocol is the interval between the familiarization session and the test session. In our studies, we set the interval time to 3−4 hours. However, the lack of significant differences between groups in **papers II and III** suggests that this interval time may have been too short to detect any differences, as all animals remembered the objects well. Furthermore, it is essential to allocate an appropriate amount of time for the test session to ensure that all animals have sufficient opportunity to explore both objects thoroughly and maintain engagement.

6.3.3 Barnes maze: mice receiving the berry mixture perform better

The Barnes maze test, included in **paper III**, further assesses hippocampal-dependent spatial learning and memory. The test principle resembles the Morris water maze, as both tests evaluate the animal's ability to learn the relationship between distal cues and a fixed escape location. Both tests have been commonly used to assess hippocampus-dependent spatial learning and memory in mouse models [204-206]. The Barnes maze test was chosen over the Morris water maze test in **paper III** because the Morris test may lead to behavioral despair in mice, which may cause ambiguity in the data interpretation [206].

The results of the Barnes maze test in **paper III** showed that all groups improved in their ability to locate the target hole over the experimental period (Figure 15). The study revealed that the HF+Berry group and the chow group performed significantly ($p < 0.01$, $p < 0.001$, respectively) better than the HF group. These results indicate that the berry mixture containing lingonberries, bilberries, and grapes can potentially improve hippocampal-dependent spatial learning and memory.

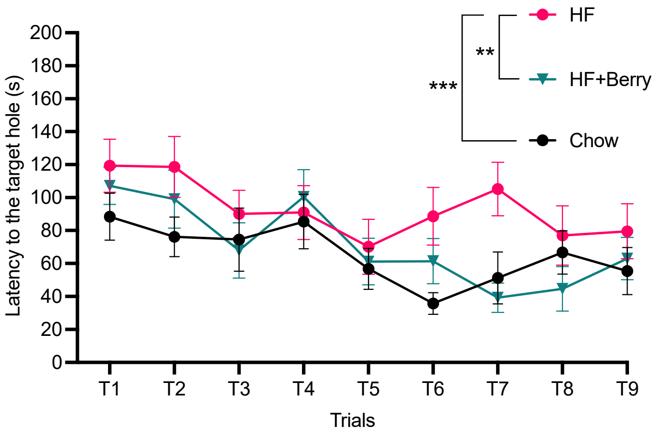


Figure 15 Barnes maze performance of the chow–, the HF– and the HF+Berry group.
The latency (s) until the mice entered the escape box during the training trials; significant differences denoted ** $p < 0.01$, *** $p < 0.001$. Values are represented as mean ± SEM for n = 12 per diet group.

However, a limitation of the study is that no impaired performance was observed in the HF group in the probe trial, as was the case for the chow group and the group fed HF with berries. This suggests that the HF diet did not severely impair spatial learning and memory, possibly due to the young age of the mice, which may have provided some protection against HF-induced cognitive impairment. It is important to note that the work of **paper III** represents a relatively mild model of cognitive dysfunction that reflects lifestyle rather than genetic or drug-induced cognitive dysfunction [62].

Overall, the behavior test results indicate that certain Nordic berries can enhance cognitive function, as evidenced by improved performance in the T-maze and Barnes maze tests.

6.4 Impact of berry intake on brain function

6.4.1 Berry intake may enhance synaptic function in the hippocampus

In **paper I**, mice fed wLB and insLB showed a significant increase ($p < 0.05$) in synaptic density in the CA1 (the first region in the hippocampal circuit) compared to the HF control. In **paper III**, the level of the phosphorylated form of glutamate A1 (pGluA) was significantly higher ($p < 0.05$) in the HF+Berry group compared to the HF group.

Immunohistochemistry research has previously revealed that synapse loss is correlated with cognitive deficits [207]. Other studies have also suggested that while GluA1 plays a crucial role in maintaining synaptic functionality, pGluA1 at specific sites can regulate the trafficking, insertion, and removal of AMPA receptors from the synaptic membrane, ultimately influencing synaptic strength and plasticity [208-211].

The findings in **papers I and III** suggest that the intake of lingonberries and the berry mixture can enhance hippocampal synaptic function, which is associated with potential improvements in cognition and learning memory.

6.4.2 Berry intake has no significant effect on hippocampal neurogenesis

Paper II found that C57BL/6J mice fed a HF diet without berries had lower levels of DCX expression, whereas the groups supplemented with lingonberry, bilberry, or blackcurrant had higher levels of DCX-expressing cells and a higher %DCX area compared to the HF group. It is worth noting that the DCX results in **paper I** were presented only in a descriptive way, as a statistical analysis was not conducted due to the limited sample size. In addition, no difference was found in the total protein levels of brain-derived neurotrophic factor (BDNF) – an important mediator of neurogenesis and neuronal plication in the formation of long-term memory [212, 213] – of the mice from different berry diet groups. **Paper III** indicated that there was no significant difference in the total protein levels of BDNF, nerve growth factor (Ngf) or DCX in the hippocampus of the C57BL/6J mice receiving HF and HF+Berry groups.

Previous studies have found that HF and refined sugar diets affect neurogenesis and neuroplasticity through a decrease in hippocampal BDNF [212, 213]. The author concluded that the berry supplementation added to a HF diet did not cause significant changes on neurogenesis in the hippocampus region.

However, it is important to remember that conclusions regarding berry intake on neurogenesis may vary depending on the specific markers and brain regions examined. While the studies discussed in **papers II and III** primarily focused on the hippocampus, it would be worthwhile to explore other brain regions to understand more about the effects of berries on neurogenesis.

6.4.3 Berry diet may reduce the level of hippocampal neuroinflammation

Neuroinflammation refers to the inflammation of nervous tissue in response to injury, infection, or disease. It involves the activation of immune cells, such as microglia and astrocytes, and the release of pro-inflammatory cytokines and chemokines. Previous research suggested that there is a correlation between neuroinflammation and cognitive impairment [68, 69].

In **paper II,** among the berries diet being studied, the levels of IL-1β, a pro-inflammatory cytokine in the hippocampus, remained unchanged between the groups. Interestingly, in **paper III**, the mice that received the HF supplemented with the berry mixture had a significantly higher level of anti-inflammatory cytokine IL-10 in the hippocampus than those fed HF diets without berries. Among all the anti-inflammatory cytokines, IL-10 is a cytokine with potent anti-inflammatory properties, repressing the expression of inflammatory cytokines such as TNF-α, IL-6, and IL-1β by activated macrophages [214]. IL-10 is synthesized mainly in microglia and astrocytes of the central nervous system and has properties affecting immunoregulation and inflammation in all tissues in response to neuroinflammation [215]. The anti-inflammatory effect of the bioactive compounds, such as polyphenols found in berries, may be due to their ability to inhibit the inflammatory response mediated by key signalling pathways, namely, mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-kB), these pathways are crucial in regulating inflammation [216-219] (Figure 16).

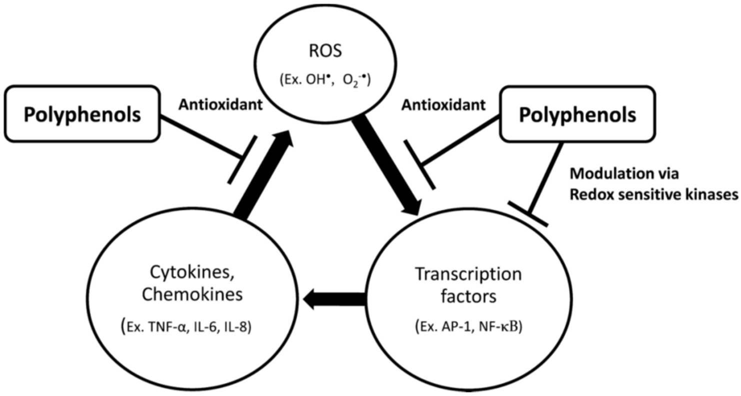


Figure 16. Schematic representation of link between antioxidative and anti-inflammatory potential of dietary polyphenols.
ROS, reactive oxygen species; $OH^{•}$, hydroxyl radical; $O_2^{-•}$, superoxide radical; AP-1, activator protein-1; NF-κB, nuclear factor-kappa B; TNF-α, tumor necrosis factor-alpha; IL, interleukin. Source of illustration was obtained from Gu, I., et al (2020), under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

The results of **papers II and III** suggest that the berry mixture may reduce neuroinflammation, however, further studies are warranted to understand how Nordic berries may affect neuroinflammation.

6.5 Impact of berry intake on cardiometabolic risk markers

6.5.1 Improved glucose response in ApoE–/– mice receiving lingonberry or its insoluble fiber fraction

The results of **paper I** showed that the ApoE–/– mice receiving the HF diet supplemented wLB and the insLB had significantly ($p < 0.0001$ and $p < 0.01$, respectively) lower levels of glucose reported by the oral glucose tolerance test (OGTT). The mice fed wLB and insLB also had significantly higher levels of HDL-cholesterol ($p < 0.05$ and $p < 0.01$, respectively) and triglyceride (TG) ($p < 0.05$ and $p < 0.01$, respectively) in plasma compared to the HF control, and there were no significant changes in total plasma cholesterol, low-density lipoprotein (LDL)-cholesterol levels, and TG/HDL ratio between the wLB–, the insLB– and the HF groups. For mice fed solLB, there were no significant differences in blood lipid profiles.

6.5.2 The mixture of berries may reduce body weight gain and fasting insulin levels in C57BL/6J mice

In **papers II and III**, the berry supplementation did not significantly affect the fasting plasma glucose, fasting insulin levels, and lipid profile, including HDL, total cholesterol, and oxidized low-density lipoprotein (OxLDL, only checked in **paper III**). However, it is worth pointing out that there was a strong trend ($p = 0.08$) toward reduced insulin levels and body weight gain ($p = 0.06$) amongst the C56BL/6J mice receiving the berry mixture in **paper III**.

Recent studies on less-known berries showed that, for example, lingonberry intake was associated with various health-promoting effects, including the prevention of body weight gain and the reduction of low-grade inflammation [118, 119]. Similarly, bilberries have been shown to prevent body weight gain, and to reduce inflammation, hypertension, and hyperglycemia [100, 220, 221]. There is also a growing body of clinical research that indicate beneficial effects of berries on metabolic health [11, 124, 125].

Taken all together, the findings from **papers I-III** suggest that consuming lingonberries, or a mixture of berries, including bilberry and lingonberry, may have a positive impact on whole-body metabolism, helping to protect against insulin resistance induced by a HF diet.

Overall, in our studies, berries appear to have cognitive and metabolic benefits regardless of age, berry doses, or animal models. However, we consider that the C57BL/6J model may be more appropriate for behavior tests, while the ApoE–/– model may be more suitable for metabolic function studies for our future studies.

6.6 Impact of berry intake on gut microbiota

6.6.1 Berry intake contributes to a high level of microbial diversity and richness in the gut

A high Shannon diversity index in the gut microbiota composition is generally considered a marker of healthy gut microbiota, indicating that many different bacterial species are present in the gut and that these species are relatively evenly distributed, which is a sign of a resilient gut microbiota [222]. Research has suggested that a high diversity of gut microbiota is associated with several health benefits, such as improved immune function [222], better nutrient absorption [223], reduced risk of inflammatory bowel disease [224] and cognitive function [225]. On the other hand, a low diversity of gut microbiota may lead to dysbiosis, which has been associated with inflammatory disorders and metabolic diseases [226].

The analysis of cecal bacterial 16S rRNA genes in **paper II** revealed that the animals fed on a HF diet supplemented with Nordic berries – lingonberry, blackcurrant, cloudberry, sea buckthorn, and blueberry – had significantly higher levels of microbial diversity as measured by the Shannon diversity index, and a greater number of different types of bacteria as measured by the total OTU richness compared to the HF control group without berries. This is presented in Figure 17.

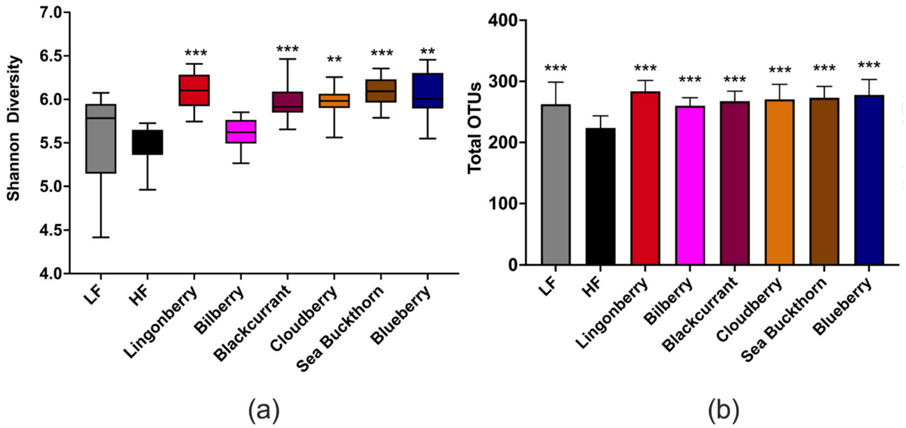


Figure 17 Alpha diversity of the gut microbiota in mice fed LF, HF, lingonberry, bilberry, blackcurrant, cloudberry, sea buckthorn, and blueberry (Paper II).
The level of diversity was measured by (a) Shannon diversity index; (b) total OTU richness. The Shannon index compared each group to the HF control group using the Kruskal–Wallis rank-sum test followed by pairwise comparisons using the Wilcoxon rank-sum test. n = 12–15 per group, ** $p < 0.01$, *** $p < 0.001$.

Interestingly, the gut microbiota data from **paper III** indicates that consuming 20% (w/w) of berries did not result in a significant increase in either the diversity or the total OTU richness of the gut microbiota. It is possible that a high dosage of berry intake may promote the growth of a specific group of bacteria, leading to a reduction in overall diversity. This is supported by a recent study in which administering moderate doses of a purified extract of blueberry polyphenols to rats increased their gut microbiota diversity, while high doses decreased the diversity in 5-month-old rats [227].

The study findings suggest that the supplementation of berries to the diet may help restore the balance of gut bacteria and promote a more diverse and resilient gut microbiota.

6.6.2 Berry intake can modulate the gut microbiota compositions

The inclusion of berry supplements in a HF diet resulted in significant changes in the composition of the cecal microbiota, as demonstrated in **papers I-III**. The gut microbiota of mice fed a berry diet differed notably from that of control groups fed a HF diet alone. The results are summarized in Table 3.

The gut microbiota of humans and mice is dominated by two major phyla, Bacteroidetes and Firmicutes. Previous studies have shown that a western diet, characterized by HF content, is associated with a significant increase in the relative abundance of Firmicutes and a significant decrease in the abundance of Bacteroidetes in the cecal community [228, 229]. This finding is consistent with the results from **paper II**, which observed that a low berry dose in the HF diet did not reverse the effect of a HF (60E%) diet on the relative abundance of Firmicutes and Bacteroidetes (Table 3). However, in **papers I and III**, where the berry dose was increased, mice fed a HF diet supplemented with lingonberries or the berry mixture did have a significant decrease in the relative abundance of Firmicutes and a significant increase in the relative abundance of Bacteroidetes (Table 3).

Lingonberry, bilberry, and the mixture of berries were found to be associated with an increase in the relative abundance of Bacteroidetes (**papers I-III**). Bacteroidetes are important in the breakdown of complex carbohydrates, such as dietary fibers, and have been found to be particularly abundant in the large intestine [230]. Previous studies have also found that many species of Bacteroidetes can help regulate energy metabolism and aid in the control of body weight. Additionally, Bacteroidetes have been linked to the maintenance of gut barrier function and immune system regulation [230-233].

The phylum Proteobacteria is a diverse group of gram-negative bacteria in the human gut microbiota. Some of the most well-known genera of *Proteobacteria* include *Escherichia, Salmonella*, and *Helicobacter*; these are common factors in human diseases [234]. A previous review has proposed that an increased prevalence of Proteobacteria is a potential diagnostic signature of dysbiosis and risk of disease [235]. Interestingly, our studies have shown that supplementation with several Nordic berries was associated with a decrease in the relative abundance of Proteobacteria in the gut across the studies (Table 3), suggesting that the

Table 3 Summary of the gut microbiota composition in the cecum of the mice fed berry diets.
Each group was compared to HF control group using two-way ANOVA and the p-values were corrected for multiple comparisons by controlling the False Discovery Rate (FDR) using the original FDR method of Benjamini and Hochberg (n = 12–15 per group). Significant differences denoted * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (# $p < 0.1$ denotes a non-significant trend)

		Phylum level						Genus level
		Firmicutes	Bacteroidetes	Deferribacteres	Desulfobacterota	Verrucomirobiota	Proteobacteria	*Akkermansia*
Paper I	wLB[1]	↓#	↑**	↓***		↑*		↑*
	insLB[2]			↓**		↑**		↑***
	solLB[3]	↑**	↓**				↓***	
Paper II	Lingonberry		↑*			↑*	↓***	↑***
	Bilberry	↓***	↑***			↑***		↑***
	Blackcurrant	↑***					↓***	
	Cloudberry	↑***					↓***	↑**
	Sea buckthorn	↑**					↓***	
	Blueberry	↑***					↓***	
Paper III	HF+Berry	↓***	↑***		↓***	↑***		↑***

1. wLB: HF (38E%) supplemented with lingonberries (26% dwb, wLB)
2. insLB: HF (38E%) supplemented with insoluble fiber fraction (20% dwb, insLB)
3. solLB: HF (38E%) supplemented with soluble fiber fraction (13.6%, solLB)

consumption of certain Nordic berries may potentially suppress the growth of opportunistic bacteria in the gut, reducing the risks of health problems.

6.6.3 Consuming berries can promote the relative abundance of potentially beneficial bacteria in the gut

The findings in **papers I-III** showed that the phylum Verrucomicrobia and the species *Akkermansia muciniphila* increased significantly after lingonberry (**papers I and II**), bilberry (**paper II**), sea buckthorn (**paper II**) and berry mixture (Figure 18, **paper III**) intake. In addition, the findings in **paper I** showed that lingonberry intake was associated with an increase in the proportion of *Akkermansia muciniphila* in the gut microbiota of mice, which was found to be correlated with higher synaptic density in the hippocampus.

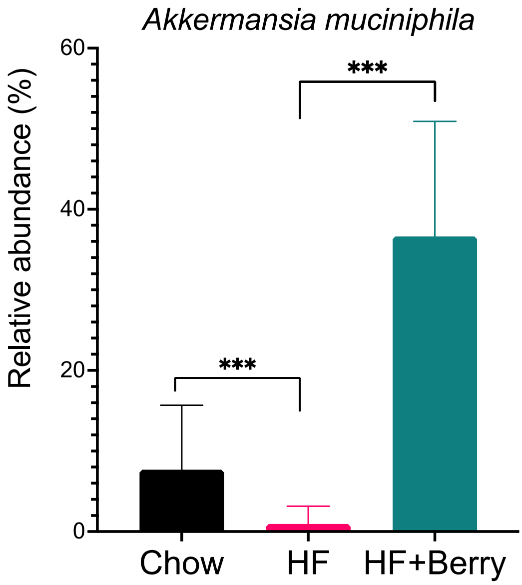


Figure 18 *Akkermanisa muciniphila in* the cecum of mice fed the chow–, the HF– or the HF+Berry diets (Paper III).
The animals were feeding experimental diets for 18 weeks (n=12 per group), represents mean ± SD. Each group were compared to HF control group using two-way ANOVA and the p-values were corrected for multiple comparisons by controlling the False Discovery Rate (FDR) using the original FDR method of Benjamini and Hochberg. Significant differences denoted *** $p < 0.001$.

Akkermansia is a gram-negative bacterium that degrades mucins as carbon, nitrogen, and energy sources to produce oligosaccharides and SCFAs for the metabolism of itself and the host [236, 237]. Studies have shown that supplementing the diet of mice with isolated proanthocyanins at levels equivalent to 1% of their total food intake led to a significant increase in the relative abundance of the bacterium *A. muciniphila*. This increase was associated with reduced low-grade systemic inflammation, decreased adiposity, and improved insulin sensitivity when the mice were on a HF diet [238, 239]. A study on an AD mouse model has also shown that restoring this bacterium, which was depleted in APPPS1 mice compared to healthy wild-type controls, was one of the parameters associated with reduced amyloid–beta pathology in the mouse brains [240]. Furthermore, two clinical studies from the same group reported that a larger proportion of Verrucomicrobia significantly correlates with better performance in cognitive tests [241, 242].

A. muciniphila was shown to play a role in immune and metabolic regulation, combined with increased gut barrier function [243, 244]. The mechanism by which *A. muciniphila* exerts beneficial effects on the host involves direct signaling of outer membrane proteins, metabolite production, and the enhancement of beneficial mucosal microbial networks [245, 246] (Figure 19). *A. muciniphila* performs several key functions related to the breakdown of mucin, including the production of acetate, propionate, and 1,2-propanediol [247]. The degradation of mucin by A. muciniphila attracts other members of the microbiota, helping to prevent the colonization of harmful pathogens [248]. These microbes may also in turn produce beneficial compounds such as butyrate, which provides primary energy source for colonocytes [249] and can help to maintain homeostasis [249].

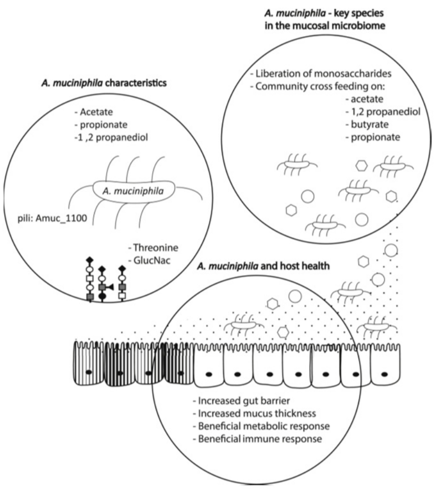


Figure 19 Three key functions of *A. muciniphila* in the microbiome that could be targets for probiotic interventions.
The mechanism by which *A. muciniphila* exerts beneficial effects on the host involves direct signaling of outer membrane proteins, metabolite production, and the enhancement of beneficial mucosal microbial networks. Source for illustration: Best Practice & Research Clinical Gastroenterology, 31(6), Ottman, N., et al., action and function of *Akkermansia muciniphila* in microbiome ecology, health and disease, Pages 637-642, Copyright (2017), with permission from Elsevier.

6.6.4 Cultivation technology and growth characteristics is revealed for *Prevotella copri* DSM 18205^T are revealed

Previous studies on the growth and the growth conditions of *P. copri* have yielded limited results in terms of bacterial characteristics and cell mass production [164]. Additionally, understanding the characteristics of *P. copri* under different conditions is crucial for increasing our knowledge of this bacterium. The research in **paper IV** provides valuable insights into the growth profile of *P. copri* through the successful development of batch cultivation strategies for viable strain inoculations in both serum bottles and a STR, without the need for an anaerobic chamber. The results of **paper IV** also showed that *P. copri* could utilize both glucose and xylose (Figure 20), as the bacteria cultivated in peptone yeast xylose (PYX) medium resulted in a comparable growth rate and metabolite production to peptone yeast glucose (PYG) in batch cultivations.

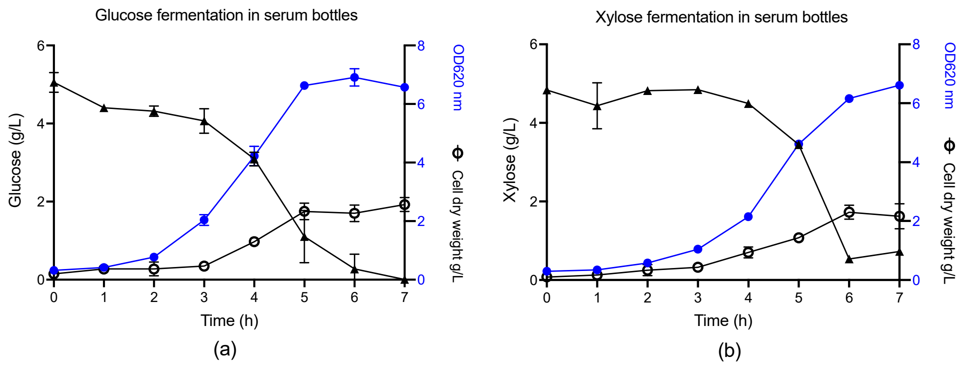
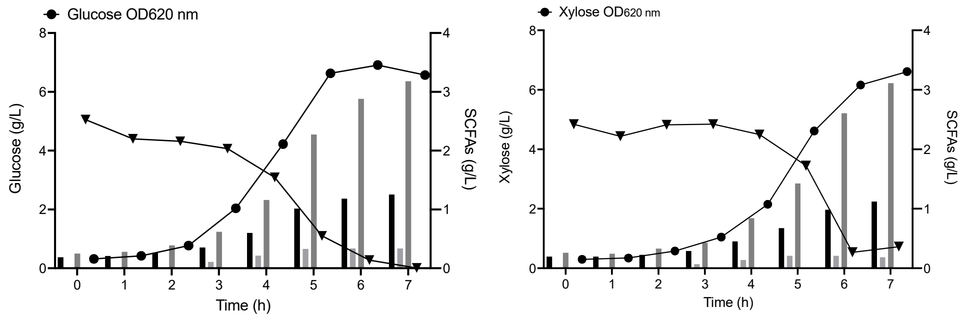


Figure 20 Growth profile of *Prevotella copri* DSM 18205^T in serum bottles (Paper IV).
Serum bottle cultivations using (a) PYG medium; (b) PYX medium. Symbols indicate (●) *P. copri* DSM 18205^T growth represented as $OD_{620\ nm}$; (○) *P. copri* DSM 18205^T cell dry weight; (▲) sugar consumption.

The production of succinic acid, acetic acid, and formic acid by the bacteria was achieved, with cell densities reaching 6–7.5. The highest yield of produced succinic acid was 0.63 ± 0.05 g/g glucose in PYG medium cultivations and 0.88 ± 0.06 g/g xylose in PYX medium cultivations (Figure 21).



(a) (b)

Figure 21 Organic acid profile and sugar consumption in anaerobic cultivation of *Prevotella copri* DSM 18205[T] (Paper IV).
Serum bottle cultivations using (a) PYG medium; (b) PYX medium. Symbols indicate: (●) *P. copri* DSM 18205[T] growth represented as $OD_{620\ nm}$; (▼) sugar consumption, black column represented acetic acid, light grey column represented formic acid, and dark grey represented succinic acid.

In addition, the findings of **paper IV** also showed, for the first time, that the *P. copri* strain could tolerate oxygen during inoculations and culture transfers. However, successful inoculations must use exponential phase cultures to maintain viable cell cultures. Furthermore, the successful scaling up of the cultivation to a stirred tank bioreactor provides a solid base for studying the bacterium and opens opportunities for more efficient production.

6.7 Concerns and reflection

Translating results from mice to humans requires considering the similarity of experimental conditions. For instance, a study with a diet uncommon in humans can make findings hard to apply. Caution must be taken when extrapolating from animal studies to humans due to potential species differences.

Additionally, research on working memory is ongoing, and its limitations, such as capacity, processing speed, and retention time, remain unclear. Minor changes in study methodology can lead to different results, and the findings may not be applicable to other situations, or may not be generalizable.

7 Concluding remarks and future perspectives

In **paper I-III,** the work delves into a novel research area by exploring the potential effects of several edible wild Nordic berry species on brain function and memory. This series of studies is the first of its kind to examine these less studied berries in relation to cognitive function. By investigating the underlying mechanisms that may lead to cognitive improvement, the research provides valuable insights into the potential benefits of consuming Nordic berries.

The findings in this thesis demonstrate that:

Among the berries being screened and studied, lingonberry (*Vaccinium vitis-idaea*), bilberry (*Vaccinium myrtillus*), blackcurrant (*Ribes nigrum*), cloudberry (*Rubus chamaemorus*), sea buckthorn (*Hippophae rhamnoides*) and blueberry (*Vaccinium ashei*) are shown to have potential positive impact on cognitive function in spatial memory as well as recognition memory in C57BL/6J mice, with varying degrees of effectiveness.

Further evaluation of the intake of a berry mixture containing lingonberries and bilberries demonstrates that these berries can also significantly enhance spatial memory performance in C57BL/6J mice fed a HF diet. The cognitive benefits of these berries may be due to their ability to modulate neuroinflammation and enhance synaptic function in the hippocampus, a crucial brain region responsible for spatial learning and memory.

Lingonberry and its soluble and insoluble fiber fractions show promising effects in reducing body weight gain caused by HF feeding in ApoE−/− mice, in addition, mice receiving lingonberry and its insoluble fraction had significantly improved glucose response. The berry mixture may help to lower body weight and fasting insulin levels in C57BL/6J mice fed a HF diet.

The intake of lingonberry, blackcurrant, cloudberry, sea buckthorn or blueberry is linked to a more diverse and robust gut microbiota, promoting a shift in gut microbiota composition. Additionally, the supplementation of lingonberry and its insoluble fiber fraction, bilberry or sea buckthorn to a HF diet resulted in a significant increase in the relative abundance of the gut bacterium *Akkermansia muciniphila*.

The research conducted in **papers I-III** contributes significantly to the expanding research on the potential benefits of berries for brain health, metabolic function, and gut microbiota research. The findings establish Nordic berries as promising candidates for dietary interventions to prevent cognitive impairment. Given the current global trend of an aging population, incorporating a diverse range of berries into one's diet could be a highly effective approach for promoting healthy aging and reducing the risk of cognitive decline.

When the research for **paper IV** was conducted, there was a lack of information regarding the bacterial phenotypic characterization, cultivation techniques, and growth conditions of *Prevotella copri* DSM 18205^T. As a result, the research conducted in **paper IV** was critical in advancing the scientific understanding of this bacterial strain. The study explored the growth conditions and characteristics of the bacterium, providing valuable information about how it behaves and responds to different environments. By conducting this research, the authors were able to fill a significant gap in knowledge about *Prevotella copri* DSM 18205^T, which has helped to further research and understanding of the bacterium's potential benefits for human health.

Future perspective:

- We have described how berry intake modifies the gut microbiota. Further studies could evaluate the impact of berry intake on the gut microbiota. This could involve assessing the production of SCFAs, conducting gut barrier permeability tests, measuring levels of gut inflammation markers, and examining the gene expression of intestinal tissues.
- Mice brain samples could be used to conduct immunohistology and examine microglia morphology and activation, which could provide further insights into the effects of berry intake on brain function and neuroinflammation.
- For future preclinical or clinical studies, the metabolomics of polyphenols in the bloodstream could be analyzed right after berry intake, both in the short and long term. This could reveal valuable information about the bioavailability and metabolism of polyphenols in the body.
- The precise characterization of the fiber content in berries could be achieved through analytical techniques such as mass spectrometry and high-performance liquid chromatography, and biochemical analysis.
- Human intervention studies could be conducted to test the effects of consuming a berry drink, both in the acute and long term. This could provide essential information about the potential health benefits of berries in humans.
- Fermenting berries with potential probiotic strains or conducting in vivo cultivation could offer further insights into the gut microbiota's response to

berry intake. This could be followed up by additional studies examining the effects of berry intake on the gut microbiota in humans.

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I still think that a brief visit to Sweden to finish the final internship in my master's program in Europe turned out to be one of the best decisions I have made, so the first thanks go to myself for that decision!

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To Johan's family **Eva & Alf, Lars & Elisabeth, Thérèse & Jacob, Mormor Barbro & Morfar Bertil, Farmor Ulla**. Thank you for welcoming me to the family! I feel extremely lucky that you all have an open mind to understand, respect, and appreciate a person like me from the another side of the world, carrying a different background, culture, and mother tongue. You show your love and care in the most genuine ways, and I am very grateful for your unwavering support and encouragement throughout these years. Thank you for being there, always. Tack till er alla!

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当我写下这些文字提及我的家人时，心中充满了感激。我的爸爸妈妈和姐姐是善良、真诚、有趣的人。在我和昕的成长过程中，我的父母总是习惯给予我们信任和鼓励。我和我的姐姐昕有着相互陪伴支持，共同成长的美好记忆。2023年，博士答辩了，疫情也过去了，真好。希望我们一家人以后都快快乐乐，健健康康，还有很多地方要一起去玩耍！

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An aging population is associated with an increased prevalence of cognitive decline and related diseases. This thesis investigates the cognitive and health benefits of Nordic berries in mice fed a high fat diet, demonstrating that various berries, especially a mix containing predominantly lingonberries and bilberries, positively impact brain function and improve spatial and learning memory. Berry supplementation also helps to reduce body weight gain and alters the gut microbiota composition in a beneficial way. Our findings suggest that incorporating berries into dietary strategies may aid in mitigating the growing prevalence of cognitive decline and related diseases among the aging global population.

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