Metabolic health, vascular function and cognition

Metabolic health, vascular function and cognition:

The effects of diet

Elske Gravesteijn

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TABLE OF CONTENTS

Chapter 1

General introduction

Obesity

Overweight and obesity are contributing to a growing global epidemic. According the World Health Organization, 39% of the adult population in 2016 was overweight and 13% was even obese [1]. In 2019, 15.6% of women and 12.6% of men in the Netherlands were obese [2]. Overweight is defined as having a body mass index (BMI) between 25 - 30 kg/m^2 and obesity as having a BMI >30 kg/m^2. Despite the fact that BMI is not a true measure of body fatness [3], subjects with an elevated BMI are generally characterized by excessive fat deposition [4]. In general, the underlying cause is an imbalance between caloric intake and energy expenditure [5].

Regarding energy intake, it could be that brain circuits related to appetite and addiction, and central hormonal signalling pathways contribute to the development of obesity [6-8]. Hyperactivation in reward circuitries in response to palatable foods may lead to overeating [9]. In obese individuals there may be an imbalance between brain circuits responsible for motivation and brain circuits responsible for inhibition [6]. The same accounts for hormones and gut peptides from adipose tissue, the pancreas, and the gastrointestinal tract that forward signals related to hunger and satiety to the hypothalamus and its various circuits. For example, with the presence of leptin resistance in obese individuals, the role of the anorexigenic hormone leptin to lower the reward value of food and to downregulate hunger, is diminished.

Both overweight and obesity are associated with an increased risk of developing non-communicable diseases such as type 2 diabetes mellitus, cardiovascular diseases, and some types of cancer [10]. Some aspects of these pathologies are criteria of the metabolic syndrome [4].

Metabolic syndrome

The metabolic syndrome is a cluster of several conditions that occur together. According the definition of the International Diabetes Federation [11], at least having abdominal obesity defined as a BMI ≥30 kg/m^2 or a waist circumference ≥94 cm (men) or ≥80 cm (women), and two or more of the following criteria: elevated fasting plasma glucose ≥5.6 mmol/L, systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, triglycerides ≥1.7 mmol/L, and low high-density lipoprotein cholesterol <1.03 mmol/L (men) or <1.29 mmol/L (women). These values may vary depending on ethnicity. Abdominal obesity and insulin resistance are the main underlying causes [12], but also age, gender, and race are related to the prevalence of metabolic syndrome [13]. In

contrast to peripheral obesity marked by an accumulation of subcutaneous adipose tissue, abdominal obesity is marked by an accumulation of visceral adipose tissue [14]. The latter adipose depot has a higher lipolytic activity and releases more free fatty acids, which results in increased glucose, contributing to the development of the metabolic syndrome [15]. The metabolic syndrome is associated with an increased risk of developing type 2 diabetes mellitus, cardiovascular diseases [12], and possibly cognitive impairment [16].

(Pre)diabetes

On a global scale, type 2 diabetes is one of the main underleading causes of death [17]. According the International Diabetes Federation, in 2019 8.3% of the population worldwide and 5.4% of the population in the Netherlands was diagnosed with type 2 diabetes. The prevalence of established type 2 diabetes in the Netherlands is 1.2 million and, in addition, an estimated 1.1 million have prediabetes [18].

Prediabetes is an intermediate state between healthy and type 2 diabetes [19]. It is characterized by an increased plasma glucose concentration and, therefore, part of the metabolic syndrome. A state of hyperglycaemia is associated with developing diabetes, but also cardiovascular diseases [19]. However, prediabetes is often not accompanied by clinical signs or symptoms which results in an underestimation of the prevalence rates [20]. An oral glucose tolerance test (OGTT) can be used for diagnosis where a fasting blood glucose sample is taken and a second one 2 hours after ingestion of 75 g oral glucose [21]. Three distinct prediabetic states are described by the World Health Organization: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or a combination of both. IFG is defined by a fasting plasma glucose concentration between 6.1 - 6.9 mmol/L and a 2-h plasma glucose concentration <7.8 mmol/L after the OGTT, whereas IGT is defined by a fasting plasma glucose concentration <7.0 mmol/L and a 2-h plasma glucose concentration between 7.7 - 11.1 mmol/L.

Insulin sensitivity

Insulin is a hormone which is secreted by the beta cells of the pancreas in both a fasted and postprandial state in order to maintain glucose homeostasis [6]. It is responsible for the uptake of glucose into various tissues where it can be either used as energy source or converted and stored as glycogen in liver and muscle or as fat in adipose tissue. Insulin

can also cross the blood-brain barrier where it plays a role in energy homeostasis and cognitive function [22].

Insulin sensitivity refers to the body's responsiveness to the expected physiological effects of insulin. Insulin sensitivity and beta-cell function are impaired in prediabetes [23]. Changes in plasma glucose concentrations during the postprandial state are normally regulated by intestinal absorption, suppression of endogenously produced glucose, and glucose uptake followed by glycogen storage [19]. However, in the development of diabetes, endogenous glucose production is linearly related to fat-free mass, which is often increased with higher BMI, and not substantially suppressed after glucose consumption [24]. Consequently, insulin secretion and beta-cell volume are increased to compensate for increased glucose concentrations [19]. Concomitantly, the prolonged excess of insulin results in insulin resistance in which insulin receptors are less responsive in opening glucose channels for glucose uptake, leading to further hyperglycaemia [20]. The next stage is the inability to compensate for insulin resistance [25], and malfunction and apoptosis of beta-cells occur [26]. Therefore, persistent hyperglycaemia that defines prediabetes is caused by beta-cell dysfunction marked by an impaired response to increased glucose concentrations [24].

Hyperinsulinemic euglycemic clamp

There are multiple methods to measure insulin sensitivity, of which the hyperinsulinemic euglycemic clamp is the gold standard [27]. During a clamp, a hyperinsulinemic state is reached by a constant insulin infusion. Euglycemia is reached upon a variable glucose infusion rate (GIR) depending on the plasma glucose concentration. The target is a steady glucose concentration of 5.0 mmol/L by adjusting the GIR. This steady state is aimed two hours after insulin infusion. The GIR equals the physiological rate of glucose utilization, or the glucose metabolized: the M-value. It can be calculated by subtracting urinary loss of glucose and the space correction from the GIR. Glucose space correction is a factor to compensate for changes in glucose concentration in the steady state. Under these conditions, the clamp is a measure of tissue sensitivity to exogenous insulin and the M-value a measure of whole-body insulin sensitivity.

Vascular function

The vascular system is responsible for the transport of blood throughout the body to deliver oxygen and nutrients, and remove cellular waste products [28]. It consists of a network of arteries and veins (macrovasculature), and arterioles, capillaries and venules

(microvasculature; [29]). The endothelium is a layer of endothelial cells that line the blood vessels. One of the many functions of the endothelial cells is to release substances, such as nitric oxide, that control vasoconstriction and vasodilation [30]. The ability of vascular dilation is related to cardiovascular health. Peripheral vascular function covers the vasculature in the periphery, while central vascular function covers the vasculature in the brain, with crosstalk between peripheral tissues and the central nervous system [31]. Systolic blood pressure is the pressure waveform peak due to ventricular contraction, whereas diastolic blood pressure is the pressure waveform trough [32]. In the condition of hypertension, blood pressure against the artery walls is increased. Obesity and diabetes are associated with hypertension [33]. In case of diabetes and hypertension combined, there is an increased risk of macrovascular and microvascular complications.

Vascular function can be disrupted in multiple ways resulting in, for example, arterial stiffness or endothelial dysfunction. Arterial stiffness is the inability to flexibly adjust to changes in blood pressure via vascular distensibility [34], whereas endothelial dysfunction is characterized as an imbalance between vasoconstrictors and vasodilators mainly caused by inflammation and oxidative stress [35]. It can contribute to the development of atherosclerosis and its complications [36]. Insulin resistance and hyperglycaemia play an important role in the pathogenesis of endothelial dysfunction [37]. Next to glucose metabolism, insulin also exerts control on the endothelium where it stimulates the production of the vasodilator nitric oxide and vasoconstrictor endothelin-1 [38]. However, because of insulin resistance there is an imbalance between these two substances contributing to endothelial dysfunction. Moreover, the accumulation of glucose causes damage to endothelial cells [39].

Pulse Wave Analysis

Arterial stiffness and endothelial function can be measured by analyzing the aortic pressure waveform with pulse wave analysis (PWA; [40]; **Figure 1.1**). The peak of the aortic pressure waveform reflects the systolic pressure, whereas the trough reflects the diastolic pressure, which together reflect the pulse pressure. As the properties of the arterial system differ, with large elastic arteries delivering blood to muscular arteries in the periphery, the waveform consists of a forward and backward wave [34]. The forward wave results from ventricular contraction, and the backward wave from amplification of the forward wave concomitant with a partial wave reflection due to the varying properties of the vascular system. The augmentation pressure is the additional pressure from arrival of the backward wave. When expressed as the percentage of pulse pressure,

the augmentation index (AIx) is derived. Since the AIx is dependent on the heart rate, it is often normalized for a heart rate of 75 beats per minute (AIxHR75). In case of arterial stiffness, the backward wave is conducted faster amplifying the pulse pressure which results in an increased AIx. The AIx is increased in individuals with obesity [41] and diabetes [42].

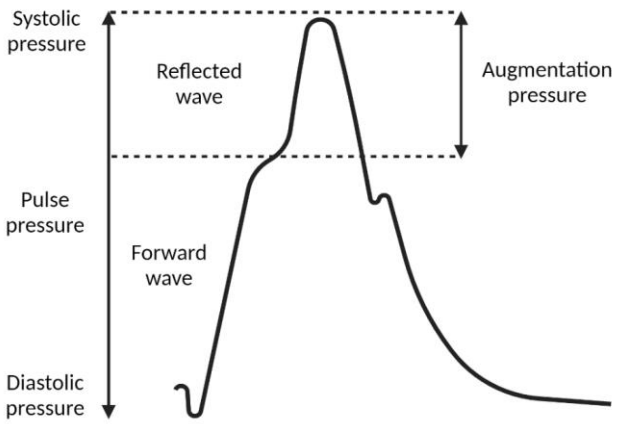

Figure 1.1 | Aortic pressure waveform. Adapted from "Assessments of arterial stiffness and endothelial function using pulse wave analysis" by L. Stoner *et al.*, 2012, *International Journal of Vascular Medicine, 2012,* p. 2. Created by BioRender.com.

Pulse Wave Velocity

PWA is considered an indirect measure of arterial stiffness [34]. However, pulse wave velocity (PWV) is a measure of regional arterial stiffness [40]. PWV is the propagation rate of the pressure pulse waveform [43], and can be measured at different sites. Carotid-to-femoral PWV ($PWV_{c\text{-}f}$) is considered the gold standard which reviews central arterial stiffness, whereas carotid-to-radial PWV ($PWV_{c\text{-}r}$) reviews peripheral muscular arterial stiffness [44]. PWV is assessed by dividing the distance by the transit time of the arterial waveform between two arterial sites, based on the foot of the pulse [34]. Due to the varying properties of the arteries, PWV changes from 4 - 6 m/s in the aorta to 8 - 9 m/s in the iliac and femoral arteries [45]. $PWV_{c\text{-}f}$ >13 m/s is a strong predictor of cardiovascular mortality [46], and is increased in individuals with (pre)diabetes [47].

Brain vascular function

The brain demands a constant blood flow to meet a healthy cerebrovascular function [48]. The cerebrovascular network shows similarities with the peripheral vascular

network, but utilizes most of the blood glucose [49]. The blood-brain barrier is at the interface of these two networks. It mainly consists of endothelial cells and limits transport by its physical and metabolic properties [50]. The vertebral arteries and the internal carotid arteries are responsible for the blood supply from the periphery to the brain [51]. A physiological marker of cerebrovascular function is cerebral blood flow (CBF; [52]), which is associated with brain activity [53]. CBF is expressed as the arterial blood volume transported to a unit of brain tissue mass per time unit, mostly milliliters of blood per 100 grams of brain tissue per minute. An average CBF is 60 mL/100 g/min [54], although perfusion decreases with age [55] as well as with obesity [56] and type 2 diabetes [57].

Arterial spin labelling

A non-invasive assessment of cerebral perfusion is arterial spin labelling (ASL) magnetic resonance imaging (MRI). This technique uses water molecules in arterial blood magnetically labelled by radiofrequency pulses to image tissue perfusion. In other words, by changing the magnetization of the blood, it functions as a tracer that signals the amount and location of the labelled blood to target tissues. The acquired image contains a signal from both labelled blood and static tissues. The signal of the labelled blood is isolated by subtracting a second control image that is acquired without prior labelling. The greater the blood flow, the higher the signal [52]. The site of labelling is in the neck where the blood flows via the arterial vessels to the capillary beds of the different brain tissues [58]. After labelling, there is a delay to let the labelled blood reach the capillaries before labelling is repeated to acquire the next image. Advantages of ASL are the accurate, absolute, and reproducible CBF measurements. In contrast, disadvantages are the low signal-to-noise ratio which increases the acquisition time, and the limited spatial resolution [59]. Besides global CBF, regional CBF can be assessed which is a valid marker of local neuronal activity [60].

Cognition

The brain is central to the nervous system and can be divided in gray and white matter. Gray matter regions are mainly found in the outer part of the cortex and comprised of neuronal cell bodies, whereas white matter regions are mainly found in the inner part of the cortex and comprised of axons that connect neurons [61]. Besides nerves, the brain contains blood vessels that together form the cerebrovascular network. Cerebrovascular function plays a central role in cognition [62]. When CBF is reduced as a result of

cerebrovascular dysfunction, cognition is impaired [48]. Cognition refers to the mental processes involved in acquiring knowledge and the ability to comprehend, such as sensation, attention, perception, memory, learning, language, problem solving, decision making, reasoning, and intelligence [63]. These higher cognitive functions are supported by the white matter in the prefrontal cortex that represents dense neural connectivity and information processing [64]. Neural transmission is underlying cognition as it covers the transfer of electrical and chemical signals which are prone to plastic changes [61].

Cognitive performance employs the objective assessment in terms of domains of functioning. The six key cognitive domains are perceptual-motor function, attention, memory, social cognition, executive function, and language ([65]; **Figure 1.2**). Each domain includes subdomains referring to elements of the cognitive processes. First, perceptual-motor function is used to understand and act upon information that is perceived from the senses. Second, attention implies the ability to focus on a specific stimulus in the environment. Third, memory is critical to learning and is responsible for the ability to encode, store, and retrieve information. Fourth, social cognition is involved in social interactions. Fifth, executive function integrates and controls more basic sensory and perceptual processes for reasoning and problem solving. Last, language includes the ability to understand and express thoughts through verbal and written communication [66]. With cognitive impairment there is loss in these cognitive abilities. The gold standard in cognitive assessments across all domains is the Cambridge Neuropsychological Test Automated Battery (CANTAB; [67, 68]), which can be applied to various clinical populations.

Brain-derived neurotrophic factor

A key molecule to cognition is brain-derived neurotrophic factor (BDNF; [69]). This protein is part of the neurotrophin family, which induces neural survival, development, function, and plasticity [70]. BDNF is mainly produced in the central nervous system, but also by peripheral tissues such as the endothelium [71]. Through a multistage process BDNF is synthesized from the precursor proneurotrophin isoform of BDNF (pro-BDNF) to the mature isoform (m-BDNF; [72]). However, both isoforms are released which contributes to different functions in different stages of brain development: during development BDNF stimulates neural survival and differentiation, whereas in adult life it regulates synaptic transmission and plasticity [73], in order to control communication efficacy between neurons. BDNF is highly expressed in the hippocampus, a memory related brain region, where it improves all forms of plasticity and, thereby, learning and memory. Upon frequent activation, synaptic strength is increased. This so-called

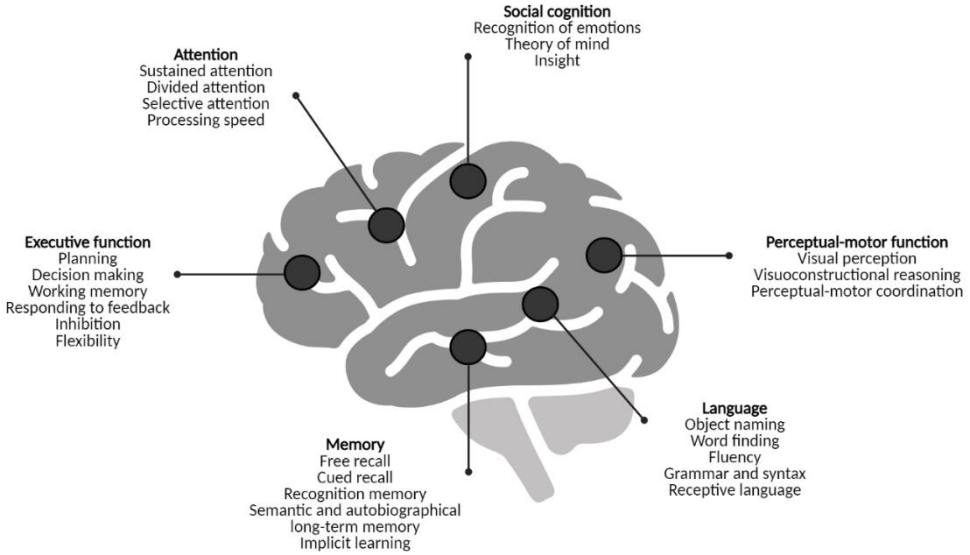

Figure 1.2 | Cognitive domains. Adapted from "Classifying neurocognitive disorders: the DSM-5 approach" by P.S. Sachdev *et al.*, 2014, *Nature Reviews Neurology, 10,* p. 636. Created by BioRender.com.

long-term potentiation is induced by BDNF-mediated pathways, supporting memory formation and maintenance [74]. Higher BDNF concentrations seem to be associated with improved cognitive performance [73].

BDNF also acts as a regulator in energy metabolism [75]. Via the receptors tropomyosin related kinase B (TrkB) and p75 neurotrophin receptor (p75NTR) it modulates energy intake, energy expenditure, and, consequently, weight [76]. This is demonstrated by hyperphagia, obesity, and hyperglycaemia with reduced BDNF concentrations. The two receptors are mainly found in the hypothalamus, a feeding related brain region, with TrkB having an anorexigenic effect and p75NTR an orexigenic effect. Beyond the brain, BDNF has peripheral actions as TrkB in the pancreas stimulates the release of insulin, and p75NTR in adipose tissue decreases lipolysis. Together with this role in insulin homeostasis, there is a role in glucose homeostasis.

There are several genetic and environmental factors that are able to influence BDNF [73]. Val66Met is a polymorphism in the BDNF gene that influences BDNF secretion [77]. Both stress and sleep deprivation decrease BDNF concentrations [78], whereas physical activity has the opposite effect [79]. Disturbances in BDNF signalling can lead to the

pathogenesis of diseases such as Alzheimer's disease, and disorders such as depression [69]. More importantly, BDNF concentrations can be modulated by nutrition [80].

Lifestyle

An unhealthy lifestyle is related to obesity [81], metabolic syndrome [82], diabetes and prediabetes [19], cardiovascular disease [83], cognitive impairment [73], and many other pathologies [84]. In order to improve public health, lifestyle interventions may be the most effective way. Lifestyle modifications can prevent, attenuate, or even reverse some pathological developments [85]. For example, a diabetes dietary intervention prevention program (lowering dietary saturated fat intake, and increasing fiber intake) combined with exercise showed a reduced diabetes risk [86]. Another intensive lifestyle intervention aimed at diet and exercise showed health effects on vascular function [87]. Furthermore, implementing a healthy diet rich in fruits and vegetables maintained cognitive health and prevented cognitive decline [88]. These are significant benefits of implementing lifestyle interventions, besides that they are cost-effective, easily accessible, and often improve general well-being [89]. In this thesis, the focus lies on the nutritional aspect of the healthy lifestyle advices. Dietary interventions can vary from nutrition education to caloric restriction and weight loss, in combination with a healthy diet. In the Netherlands, the evidence-based dietary guidelines are practically translated into the Wheel of Five [90]. In the latest version, the focus shifted from nutrients to foods and whole diets. Macronutrients are the main components of food consisting of fat, carbohydrates, and protein. According to the National Institute for Health and Environment, the composition of an average Dutch diet in 2016 was 35 En% fat, 45 En% carbohydrates, 15 En% protein, and the remaining 5 En% from fiber and alcohol. The European Food Safety Authority's (EFSA), provide the following dietary reference values: 20 - 35 En% fat, 45 - 60 En% carbohydrates, and 12 - 20 En% protein.

Dietary guidelines recommend to consume a handful of unsalted nuts every day. Nut consumption is encouraged from a sustainable perspective as alternative protein source, but also for cardiovascular prevention [91]. Nuts are predominantly composed of fats, with a high mono- and polyunsaturated fatty acid content, and a low saturated fatty acid content [92]. Furthermore, they are rich in fiber, vitamins, minerals, and polyphenols. Specifically almonds (*Prunus dulcis*) contain high amounts of protein, fiber, riboflavin, niacin, α-tocopherol, and calcium [93]. The health benefits of almonds seem to positively impact satiety and weight management [94], lipid metabolism [95], and possibly glucose metabolism [96]. Besides the dietary guidelines there are also several functional food ingredients. Functional foods are foods that have a positive health effect, besides

providing nutrients and energy. The egg protein hydrolysate NWT-03 is considered a functional food, which seems to improve cardiovascular health [97].

Thesis outline

The aim of this thesis was to investigate the effects of nutritional interventions on insulin sensitivity, vascular function, and cognition in humans. Moreover, the relations between these outcomes are investigated. In chapter 2 the existing literature on the effects of nutritional interventions on BDNF concentrations have been systematically reviewed. By targeting BDNF changes via nutrition, cognitive and metabolic health could be improved. In chapter 3 results are presented of a randomized controlled trial evaluating the effects of an egg protein hydrolysate (NWT-03) on cognitive function. Consuming this functional food ingredient already showed improvements in peripheral vascular function. This research was performed in individuals with the metabolic syndrome. In chapter 4 results are presented of another randomized controlled trial comparing the effects of the three dietary macronutrients side by side on BDNF concentrations, with a focus on acute postprandial effects. This research was performed in overweight and obese individuals. In chapter 5 the relations are described between insulin sensitivity, vascular function, and cognition. Insulin sensitivity was measured with a hyperinsulinemic euglycemic clamp. Data were analyzed from the control period of a randomized controlled trial on almonds in individuals with prediabetes in order to investigate whether this state is already accompanied by pathologies. In chapter 6 the results are presented of this randomized controlled trial examining the effects of almonds on various aspects of glucose metabolism such as whole-body insulin sensitivity, postprandial glucose responses, and fee-living glucose patterns. In chapter 7 an overview is provided of the main research findings of this thesis and combined to a general discussion.

REFERENCES

1. World Health Organization. Obesity and overweight fact sheet. June 2021. [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. [Accessed on 17/08/2021].
2. Statista. Prevalence of obesity among adults by country. August 2021. [Internet]. Available from: https://www.statista.com/statistics/236823/prevalence-of-obesity-among-adults-by-country/. [Accessed on 21/09/2021].
3. Liu P, Ma F, Lou H, Liu Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. BMC Public Health. 2013;13(629).
4. Gonzalez-Muniesa P, Martinez-Gonzalez MA, Hu FB, Despres JP, Matsuzawa Y, Loos RJF, *et al*. Obesity. Nature Reviews Disease Primers. 2017;3:17034.
5. Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012;126(1):126-32.
6. Zhang Y, Liu J, Yao J, Ji G, Qian L, Wang J, *et al*. Obesity: pathophysiology and intervention. Nutrients. 2014;6(11):5153-83.
7. Yeung AY, Tadi P. Physiology, Obesity Neurohormonal Appetite and Satiety Control. November 2020. In: StatPearls. [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555906/.
8. Han Y, Xia G, He Y, He Y, Farias M, Xu Y, *et al*. A hindbrain dopaminergic neural circuit prevents weight gain by reinforcing food satiation. Science Advances. 2021;7.
9. Kenny PJ. Reward mechanisms in obesity: New insights and future directions. Neuron. 2011;69(4):664-79.
10. Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. Overweight and obesity: Prevalence, consequences, and causes of a growing public health problem. Current Obesity Reports. 2015;4(3):363-70.
11. International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome. July 2020. [Internet]. Available from: https://idf.org/our-activities/advocacy-awareness/resources-and-tools/60:idfconsensus-worldwide-definitionof-the-metabolic-syndrome.html. [Accessed on 18/08/2021].
12. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112(17):2735-52.
13. Razzouk L, P. M. Ethnic, gender, and age-related differences in patients with the metabolic syndrome. Current Hypertension Reports. 2009;11:127-32.
14. Wang R, Li X. Different adipose tissue depots and metabolic syndrome in human. Acta Physiologica Sinica. 2017;69(3):357-65.
15. Bjørndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. Journal of Obesity. 2011;2011:490650.
16. Assuncao N, Sudo FK, Drummond C, de Felice FG, Mattos P. Metabolic Syndrome and cognitive decline in the elderly: A systematic review. PLoS One. 2018;13(3):e0194990.
17. World Health Organization. The top 10 causes of death. December 2020. [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. [Accessed on 24/08/2021].
18. Diabetes Fonds. Diabetes in cijfers (in Dutch). 2019. [Internet]. Available from: https://www.diabetesfonds.nl/over-diabetes/diabetes-in-het-algemeen/diabetes-in-cijfers. [Accessed on 24/08/2021].
19. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. The Lancet. 2012;379(9833):2279-90.

20. Alvarez S, Coffey R, Algotar AM. Prediabetes. StatPearls Publishing; 2021.
21. Eyth E, Basit H, Smith CJ. Glucose Tolerance Test. Treasure Island (FL): StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532915/.
22. Cetinkalp S, Simsir IY, Ertek S. Insulin resistance in brain and possible therapeutic approaches. Current Vascular Pharmacology. 2014;12(4):553-64.
23. Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of β-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care. 2006;29(5):1130-9.
24. Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of prediabetes. Medical Clinics of North America. 2011;95(2):327-39, vii-viii.
25. Bergman M. Pathophysiology of prediabetes and treatment implications for the prevention of type 2 diabetes mellitus. Endocrine. 2013;43:504-13.
26. Maedler K, Donath MY. Beta-cells in type 2 diabetes: a loss of function and mass. Hormone Research. 2004;62 Suppl 3:67-73.
27. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. American Journal of Physiology. 1979;237(3):E214-23.
28. Pugsley MK, Tabrizchi R. The vascular system. An overview of structure and function. Journal of Pharmacological and Toxicological Methods. 2000;44:333-40.
29. Yuan SY, Rigor RR. Regulation of endothelial barrier function. Chapter 2, Structure and Function of Exchange Microvessels. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54123/.
30. Hanson BE, Proffit M, Gifford JR. Vascular function is related to blood flow during high-intensity, but not low-intensity, knee extension exercise. Journal of Applied Physiology. 2020;128(3):698-708.
31. Goncalves RA, De Felice FG. The crosstalk between brain and periphery: Implications for brain health and disease. Neuropharmacology. 2021;197:108728.
32. Terentes-Printzios D, Gardikioti V, Vlachopoulos C. Central over peripheral blood pressure: An emerging issue in hypertension research. Heart, Lung and Circulation. 2021;30(11):1667-74.
33. Zanella MT, Kohlmann O, Ribeiro AB. Treatment of obesity hypertension and diabetes syndrome. Hypertension. 2001;38:705-8.
34. Shirwany NA, Zou MH. Arterial stiffness: a brief review. Acta Pharmacoligica Sinica. 2010;31(10):1267-76.
35. Hadi HAR, Carr CS, Al Suwaidi J. Endothelial dysfunction, cardiovascular risk factors, therapy, and outcome. Vascular Health and Risk Management. 2005;1(3):183-98.
36. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: A marker of atherosclerotic risk. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23(2):168-75.
37. Viigimaa M, Sachinidis A, Toumpourleka M, Koutsampasopoulos K, Alliksoo S, Titma T. Macrovascular complications of type 2 diabetes mellitus. Current Vascular Pharmacology. 2020;18(2):110-6.
38. De Souza AL, Batista GA, Alegre SM. Assessment of insulin sensitivity by the hyperinsulinemic euglycemic clamp: Comparison with the spectral analysis of photoplethysmography. Journal of Diabetes and its Complications. 2017;31(1):128-33.
39. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. Chronic complications of diabetes mellitus: A mini review. Current Diabetes Reviews. 2017;13(1):3-10.
40. Stoner L, Young JM, Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. International Journal of Vascular Medicine. 2012;2012:903107.
41. Tabara Y, Kohara K, Nakagawa S, Handa J, Hayashi M, Hamada C, *et al*. Effects of obesity and smoking on mental stress-induced blood pressure and augmentation index responses in normotensive young males: The J-SHIPP study. Hypertension Research. 2008;31(6):1219-24.

42. Ravikumar R, Deepa R, Shanthirani CS, Mohan V. Comparison of carotid intima-media thickness, arterial stiffness, and brachial artery flow mediated dilatation in diabetic and nondiabetic subjects. American Journal of Cardiology. 2002;90:702-7.
43. Yu S, McEniery CM. Central versus peripheral artery stiffening and cardiovascular risk. Arteriosclerosis, Thrombosis, and Vascular Biology. 2020;40(5):1028-33.
44. Gorgui J, Doonan RJ, Gomez YH, Kwong C, Daskalopoulou SS. Carotid endarterectomy improves peripheral but not central arterial stiffness. European Journal of Vascular and Endovascular Surgery. 2013;45(6):548-53.
45. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, *et al*. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European Heart Journal. 2006;27(21):2588-605.
46. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Hypertension. 1999;33:1111-7.
47. Stehouwer CD, Henry RM, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia. 2008;51(4):527-39.
48. Fouda AY, Fagan SC, Ergul A. Brain vasculature and cognition. Renin-angiotensin system, endothelin, and beyond. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019;39(4):593-602.
49. Tam SJ, Watts RJ. Connecting vascular and nervous system development: angiogenesis and the blood-brain barrier. Annual Review of Neuroscience. 2010;33:379-408.
50. Correale J, Villa A. Cellular elements of the blood-brain barrier. Neurochemical Research. 2009;34(12):2067-77.
51. Purves D, Augustine GJ, Fitzpatrick D, *et al*. Neuroscience. The Blood Supply of the Brain and Spinal Cord. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11042/.
52. Liu TT, Brown GG. Measurement of cerebral perfusion with arterial spin labeling: Part 1. Methods. Journal of the International Neuropsychological Society. 2007;13(3):517-25.
53. Bentourkia M, Bol A, Ivanoiu A, Labar D, Sibomana M, Coppens A, *et al*. Comparison of regional cerebral blood flow and glucose metabolism in the normal brain: effect of aging. Journal of the Neurological Sciences. 2000;181:19-28.
54. Joris PJ, Mensink RP, Adam TC, Liu TT. Cerebral blood flow measurements in adults: A review on the effects of dietary factors and exercise. Nutrients. 2018;10(5).
55. Parkes LM, Rashid W, Chard DT, Tofts PS. Normal cerebral perfusion measurements using arterial spin labeling: reproducibility, stability, and age and gender effects. Magnetic Resonance in Medicine. 2004;51(4):736-43.
56. Selim M, Jones R, Novak P, Zhao P, Novak V. The effects of body mass index on cerebral blood flow velocity. Clinical Autonomic Research. 2008;18(6):331-8.
57. Chau ACM, Cheung EYW, Chan KH, Chow WS, Shea YF, Chiu PKC, *et al*. Impaired cerebral blood flow in type 2 diabetes mellitus - A comparative study with subjective cognitive decline, vascular dementia and Alzheimer's disease subjects. NeuroImage Clinical. 2020;27:102302.
58. Ferre JC, Bannier E, Raoult H, Mineur G, Carsin-Nicol B, Gauvrit JY. Arterial spin labeling (ASL) perfusion: techniques and clinical use. Diagnostic and Interventional Imaging. 2013;94(12):1211-23.
59. Grade M, Hernandez Tamames JA, Pizzini FB, Achten E, Golay X, Smits M. A neuroradiologist's guide to arterial spin labeling MRI in clinical practice. Neuroradiology. 2015;57(12):1181-202.
60. Rosario de Natale E, Wilson H, Pagano G, Politis M. International Review of Neurobiology. Chapter Seven – Imaging Transplantation in Movement Disorders. 143. 2018. p. 213-63. [Internet]. Available from: https://doi.org/10.1016/bs.irn.2018.10.002.

61. Barha CK, Nagamatsu LS, Liu-Ambrose T. Handbook of Clinical Neurology. Chapter 4 – Basics of Neuroanatomy and Neurophysiology. In: Aminoff MJ, Boller F, & Swaab DF, editor; 2016. p. 53-68. [Internet]. Available from: https://doi.org/10.1016/B978-0-12-802973-2.00004-5.
62. Ozturk ED, Tan CO. Human cerebrovascular function in health and disease: insights from integrative approaches. Journal of Physiological Anthropology. 2018;37(1):4.
63. Smith A, Kelly A. The Encyclopedia of Adulthood and Aging. Cognitive Processes: Major Reference Works. 2016. 1-118-52892-1. 10.1002/9781118521373.
64. Schoenemann PT, Sheehan MJ, Glotzer LD. Prefrontal white matter volume is disproportionately larger in humans than in other primates. Nature Neuroscience. 2005;8(2):242-52.
65. Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, *et al*. Classifying neurocognitive disorders: the DSM-5 approach. Nature Reviews Neurology. 2014;10(11):634-42.
66. Harvey PD. Domains of cognition and their assessment. Dialogues in Clinical Neuroscience. 2019;21(3):227-37.
67. Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dementia. 1994;5:226-81.
68. Smith PJ, Need AC, Cirulli ET, Chiba-Falek O, Attix DK. A comparison of the Cambridge Automated Neuropsychological Test Battery (CANTAB) with "traditional" neuropsychological testing instruments. Journal of Clinical and Experimental Neuropsychology. 2013;35(3):319-28.
69. Lu B, Nagappan G, Lu Y. Handbook of experimental pharmacology. BDNF and synaptic plasticity, cognitive function, and dysfunction. Neurotrophic Factors. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. [Internet]. Available from: https://doi.org/10.1007/978-3-642-45106-5_9.
70. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annual Review of Neuroscience. 2001;24:677-736.
71. Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, *et al*. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Journal of Thrombosis and Haemostasis. 2002;87:728-34.
72. Kowiański P, Lietzau G, Czuba E, Waskow M, Steliga A, Morys J. BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. Cellular and Molecular Neurobiology. 2018;38(3):579-93.
73. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. Frontiers in Cellular Neuroscience. 2019;13:363.
74. Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? Frontiers in Molecular Neuroscience. 2010;3:1.
75. Marosi K, Mattson MP. BDNF mediates adaptive brain and body responses to energetic challenges. Trends in Endocrinology & Metabolism. 2014;25(2):89-98.
76. Podyma B, Parekh K, Guler AD, Deppmann CD. Metabolic homeostasis via BDNF and its receptors. Trends in Endocrinology and Metabolism. 2021;32(7):488-99.
77. Kuczewski N, Porcher C, Gaiarsa JL. Activity-dependent dendritic secretion of brain-derived neurotrophic factor modulates synaptic plasticity. European Journal of Neuroscience. 2010;32(8):1239-44.
78. Eckert A, Karen S, Beck J, Brand S, Hemmeter U, Hatzinger M, *et al*. The link between sleep, stress and BDNF. European Psychiatry. 2020;41(S1):S282-S.
79. Neeper SA, Gómez-Pinilla F, Choi J, Cotman C. Exercise and brain neurotrophins. Nature. 1995;373.

80. Gravesteijn E, Mensink RP, Plat J. Effects of nutritional interventions on BDNF concentrations in humans: A systematic review. Nutritional Neuroscience. 2021:1-12.
81. Baillot A, Romain AJ, Boisvert-Vigneault K, Audet M, Baillargeon JP, Dionne IJ, *et al*. Effects of lifestyle interventions that include a physical activity component in class II and III obese individuals: a systematic review and meta-analysis. PLoS One. 2015;10(4):e0119017.
82. Bo S, Ciccone G, Baldi C, Benini L, Dusio F, Forastiere G, *et al*. Effectiveness of a lifestyle intervention on metabolic syndrome. A randomized controlled trial. Journal of General Internal Medicine. 2007;22(12):1695-703.
83. Khanji MY, van Waardhuizen CN, Bicalho VVS, Ferket BS, Hunink MGM, Petersen SE. Lifestyle advice and interventions for cardiovascular risk reduction: A systematic review of guidelines. International Journal of Cardiology. 2018;263:142-51.
84. Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, *et al*. American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. Cancer Journal for Clinicians. 2012;62(1):30-67.
85. Barnes JN, Corkery AT. Exercise improves vascular function, but does this translate to the brain? Brain Plasticity. 2018;4(1):65-79.
86. Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, *et al*. The Finnish Diabetes Prevention Study (DPS) Lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care. 2003;26:3230-6.
87. Vamvakis A, Gkaliagkousi E, Lazaridis A, Grammatikopoulou MG, Triantafyllou A, Nikolaidou B, *et al*. Impact of intensive lifestyle treatment (diet plus exercise) on endothelial and vascular function, arterial stiffness and blood pressure in stage 1 hypertension: Results of the HINTreat randomized controlled trial. Nutrients. 2020;12(5).
88. Klimova B, Dziuba S, Cierniak-Emerych A. The effect of healthy diet on cognitive performance among healthy seniors - A mini review. Frontiers in Human Neuroscience. 2020;14:325.
89. Walsh R. Lifestyle and mental health. American Psychologist. 2011;66(7):579-92.
90. Brink EJ, Postma-Smeets A, Stafleu A, Wolvers D. The Wheel of Five (in Dutch). The Hague: Netherlands Nutrition Centre; 2017.
91. Ros E. Nuts and CVD. British Journal of Nutrition. 2015;113 Suppl 2:S111-20.
92. Kim Y, Keogh J, Clifton PM. Nuts and cardio-metabolic disease: A review of meta-analyses. Nutrients. 2018;10(12).
93. Kalita S, Khandelwal S, Madan J, Pandya H, Sesikeran B, Krishnaswamy K. Almonds and Cardiovascular Health: A Review. Nutrients. 2018;10(4).
94. Abazarfard Z, Salehi M, Keshavarzi S. The effect of almonds on anthropometric measurements and lipid profile in overweight and obese females in a weight reduction program: A randomized controlled clinical trial. Journal of Research in Medical Sciences. 2013;19:457-64.
95. Musa-Veloso K, Paulionis L, Poon T, Lee HY. The effects of almond consumption on fasting blood lipid levels: A systematic review and meta-analysis of randomised controlled trials. Journal of Nutritional Science. 2016;5:e34.
96. Ntzouvani A, Antonopoulou S, Nomikos T. Effects of nut and seed consumption on markers of glucose metabolism in adults with prediabetes: A systematic review of randomised controlled trials. British Journal of Nutrition. 2019;122(4):361-75.
97. Plat J, Severins N, Morrison S, Mensink RP. Effects of NWT-03, an egg-protein hydrolysate, on blood pressure in normotensive, high-normotensive and mild-hypertensive men and women: a dose-finding study. British Journal of Nutrition. 2017;117(7):942-50.

Chapter 2

Effects of nutritional interventions on BDNF concentrations in humans: A systematic review

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) plays an essential role in brain and metabolic health. The fact that higher concentrations are associated with improved cognitive performance has resulted in numerous intervention trials that aim at elevating BDNF levels. This systematic review provides an overview of the relation between various nutritional factors and BDNF concentrations in controlled human intervention studies.

Methods: A systematic search in May 2020 identified 48 articles that examined the effects of dietary patterns or foods (n = 3), diets based on energy intake (n = 7), vitamins and minerals (n = 7), polyphenols (n = 11), long-chain omega-3 polyunsaturated fatty acids (n = 5), probiotics (n = 8), and miscellaneous food supplements (n = 7).

Results: In particular, studies with dietary patterns or foods showed increased peripheral BDNF concentrations. There are also strong indications that polyphenols tend to have a positive effect on BDNF concentrations. Four of the 11 included studies with a polyphenol intervention showed a significant increase in BDNF concentrations, one study showed an increase but this was not statistically analyzed, and two studies showed a trend to an increase.

Conclusion: The two polyphenol classes, phenolic acids, and other phenolic compounds were responsible for the significant effects. No clear effect was found for the other dietary factors, which might also be related to whether serum or plasma was used for BDNF analysis. More work is needed to understand the relation between peripheral and central BDNF concentrations.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a protein that is mainly produced in the central nervous system [1]. Its function depends on the stage of brain development. In early life, BDNF plays an important role in neural development and functioning, whereas in adult life it is involved in processes such as synaptic transmission and synaptic plasticity, which contribute to cognitive function [2]. Indeed, previous studies have shown that lower BDNF concentrations are associated with cognitive impairment [3] and higher BDNF concentrations with improved cognitive performance [4]. It is well-known that lifestyle factors such as diet are contributing to mental and cognitive health [5]. Therefore, specific dietary changes could be an effective way to exert an effect on BDNF and thereby preserving and improving cognitive and metabolic health. In more detail, it seems that dietary factors can influence cognition via pathways related to energy metabolism and synaptic plasticity [6], in which BDNF could play a role.

BDNF can also be found in the periphery since it can cross the blood–brain barrier, albeit this has only been demonstrated in animal studies [7]. However, this circulating peripheral BDNF is not only brain-derived since it can also be produced by peripheral tissues including muscle, thymus, heart, liver, vascular smooth muscle cells, lung, and spleen [8]. This peripheral BDNF production seems to be similar between animals and humans [9]. In the periphery, the majority of BDNF is stored in platelets while the rest circulates in plasma [8]. There are indications that peripheral and central BDNF concentrations are positively associated [10], which suggests that concentrations of BDNF in the blood can be regarded as indicative of concentrations of BDNF in the central nervous system, though this conclusion remains open for discussion.

Although it has received the most attention, BDNF is not only relevant from the cognitive health perspective as evidence is also growing that BDNF also plays a role in other more peripheral oriented processes. Lower peripheral BDNF concentrations are not only associated with an impaired cognitive performance [11], but, for example, also with a higher body weight [12]. In this context, a combined central and peripheral role for BDNF was found in energy homeostasis, a finding which was first demonstrated in rats [13]. In that study, intraventricularly administered BDNF resulted in decreased energy intake and consequently body weight loss. More recently, it has been suggested that BDNF also acts as a metabolic modulator in humans by controlling and affecting patterns of food intake, physical activity, and glucose metabolism [14]. Moreover, BDNF mediates energy metabolism not only via the brain but also via peripheral neurons and target organs that are involved in maintaining energy balance. These various sites of action and functions

imply that BDNF could influence appetite, insulin sensitivity, and parasympathetic cardiovascular tone. If that is indeed the case, then there is a possibility that elevated BDNF concentrations could counteract the development of obesity and the metabolic syndrome [15].

Interventions aimed at increasing BDNF concentrations seem an attractive target to maintain or even improve cognitive performance as well as metabolic health. Despite numerous observations that specific dietary ingredients affect BDNF concentrations, the question remains whether these dietary factors indeed modulate BDNF concentrations. In this systematic review, we provide an overview of the potential relation between the consumption of different dietary factors and BDNF concentrations in controlled human intervention studies.

METHODS

Search strategy

The systematic review was based on the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) checklist [16]. This systematic review was not pre-registered with PROSPERO. A systematic literature search was performed on May 27th 2020 in three databases: Cochrane Central Register of Clinical Trials, Ovid MEDLINE, and Embase. Search terms consisted of (brain-derived neurotrophic factor or BDNF) combined with (diet or dietary or food or nutrient or nutrition or nutritional or supplement or supplements or supplementation or intake). Duplicates were removed and those studies that only included human were filtered out. However, this filter was impossible in Cochrane where all records had to be retained.

Selection criteria

The first phase of the selection procedure consisted of screening the titles and abstracts. This selection was performed independently by two researchers. When inconclusive, articles' eligibility was discussed by both researchers until agreement was reached. Articles were included if they met the following criteria: (1) intervention studies performed in human adult subjects (aged ≥ 18 years); (2) intervention with a nutritional component; (3) plasma or serum concentrations of BDNF are provided; (4) original research (*i.e.*, no letters, conference proceedings or reviews); (5) written in English; (6) no duplicates. The second phase of the selection procedure consisted of reading full

texts to assess their eligibility. Articles were excluded when no control group had been used.

Data collection

The data from the selected articles were extracted to create an overview that included: publication information (year of publication, first author); study and subject characteristics (design, sample size, specification of subgroups, mean age, gender); characteristics of the BDNF measurement (assay, unit, plasma or serum); and specifications of the intervention. If BDNF concentrations were expressed in ng/ml, units were converted to pg/ml. A software caliper package was used when data were only displayed in graphs (Onde Rulers; Ondesoft, Beijing, China). The intervention effect of studies with parallel designs was defined as the difference between the changes from baseline and intervention in the experimental and placebo groups. The intervention effect of crossover studies was defined as the difference between values obtained at the end of the experimental and control periods. In order to assess the methodological quality of the selected intervention studies, the Jadad score was calculated [17]. This five-point scale reviews the randomization process, blinding and the description of withdrawals (**Supplemental Table 2.1**).

RESULTS

Search results

The literature search retrieved 3641articles from the selected databases. Screening of titles and abstracts resulted in the exclusion of 2974 articles based on the predefined selection criteria. After reviewing the full texts of the remaining 132 articles, another 89 articles were excluded as they did not meet the selection criteria. Based on a search through references of the included articles five additional articles were included. Ultimately 48 articles met the inclusion criteria (**Figure 2.1**), which were then clustered based on the type of nutritional intervention. This resulted in the following clusters: dietary patterns or foods (n = 3); diets based on energy intake (n = 7); and supplements (n = 38). The latter category was further divided into the following sub-clusters: vitamins and minerals (n = 7); polyphenols (n = 11); long-chain omega-3 polyunsaturated fatty acids (n = 5); probiotics (n = 8); and miscellaneous: protein (extracts) or lipids (n = 7).

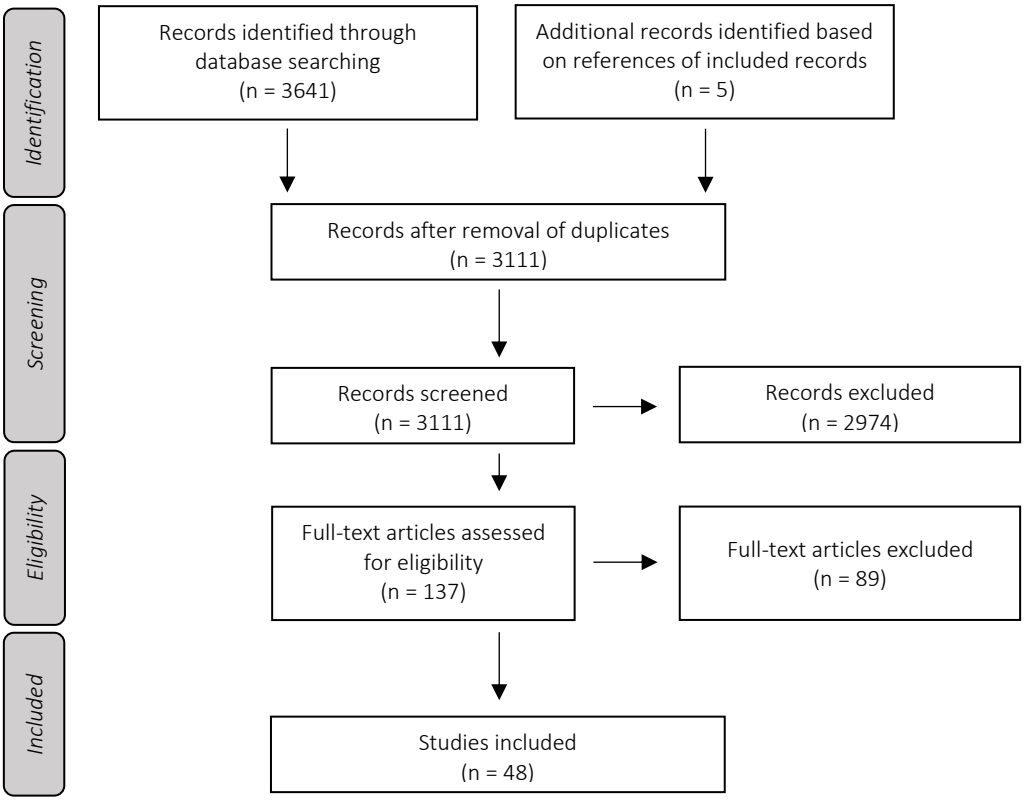

Figure 2.1 | PRISMA flowchart of the selection process.

Dietary patterns & foods

One study evaluated changes in BDNF concentrations based on dietary patterns, while two studies evaluated changes based on whole foods (**Table 2.1**). In a study by Sánchez-Villegas *et al.* [18], subjects with a high cardiovascular risk consumed a Mediterranean diet supplemented with virgin olive oil or mixed nuts or a low-fat control diet. Energy intake was not controlled for and subjects were divided into two groups according to the observed weight changes. No significant differences in plasma BDNF concentrations were observed between the three diets, and these effects did not depend on changes in body weight. In a second study, Sandberg *et al.* [19] showed that consuming a whole grain rye kernel-based bread increased plasma BDNF concentrations in apparently healthy subjects when compared with white wheat flour-based control bread. Finally, a study in mild cognitively impaired women revealed that consuming

mold-fermented cheese as compared with non-mold-fermented cheese led to a significant increase in serum BDNF concentrations [20].

Energy intake

In seven studies, energy intake was changed by various types of fasting and caloric restrictions (Table 2.1). The interventions were conducted in apparently healthy subjects [21–23], subjects with overweight [24] or obesity [25,26], and subjects with a mental disorder [27]. Only in this last study did a hypocaloric diet significantly increase serum BDNF concentrations, with the other six studies observing no effects.

Supplements

Vitamins and Minerals

Seven studies were identified that had evaluated the effects of supplementation with either vitamins or minerals (Table 2.1). Four studies examined the effects of zinc but all in different populations: in women with premenstrual syndrome [28], diabetic subjects [29], subjects diagnosed with depression [30], and obese subjects [31]. Zinc supplementation showed a significant increase in serum BDNF concentrations in the premenstrual syndrome and obese population, while the other two populations did not appear to respond significantly to zinc. Two studies examined the effect of vitamin D3 in apparently healthy subjects. No significant differences between the intervention and control groups were found by Pirotta *et al.* [32], but a significant decrease in serum BDNF concentrations was observed in the supplemented group in the study by Walentukiewicz *et al.* [33]. In the latter study, however, the difference in changes between the intervention and control arms was not statistically analyzed. Finally, one study examined the separate and combined effects of both zinc and vitamin D3 in subjects with depressive symptoms [34]. Here, both the zinc arm and the combined zinc and vitamin D3 arm showed a trend to decreased serum BDNF concentrations, but again the study, unfortunately, did not statistically analyze the difference in changes between the groups.

Polyphenols

The 11 studies included in the polyphenol cluster investigated the effect of supplementation of foods or supplements rich in phenols or flavonols (Table 2.1). Therefore, the type of polyphenols studied was markedly different. One study examined

Table 2.1 | Characteristics of the studies included per cluster in the systematic review.

First author (year)	Population	Experimental group / control (N)	Intervention	Duration	Effects on BDNF*
Dietary Patterns & Foods					
Sánchez-Villegas (2011)	At high cardiovascular risk	91 / 77	Mediterranean diet with virgin olive oil	3 y	=
		75 / 77	Mediterranean diet with mixed nuts		=
Sandberg (2018)	Healthy	19	Whole grain rye kernel-based bread	1-3 d	↑
Suzuki (2019)	Mild cognitive impaired	35	Mold fermented cheese	12 wks	↑
Diets Based on Energy Intake					
Carlson (2007)	Healthy	21	Reduced meal frequency	8 wks	=
Catenacci (2016)	Obese	15 / 14	Zero-calorie alternate-day fasting	8 wks	=
Cherif (2017)	Healthy	22	Islamic intermittent fasting	3 d	=
Khoshandam Ghashang (2019)	Healthy	25 / 25	Ramadan fasting	29 d	=
Guimaraes (2008)	Schizophrenic	25 / 42	Hypocaloric diet	11.5-26 mo	↑
Harvie (2011)	Obese	54 / 53	Continuous energy restriction	26 wks	=
Schübel (2018)	Overweight	47 / 51	Intermittent calorie restriction	12 wks	=
		46 / 51	Continuous calorie restriction		
Supplements					
Vitamins & Minerals					
Jafari (2019)	Premenstrual syndrome	27 / 30	Zinc gluconate	12 wks	↑
Kheirouri (2018)	Diabetic	25 / 25	Zinc gluconate	12 wks	=
Pirotta (2014)	Healthy	13 / 13	Vitamin D3	10 wks	=

Table 2.1 (continued) | Characteristics of the studies included per cluster in the systematic review

First author (year)	Population	Experimental group / control (N)	Intervention	Duration	Effects on BDNF*
Ranjbar (2014)	Depressed	22 / 22	Zinc sulfate	12 wks	=
Solati (2015)	Obese	24 / 26	Zinc gluconate	12 wks	↑
Walentukiewicz (2018)	Healthy	27 / 30	Vitamin D3	12 wks	-
Yosaee (2020)	Depressed	24 / 22	Zinc gluconate	12 wks	-
		27 / 22	Vitamin D3		-
		25 / 22	Zinc gluconate + vitamin D3		-
Polyphenols					
Choi (2016)	Healthy	40 / 40	*Eriobotrya japonica* Lindley	12 wks	=
Choi (2016)	Healthy	10 / 11	Fermented *Laminaria japonica*	8 wks	↑
Huhn (2018)	Healthy	30 / 30	Resveratrol	26 wks	=
Liu (2018)	Healthy	37 / 39	Ellagic acid	12 wks	=
	Overweight	38 / 36			↑
Osali (2020)	Metabolic syndrome	11 / 11 [a]	Nano-curcumin	6 wks	-
Reid (2018)	Healthy	32 / 28	Fermented *Laminaria japonica* A.	6 wks	↑
Sadowska-Krepa (2017)	Healthy	9 / 9	*Ginkgo biloba* extract	6 wks	=
Sadowska-Krepa (2019)	Healthy	8 / 8	Green tea extract	6 wks	-
Sumiyoshi (2019)	Healthy	10 / 8	Dark chocolate	4 wks	=
Witte (2014)	Overweight	23 / 23	Resveratrol	26 wks	=
Yu (2015)	Depressed	50 / 50	Curcumin	6 wks	↑

Table 2.1 (continued) | Characteristics of the studies included per cluster in the systematic review

First author (year)	Population	Experimental group / control (N)	Intervention	Duration	Effects on BDNF*
Long-Chain Omega-3 Polyunsaturated Fatty Acids					
Bot (2011)	Diabetic	13 / 12	E-EPA	12 wks	=
Burg, van der (2019)	Depressed	42 / 41	EPA + DHA	8 wks	=
Matsuoka (2015)	Traumatized	53 / 57	EPA + DHA	12 wks	=
Sedláček (2018)	Overweight	11 / 10	EPA + DHA	12 wks	=
Witte (2014)	Healthy	40 / 40	LC-n3-FA	26 wks	-
Probiotics					
Chung (2014)	Healthy	26 / 10	*L. helveticus* IDCC3801-fermented milk	12 wks	=
Haghighat (2019)	Hemodialysis + depressed	25 / 25	Prebiotics + *L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, *B. longum* BIA-8	12 wks	↑
		25 / 25	*L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, *B. longum* BIA-8		=
Hwang (2019)	Mild cognitive impaired	50 / 50	*L. plantarum* C29-fermented soybean	12 wks	-
Kim (2020)	Healthy	63	*B. bifidum* BGN4 + *B. longum* BORI	12 wks	↑
Pinto-Sanchez (2017)	Irritable bowel syndrome	22 / 22	*B. longum* NCC3001	6 wks	=
Riezzo (2018)	Constipated	28 / 28	*L. reuteri* DSM-17938	15 wks	-
Romijn (2017)	Depressed	28 / 36	*L. helveticus* + *B. longum*	8 wks	=

Table 2.1 (continued) | Characteristics of the studies included per cluster in the systematic review

First author (year)	Population	Experimental group / control (N)	Intervention	Duration	Effects on BDNF*
West (2014)	Healthy	46 / 51	*B. animalis* subsp. *lactis* BI-04	21 wks	=
		47 / 51	*L. acidophilus* NCFM + *B. animalis* subsp. *lactis* BI-07		=
Miscellaneous: Protein (Extracts) & Lipids					
Almeida, de (2019)	Healthy + depressed	35 / 34	Ayahuasca	1 d	↑
Chui (2014)	Healthy	24 / 24	LD-1227 marine nutraceutical	9 wks	↑
Kim (2015)	Frail elderly	32 / 33	Milk fat globule membrane	13 wks	=
Petelin (2019)	Overweight	30 / 30	Royal jelly	8 wks	=
Sarris (2019)	Depressed	81 / 77	S-adenosyl methionine + folinic acid + EPA + DHA + 5-HTP + zinc picolinate + co-factor vitamins	8 wks	=
Stringham (2019)	Healthy	49 / 10	Macular xanthophyll	26 wks	↑
Varanoske (2020)	Healthy	10 / 9	β-alanine	2 wks	=

*Notes: * compared to control; [a] assuming that subjects were equally divided into groups; = no statistically significant effect; ↑ statistically significant increase; - significance level missing; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; E-EPA: ethyl-eicosapentaenoic acid; LC-n3-FA: long-chain omega-3 polyunsaturated fatty acids*

polyphenols from Eriobotrya japonica Lindley [35], two studies seaweed rich in polyphenols [36,37], two studies resveratrol [38,39], one study ellagic acid [40], two studies (nano-)curcumin [41,42], one study Ginkgo Biloba [43], one study green tea [44], and one study dark chocolate [45]. A combination of resveratrol and quercetin showed no effect on serum BDNF concentrations in overweight subjects [39]. The remaining interventions were mainly performed in apparently healthy subjects in whom again resveratrol [38], but also a Ginkgo biloba extract [43] showed no significant change in serum BDNF concentrations, neither did dark chocolate [45]. Although the study evaluating the effect of green tea did not statistically analyze the difference in changes between the groups, evaluating the data as such indicated that the intervention had no effect on serum BDNF concentrations [44]. In contrast, supplementation with a plant extract of leaves of Eriobotrya japonica Lindley in apparently healthy subjects [35] and supplementation with nano-curcumin in subjects with metabolic syndrome [41] showed a trend to increased serum and plasma BDNF concentrations, respectively. Furthermore, supplementation with seaweed in apparently healthy subjects resulted in a significant increase in serum BDNF concentrations [36,37]. Supplementation with ellagic acid significantly increased plasma BDNF concentrations in overweight subjects but not in healthy subjects [40]. Finally, curcumin supplementation was found to increase plasma BDNF concentrations in subjects with major depressive disorder [42].

Long-chain omega-3 polyunsaturated fatty acids

Five studies have been identified that examined the effects of long-chain omega-3 polyunsaturated fatty acid supplements (Table 2.1). No significant differences in serum BDNF concentrations were found after the interventions with the different long-chain omega-3 polyunsaturated fatty acids in subjects with diabetes [46], subjects with depression [47], trauma patients [48], and overweight subjects [49]. The study in apparently healthy subjects [50] did not statistically analyze the difference in changes between the intervention and control arms, but as the change in serum BDNF concentrations in both groups was similar and small in relation to the reported group averages and standard deviations, this would likely not have reached significance.

Long-chain omega-3 polyunsaturated fatty acids

The eight studies in the probiotics cluster evaluated the effects of various bacterial supplements (Table 2.1). Pinto-Sanchez *et al.* [51] and Riezzo *et al.* [52] found no significant effects of a single strain Bifidobacterium longum NCC3001 or Lactobacillus

reuteri DSM-17938 probiotic approach on serum BDNF concentrations in subjects with irritable bowel syndrome or constipation, respectively, though Riezzo *et al.* [52] did not statistically analyze the changes versus control. West *et al.* [53] investigated both the single strain (Bifidobacterium animalis subsp. lactis BI-04) and double strain (Lactobacillus acidophilus NCFM and Bifidobacterium animalis subsp. lactis BI-07) probiotic approach in apparently healthy subjects, and similarly found no significant changes in plasma BDNF concentrations. Haghighat *et al.* [54] examined both synbiotics (prebiotics and probiotics) and probiotics in hemodialysis patients with depressive symptoms. However, only in the synbiotics supplemented group they did find a significant increase in serum BDNF concentrations. Probiotic supplementation with Lactobacillus plantarum C29 did not affect serum BDNF concentrations in mild cognitive impaired subjects, but again no statistical test was applied to compare the difference in change [55]. Probiotic supplementation with different doses of Lactobacillus helveticus IDCC3801- fermented milk showed no overall effect on plasma BDNF concentrations in apparently healthy subjects [56]. Finally, probiotic supplementation with Bifidobacterium bifidum BGN4 and Bifidobacterium longum BORI in apparently healthy subjects did result in a significant increase in serum BDNF concentrations [57], but supplementation with Lactobacillus helveticus and Bifidobacterium longum in subjects with depressive symptoms did not [58].

Miscellaneous

The remaining cluster included seven studies with plant and protein extracts or supplements rich in lipids (Table 2.1). A single dose of ayahuasca increased serum BDNF concentrations in a combined population of apparently healthy subjects and depressed subjects [59]. A blend of high-purity caviar-derived DNA, collagen elastin and protein extracts from sturgeon did change serum BDNF concentrations in apparently healthy subjects [60]. Frail older persons who consumed milk fat globule membrane supplements showed no significant changes in serum BDNF concentrations [61]. Furthermore, supplementation with royal jelly did not significantly increase serum BDNF concentrations in overweight subjects [62], nor did the nutraceutical combination used in depressed subjects [63]. However, a study with two different doses of macular xanthophyll in apparently healthy subjects revealed a significant increase in serum BDNF concentrations when the two groups were combined [64]. Finally, another study in apparently healthy subjects found β-alanine supplementation to have no significant effect on plasma BDNF concentrations [65].

Risk of bias assessment

The assessment regarding the methodological quality of the studies resulted in sufficient Jadad scores for the dietary patterns and foods cluster and poor Jadad scores for the energy intake cluster (Supplemental Table 2.1). Regarding the poor scores for the studies in these clusters, it should be taking into account that no points could be awarded for blinding regarding the intervention with dietary patterns or whole foods, or restriction in energy intake. Therefore, it can be questioned if this outcome is a real representation of quality for these particular clusters. The overall quality of the supplement studies in the vitamins and minerals sub-cluster is good, in the polyphenol sub-cluster sufficient to good with two studies scoring below average, in the long-chain omega-3 polyunsaturated fatty acids sub-cluster good except for one study, in the probiotics sub-cluster good, and in the miscellaneous sub-cluster one sufficient and the remaining studies good.

DISCUSSION

Higher BDNF concentrations have been associated with both improved brain health [6] and metabolic health [14]. Therefore, interventions that could potentially elevate BDNF concentrations have attracted considerable attention. We have evaluated here the possible relation between consuming different nutrients and BDNF concentrations in controlled human intervention studies. To do so, we have categorized the available literature into seven (sub-)clusters. In general, two of three studies in the whole foods cluster and four of eleven studies in the polyphenol sub-cluster showed significant positive effects on BDNF concentrations. The studies in the cluster based on energy intake, and the supplement sub-clusters for vitamins and minerals, long-chain omega-3 polyunsaturated fatty acids, probiotics, and miscellaneous showed no consistent effects.

Four of the 11 studies in the polyphenol cluster showed a significant increase in BDNF concentrations, one study showed an increase in BDNF concentrations but did not statistically analyze this, and two studies showed a trend to an increase. The overall quality of the studies in this cluster was sufficient to good based on the Jadad scores (Supplemental Table 2.1). It thus seems that polyphenols are molecules of interest which could have a potentially positive effect on BDNF. Epidemiological studies and randomized controlled trials have indeed shown that an elevated intake of polyphenols can be linked to neuroprotective effects associated with improved cognitive function [66]. Based on polyphenols' chemical structure, they can be classified into flavonoids,

phenolic acids, stilbenes, lignans, and other phenolic compounds [67]. Of the 11 polyphenol studies included here, four evaluated the effects of flavonoids (Eriobotrya japonica Lindley, Ginkgo biloba, green tea, dark chocolate), one evaluated the effects of phenolic acids (ellagic acid), two evaluated the effects of stilbenes (resveratrol), and five evaluated the effects of other phenolic compounds (fermented Laminaria japonica A., (nano-)curcumin).

Phenolic acids and other phenolic compounds were responsible for the significant changes in BDNF concentrations. A potential mechanism underlying the beneficial effects of polyphenols might be a direct modulation of the cAMP-response element-binding protein (CREB) signaling pathways which are linked to BDNF expression [68]. However, activation of this CREB-mediated mechanism of action has generally been ascribed to flavonoids [69]. Although Eriobotrya japonica Lindley showed no significant effect on BDNF, it did show a trend to increased BDNF concentrations [35]. However, the other studies that applied the other flavonoids, Ginkgo biloba, green tea, and dark chocolate, showed no effect on BDNF concentrations [43,45]. The reason for this lack of an effect could be due to the low bioavailability of flavonoids in humans [70].

Another neurotrophic signaling pathway that can be modulated by polyphenols, in particular phenolic acids, is the Akt pathway, which ultimately leads to an increase in BDNF concentrations [71]. This pathway could be the mechanism of action of ellagic acid which showed an effect on BDNF concentrations, at least in overweight subjects [40]. Both the Akt and CREB pathway are stimulated by curcumin [72], resulting in an expected increase in BDNF concentration as observed in depressed subjects in the study by Yu *et al.* [42] and a trend to an increase in metabolic syndrome subjects in the study by Osali *et al.* [41]. Despite several studies highlighting the positive effect of resveratrol supplementation on cognitive improvement [73], it does not seem to be mediated via elevated BDNF concentrations [38,39].

Of the active polyphenol compounds responsible for the significant effect on BDNF concentration upon supplementation with fermented Laminaria japonica A. [36,37], the seaweed specific phlorotannins could play a role [67] as the same pathway is stimulated as is seen after flavonoid exposure [74]. However, since a specialized fermentation process had been applied to the seaweed, the supplements not only contained the phlorotannin polyphenols but were also enriched with gamma-aminobutyric acid (GABA; [75]). GABA has been recognized as stimulating BDNF expression via the same CREB mechanism, at least during early development [76]. It is possible therefore that besides the phlorotannin polyphenols, the GABA content in seaweed could also have played a role in increasing BDNF concentrations, yet it has not been shown that GABA has the

same effect on BDNF expression in adult life. Furthermore, the high fiber content in seaweed [77] may also have contributed to the positive effects on BDNF concentration, since Sandberg *et al.* [19], as presented in the dietary patterns and foods cluster, showed that a fiber-rich di*et al*so elevated BDNF concentrations. Another mechanism that could be of importance is adult hippocampal neurogenesis since both BDNF and various dietary interventions have been shown to affect the level of neurogenesis in the adult hippocampus [78].

Regarding the dose of the polyphenol supplements used in the interventions, it is notable that those studies that did not show an effect on BDNF concentrations used a lower dose (38-575 mg) than the studies that did show an effect (>1000 mg; Supplemental Table 2.2). The doses in those studies that did show an effect were comparable to the average dietary polyphenol intake in Europe [79], suggesting that the negative findings in the other studies may have been related to the low doses used. In summary, based on the included studies in this cluster it can be postulated that polyphenols, more specifically the phenolic acids and other phenolic compounds, have a beneficial effect on neurotrophic factors such as BDNF. The underlying mechanisms, as far as evaluated in humans, appear to be similar to the CREB and Akt pathways as described in cell and animal studies [71].

As well as the polyphenol sub-cluster, interesting observations have also been found in the dietary patterns and foods cluster. The quality of the studies in this cluster was sufficient based on the Jadad scores (Supplemental Table 2.1), when taking into account no points could be awarded for blinding regarding the intervention with dietary patterns or whole foods. The intake of whole-grain rye kernel-based bread resulted in increased plasma BDNF concentrations despite the short duration of the intervention [19]. It could be that the gut-brain axis is involved in increasing BDNF, amongst others because the microbiota composition changed after rye kernel bread had been consumed, which was positively correlated with the increase in circulating BDNF concentrations [80]. This is supported by the increase in short-chain fatty acids in response to rye-based products [81], as well as by improved cognitive performance that is correlated to the amount of fiber in the diet [82]. Similarly, supplementation with a mixed-grain diet in adolescents has also been seen to result in increased plasma BDNF concentrations [83], though unfortunately changes in microbiota were not analyzed. The suggestion that changes in the diet could affect BDNF via modulating microbiota composition is appealing and is supported by several studies that have shown a link between microbiota composition, BDNF concentrations [84], and cognition [85]. This link can also be seen in the respective studies from Haghighat *et al.* [54] and Kim *et al.* [57] where the microbiota populations

changed upon supplementation. In this context, it is significant that the studies in the probiotics sub-cluster showed no significant changes in BDNF concentrations [51,53]. However, it should be acknowledged that in those interventions the microbiota profiles also remained stable [51], except for one genus [55].

When evaluating the observed effects on BDNF concentrations in these intervention studies, it should be noted that a number of issues make interpreting the data complex. First, there is enormous variation in peripheral BDNF concentrations between studies, something which could depend on whether it was measured either in serum or in plasma (Supplemental Table 2.2). Since platelets release BDNF during the clotting process, serum contains higher concentrations of BDNF than plasma [8]. These differences in BDNF concentrations between serum and plasma can be up to 100- to 200-fold [86]. Moreover, clotting time also affects serum BDNF concentrations, whereas the centrifugation protocol also affects plasma BDNF concentrations [87]. Due to the low association between serum and plasma BDNF concentrations, it is difficult to generalize the findings of peripheral measurements [10]. Differences between plasma and serum BDNF analysis might also explain the rather unexpected finding that the studies in the long-chain omega-3 polyunsaturated fatty acid sub-cluster showed no effect on BDNF concentrations, even though omega-3 fatty acids have been found to have neuroprotective effects [88]. In addition, the expected link between dietary omega-3 fatty acid intake and BDNF concentrations has been shown in rat studies [89], and more recently in humans [90]. Interestingly, in the latter study, BDNF concentrations were measured in plasma unlike those interventions described in the sub-cluster where BDNF was always analyzed in serum. As fish oil lowers platelet aggregation [91], it could be that the BDNF stored in platelets was not secreted as easily during the clotting procedure after fish oil supplementation and, therefore, any potential increase in BDNF concentrations would not have become visible when analyzed in serum. Such a reduced BDNF release from platelets has also been shown upon an oral dose of the anticoagulant drug clopidogrel [92]. In addition to this potential serum effect, the absence of an effect in the reviewed fish oil intervention studies could also be related to the different populations studied. Fish oil supplements improved cognitive function in subjects with cognitive decline but not in healthy subjects [93], which could explain why Witte *et al.* [50] found no changes in BDNF concentrations.

Second, the majority of the immunoassays used to analyze BDNF concentrations cannot distinguish between precursor BDNF (proBDNF) and mature BDNF (mBDNF; [94]), despite the fact that the two variants are functionally different [2]. Both are biologically active, yet proBDNF has a negative influence and mBDNF a positive influence on synaptic

plasticity and, ultimately, memory and cognition. It could be that either one mediates the dietary effect on cognition [95]. For example, extracellular zinc activated metalloproteinase is involved in cleaving proBDNF into mBDNF [96].

Third, it is unclear whether peripheral BDNF concentrations as measured in the intervention studies included in this systematic review reflect central BDNF concentrations. There are certainly indications for such a relation since animal studies have shown positive correlations between peripheral and central BDNF concentrations, which was apparently evolutionary conserved across species [97]. However, from a structural and functional perspective, the blood–brain barrier in humans does not resemble those in animals [98]. Although evidence from human samples is limited, a positive correlation has been found between BDNF concentrations in cerebrospinal fluid and plasma in psychotic subjects [99]. Future research should focus on analyzing both peripheral (serum and plasma) and central BDNF to determine whether it is possible to estimate central BDNF from peripheral BDNF concentrations in other populations. There is also a pressing need to understand whether interventions induce similar changes in both compartments and whether there is a difference between peripheral plasma and serum concentrations in these correlations.

In conclusion, we have demonstrated that dietary interventions can elevate circulating BDNF concentrations. In particular, certain polyphenols, such as phenolic acids and phenolic compounds, seem to have this effect, which could be beneficial in improving metabolic and cognitive health. The effect of dietary ingredients from other (sub-)clusters was, however, inconsistent. Notwithstanding, it should be noted that reviewing studies that describe such effects on BDNF concentrations is highly complex, with findings varying depending on the type of material sampled for BDNF analysis and the BDNF variant measured. Further evaluations of nutritional interventions targeting an increase in BDNF concentrations, particularly focusing on the link between peripheral and central effects, are warranted.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

SUPPLEMENTAL MATERIAL

Supplemental Table 2.1 | Quality assessment based on Jadad scale

First author (year)	Intervention	Q1	Q2	Q3	Q4	Q5	Total score
Dietary Patterns & Foods							
Sánchez-Villegas (2011)	Mediterranean diet with virgin olive oil or mixed nuts	1	0	0	0	0	1
Sandberg (2018)	Whole grain rye kernel-based bread	1	1	0	0	1	3
Suzuki (2019)	Mold fermented cheese	1	1	0	0	1	3
Diets Based on Energy Intake							
Carlson (2007)	Reduced meal frequency	1	0	0	0	0	1
Catenacci (2016)	Zero-calorie alternate-day fasting	1	0	0	0	1	2
Cherif (2017)	Islamic intermittent fasting	1	0	0	0	1	2
Khoshandam Ghashang (2019)	Ramadan fasting	0	0	0	0	0	0
Guimaraes (2008)	Hypocaloric diet	0	0	0	0	0	0
Harvie (2011)	Continuous energy restriction	1	0	0	0	1	2
Schübel (2018)	Intermittent or continuous calorie restriction	1	1	0	0	1	3
Supplements							
Vitamins & Minerals							
Jafari (2019)	Zinc gluconate	1	1	1	1	1	5
Kheirouri (2018)	Zinc gluconate	1	1	1	1	1	5
Pirotta (2014)	Vitamin D3	1	1	1	1	1	5
Ranjbar (2014)	Zinc sulfate	1	1	1	1	1	5
Solati (2015)	Zinc gluconate	1	1	1	1	1	5
Walentukiewicz (2018)	Vitamin D3	1	0	0	0	1	2
Yosaee (2020)	Zinc gluconate and/or vitamin D3	1	1	1	1	1	5
Polyphenols							
Choi (2016)	*Eriobotrya japonica* Lindley	1	1	1	1	1	5
Choi (2016)	Fermented *Laminaria japonica*	1	0	1	1	0	3
Huhn (2018)	Resveratrol	1	1	1	1	1	5
Liu (2018)	Ellagic acid	1	0	1	1	0	3
Osali (2020)	Nano-curcumin	1	0	1	1	0	3
Reid (2018)	Fermented *Laminaria japonica* A.	1	1	1	1	1	5

Supplemental Table 2.1 (continued) | Quality assessment based on Jadad scale

First author (year)	Intervention	Q1	Q2	Q3	Q4	Q5	Total score
Sadowska-Krepa (2017)	*Ginkgo biloba* extract	1	0	0	0	1	2
Sadowska-Krepa (2019)	Green tea extract	1	0	0	0	1	2
Sumiyoshi (2019)	Dark chocolate	1	1	0	0	1	3
Witte (2014)	Resveratrol	1	0	1	1	1	4
Yu (2015)	Curcumin	1	1	1	1	1	5
Long-Chain Omega-3 Polyunsaturated Fatty Acids							
Bot (2011)	E-EPA	1	1	1	1	1	5
Burg, van der (2019)	EPA + DHA	1	0	1	1	1	4
Matsuoka (2015)	EPA + DHA	1	1	1	1	1	5
Sedláček (2018)*	EPA + DHA	1	0	1	0	1	1
Witte (2014)	LC-n3-FA	1	0	1	1	1	4
Probiotics							
Chung (2014)	*L. helveticus* IDCC3801-fermented milk	1	1	1	1	1	5
Haghighat (2019)	Prebiotics and/or *L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, *B. longum* BIA-8	1	1	1	1	1	5
Hwang (2019)	*L. plantarum* C29-fermented soybean	1	1	1	1	1	5
Kim (2020)	*B. bifidum* BGN4 + *B. longum* BORI	1	1	1	1	1	5
Pinto-Sanchez (2017)	*B. longum* NCC3001	1	1	1	1	1	5
Riezzo (2018)	*L. reuteri* DSM-17938	1	1	1	1	1	5
Romijn (2017)	*L. helveticus* + *B. longum*	1	1	1	1	1	5
West (2014)	*L. acidophilus* NCFM and/or *B. animalis* subsp. *lactis* BI-07	1	1	1	1	1	5
Miscellaneous: Protein (Extracts) & Lipids							
Almeida, de (2019)	Ayahuasca	1	0	1	0	1	3
Chui (2014)	LD-1227 marine nutraceutical	1	1	1	1	1	5
Kim (2015)*	Milk fat globule membrane	1	1	1	1	1	4
Petelin (2019)	Royal jelly	1	1	1	1	0	4
Sarris (2019)	S-adenosyl methionine, folinic acid, EPA, DHA, 5-HTP, zinc picolinate, co-factor vitamins	1	1	1	1	1	5

Supplemental Table 2.1 (continued) | Quality assessment based on Jadad scale

First author (year)	Intervention	Q1	Q2	Q3	Q4	Q5	Total score
Stringham (2019)	Macular xanthophyll	1	1	1	1	1	5
Varanoske (2020)	β-alanine	1	0	1	1	1	4

*Q1 = Was the study described as randomized?; Q2 = Was the method used to generate the sequence of randomization described and appropriate?; Q3 = Was the study described as double blind?; Q4 = Was the method of double blinding described and appropriate?; Q5 = Was there a description of withdrawals and dropouts?; * Points were deducted in the following cases: if the method used to generate the sequence of randomization was described and it was inappropriate or if the study was described as double blind but the method of blinding was inappropriate; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; E-EPA: ethyl-eicosapentaenoic acid; LC-n3-FA: long-chain omega-3 polyunsaturated fatty acids*

Supplemental Table 2.2 | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Dietary Patterns & Foods											
Sánchez-Villegas (2011)	Parallel	At high cardiovascular risk	91	Mediterranean diet with virgin olive oil	3 y	143 ml	Plasma	-	24660000	-	ns [a]
			75	Mediterranean diet with mixed nuts		30 g		-	24870000	-	ns [a]
			77	Low-fat diet				-	23370000	-	
Sandberg (2018)	Crossover	Healthy	19	Whole grain rye kernel-based bread	1-3 d	143 g	Plasma	-	490	-	0.001
				White wheat flour-based bread				-	386	-	
Suzuki (2019)	Crossover	Mild cognitive impaired	35	Mold-fermented cheese	12 wks	121 g	Serum	27000	27500	500	0.039
				Non-mold-fermented cheese				26000	25700	-300	
Diets Based on Energy Intake											
Carlson (2007)	Crossover	Healthy	21	Reduced meal frequency	8 wks		Plasma	-	141.7	-	ns
				Normal meal frequency				-	148.1	-	
Catenacci (2016)	Parallel	Obese	15	Zero-calorie alternate-day fasting	8 wks		Serum	20935	20165	-769	ns
			14	Daily caloric restriction				22552	18510	-4042	
Cherif (2017)	Crossover	Healthy	22	Islamic intermittent fasting	3 d		Serum	-	40363	-	ns
				No intermittent fasting				-	53968	-	
Khoshandam Ghashang (2019)	Parallel	Healthy	25	Fasting Ramadan	29 d		Serum	$5729x10^6$	$6561x10^6$	$832x10^6$	ns
			25	Non-fasting				$4897x10^6$	$5368x10^6$	$471x10^6$	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Guimaraes (2008)	Parallel	Schizophrenic	25	Hypocaloric diet	11.5-26 mo		Serum	-	11.0 [b]	-	0.023
			42	Regular diet				-	7.2 [b]	-	
Harvie (2011)	Parallel	Obese	54	Continuous energy restriction	26 wks		Serum	9898	9528	-370	ns
			53	Intermittent energy restriction				9539	9214	-325	
Schübel (2018)	Parallel	Overweight	47	Intermittent calorie restriction	12 wks		Serum	2100	1900	-200	ns [a]
			46	Continuous calorie restriction				2200	2100	-100	ns [a]
			51	No calorie restriction				2100	2000	-100	
Supplements											
Vitamins & Minerals											
Jafari (2019)	Parallel	Premenstrual syndrome	27	Zinc gluconate	12 wks	30 mg	Serum	870	1100	230	< 0.01
			30	Placebo				1170	1090	70	
Kheirouri (2018)	Parallel	Diabetic	25	Zinc gluconate	12 wks	30 mg	Serum	2300	2530	230	ns
			25	Placebo				1880	1950	70	
Pirotta (2014)	Parallel	Healthy	13	Vitamin D3	10 wks	0.05 mg	Serum	22300	-	4.3% [c]	ns
			13	Placebo				20900	-	-0.1% [c]	
Ranjbar (2014)	Parallel	Depressed	22	Zinc sulfate	12 wks	25 mg	Plasma	2.2	3.6	1.4	ns
			22	Placebo				2.1	3.7	1.6	
Solati (2015)	Parallel	Obese	24	Zinc gluconate	12 wks	30 mg	Serum	15370	21840	6470	0.003
			26	Placebo				16200	15660	-540	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Walentukiewicz (2018)	Parallel	Healthy	27	Vitamin D3	12 wks	4000 IU	Serum	37750	16940	-20810	-
			34	Placebo				33520	28960	-4560	
Yosaee (2020)	Parallel	Depressed	24	Zinc gluconate	12 wks	2000 IU	Serum	-	-	-110	ns [a]
			27	Vitamin D3		30 mg		-	-	-190	ns [a]
			25	Zinc gluconate + vitamin D3		2000 IU + 30 mg		-	-	190	ns [a]
			22	Placebo				-	-	-170	
Polyphenols											
Choi (2016)	Parallel	Healthy	40	*Eriobotrya japonica* Lindley	12 wks	1500 mg	Serum	25597	27816	2219	ns
			40	Placebo				25008	24445	-563	
Choi (2016)	Parallel	Healthy	10	Fermented *Laminaria japonica*	8 wks	1000 mg	Serum	19951	25037	5086	< 0.001
			11	Placebo				20346	19062	-1284	
Huhn (2018)	Parallel	Healthy	30	Resveratrol	26 wks	200 mg	Serum	22210	25480	3270	ns
			30	Placebo				26300	29600	3300	
Liu (2018)	Parallel	Healthy	37	Ellagic acid	12 wks	50 mg	Plasma	-	413	-	ns
			39	Placebo				-	420	-	
		Overweight	38	Ellagic acid		50 mg		-	379	-	< 0.01
			36	Placebo				-	312	-	
Osali (2020)	Parallel	Metabolic syndrome	11 [d]	Nano-curcumin	6 wks	80 mg	Plasma	111	199	88	-
			11 [d]	Placebo				107	105	-2	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Reid (2018)	Parallel	Healthy	32	Fermented *Laminaria japonica* A.	6 wks	1500 mg	Serum	28152	31033	2880	< 0.05
			28	Placebo				29891	26957	-2935	
Sadowska-Krepa (2017)	Parallel	Healthy	9	Polyphenols *Ginkgo biloba* extract	6 wks	38 mg	Serum	-	13116	-	ns
			9	Placebo				-	13116	-	
Sadowska-Krepa (2019)	Parallel	Healthy	8	Polyphenols green tea extract	6 wks	490 mg	Serum	10780	12790	2010	-
			8	Placebo				9970	11020	1050	
Sumiyoshi (2019)	Parallel	Healthy	10	Polyphenols dark chocolate	4 wks	575 mg	Plasma	543	590	48	ns
			8	Cacao-free white chocolate				585	501	-85	
Witte (2014)	Parallel	Overweight	23	Resveratrol + quercetin	26 wks	200 + 320 mg	Serum	4082	3994	-88	ns
			23	Placebo				4062	4140	78	
Yu (2015)	Parallel	Depressed	50	Curcumin	6 wks	1000 mg	Plasma	-	407	-	< 0.001
			50	Placebo				-	310	-	
Long-Chain Omega-3 Polyunsaturated Fatty Acids											
Bot (2011)	Parallel	Diabetic	13	E-EPA	12 wks	1000 mg	Serum	33700	33500	-200	ns
			12	Placebo				31900	32900	1000	
Burg, van der (2019)	Parallel	Depressed	42	EPA + DHA	8 wks	1000 + 656 mg	Serum	25400	23300	-2100	ns
			41	Placebo				21200	20900	-300	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collection	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Matsuoka (2015)	Parallel	Traumatized	53	EPA + DHA	12 wks	1470 + 147 mg	Serum	16000	20000	4000	ns
			57	Placebo				16400	20000	3600	
Sedláček (2018)	Parallel	Overweight	11	Lifestyle modification + EPA + DHA	12 wks	~0.6 g	Serum	-	-	-93630 [b]	ns
			10	Lifestyle modification				-	-	-94460 [b]	
Witte (2014)	Parallel	Healthy	40	LC-n3-FA	26 wks	2.2 g	Serum	4052	4316	264	-
			40	Placebo				4104	4379	275	
Probiotics											
Chung (2014)	Parallel	Healthy	10	*L. helveticus* IDCC3801-fermented milk 500mg	12 wks	125 mg	Plasma	8553	7890	192	ns [a]
			7	*L. helveticus* IDCC3801-fermented milk 1000mg		250 mg		12088	8170	-3918	ns [a]
			9	*L. helveticus* IDCC3801-fermented milk 2000mg		500 mg		10053	6475	-3578	ns [a]
			10	Placebo				7567	9433	1866	
Haghighat (2019)	Parallel	Hemodialyis + depressed	25	Prebiotics + *L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, *B. longum* BIA-8	12 wks	15 + 20 g	Serum	2330	2760	430	0.005
			25	*L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, *B. longum* BIA-8		20 g		2250	2230	-20	ns
			25	Placebo				2400	2240	-160	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Hwang (2019)	Parallel	Mild cognitive impaired	50	*L. plantarum* C29-fermented soybean	12 wks	800 mg	Serum	-	-	413	-
			50	Placebo				-	-	-1034	
Kim (2020)	Crossover	Healthy	63	*B. bifidum* BGN4 + *B. longum* BORI	12 wks	$1x10^9$ cfu	Serum	-	-	3680	< 0.05
				Placebo				-	-	-3320	
Pinto-Sanchez (2017)	Parallel	Irritable bowel syndrome	22	*B. longum* NCC3001	6 wks	$1x10^{10}$ cfu	Serum	6610	7000	390	ns
			22	Placebo				4870	5830	960	
Riezzo (2018)	Parallel	Constipated	28	*L. reuteri* DSM-17938	15 wks	$4x10^8$ cfu	Serum	25.3	22.0	-3.3	-
			28	Placebo				24.7	24.7	0	
Romijn (2017)	Parallel	Depressed	28	*L. helveticus* + *B. longum*	8 wk	$\geqslant 3\times10^9$ cfu	Serum	27600	27700	950	ns
			36	Placebo				25700	25600	-790	
West (2014)	Parallel	Healthy	46	*B. animalis* subsp. *lactis* BI-04	21 wks	$2x10^9$ cfu	Plasma	14104	13141	-963	ns
			47	*L. acidophilus* NCFM + *B. animalis* subsp. *lactis* BI-07		$5x10^9$ + $5x10^9$ cfu		13228	13693	465	ns
			51	Placebo				14029	14764	735	
Miscellaneous: Protein (Extracts) & Lipids											
Almeida, de (2019)	Parallel	Healthy + depressed	35	Ayahuasca	1 d	1 ml/kg	Serum	12466	11796	-670	0.03
			34	Placebo				10777	10223	-553	

Supplemental Table 2.2 (continued) | BDNF concentrations and effect values of the studies included per cluster.

First author (year)	Study design	Population	N	Intervention	Duration	Daily intake	BDNF collec-tion	BDNF baseline (pg/ml)	BDNF end (pg/ml)	Change in BDNF (pg/ml) *	BDNF effect value **
Chui (2014)	Parallel	Healthy	24	LD-1227 marine nutraceutical	9 wks	400 mg	Serum	5856	8056	2200	< 0.01
			24	Placebo				4611	4856	245	
Kim (2015)	Parallel	Frail elderly	32	Milk fat globule membrane	13 wks	1 g	Serum	6970	7110	140	ns [a]
			33	Placebo				6100	6360	260	
Petelin (2019)	Parallel	Overweight	30	Royal jelly	8 wks	666 mg	Serum	1712	1797	85	ns
			30	Placebo				1823	1812	-11	
Sarris (2019)	Parallel	Depressed	81	S-adenosyl methionine + folinic acid + EPA + DHA + 5-HTP + zinc picolinate + co-factor vitamins	8 wks	800 + 0.5 + 1000 + 656 + 200 + 30 + 200 mg + 40 IU	Serum	-	-	2290	ns
			77	Placebo				-	-	601	
Stringham (2019)	Parallel	Healthy	49	Macular xanthophyll [e]	26 wks	13 / 27 mg	Serum	14672	16017	1345	< 0.05
			10	Placebo				15101	14648	-453	
Varanoske (2020)	Parallel	Healthy	10	β-alanine	2 wks	12 g	Plasma	976	1065	89	ns
			9	Placebo				789	991	202	

** change in BDNF concentrations within group; ** compared to control; [a] no overall diet effect; [b] median; [c] percentage changes in log-transformed data; [d] assuming that subjects were equally divided into groups; [e] supplement groups combined; - significance level missing; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; E-EPA: ethyl-eicosapentaenoic acid; LC-n3-FA: long-chain omega-3 polyunsaturated fatty acids*

REFERENCES

1. Sakharnova TA, Vedunova MV, Mukhina IV. Brain-Derived Neurotrophic Factor (BDNF) and its Role in the Functioning of the Central Nervous System. Neurochemical Journal. 2012;6(4):251-9.
2. Kowianski P, Lietzau G, Czuba E, Waskow M, Steliga A, Morys J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. Cellular Molecular Neurobiology. 2018;38(3):579-93.
3. Yu H, Zhang Z, Shi Y, Bai F, Xie C, Qian Y, *et al.* Association Study of the Decreased Serum BDNF Concentrations in Amnestic Mild Cognitive Impairment and the Val66Met Polymorphism in Chinese Han. The Journal of Clinical Psychiatry. 2008;69:1104-11.
4. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. Frontiers in Cellular Neuroscience. 2019;13:363.
5. Walsh R. Lifestyle and Mental Health. American Psychologist. 2011;66(7):579-92.
6. Gomez-Pinilla F, Tyagi E. Diet and Cognition: Interplay between Cell Metabolism and Neuronal Plasticity. Current Opinion in Clinical Nutrition and Metabolic Care. 2013;16(6):726-33.
7. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of Brain-Derived Neurotrophic Factor across the Blood–Brain Barrier. Neuropharmacology. 1998;37:1553-61.
8. Fujimara H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, *et al.* Brain-derived Neurotrophic Factor is Stored in Human Platelets and Released by Agonist Stimulation. Thrombosis and Haemostasis. 2002;87:728-34.
9. Maisonpierre PC, Le Beau MM, Espinosa III R, Ip NY, Belluscio L, De la Monte SM, *et al.* Human and Rat Brain-Derived Neurotrophic Factor and Neurotrophin-3, Gene Structures, Distributions, and Chromosomal Localizations. Genomics. 1991;10:558-68.
10. Serra-Millas M. Are the Changes in the Peripheral Brain-Derived Neurotrophic Factor Levels Due to Platelet Activation? World Journal of Psychiatry. 2016;6(1):84-101.
11. Prickett C, Brennan L, Stolwyk R. Examining the Relationship between Obesity and Cognitive Function: A Systematic Literature Review. Obesity Resarch & Clinical Practice. 2015;9(2):93-113.
12. Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, *et al.* The Impact of Age, Weight and Gender on BDNF Levels in Human Platelets and Plasma. Neurobiology of Aging. 2005;26(1):115-23.
13. Lapchak PA, Hefti F. BDNF and NGF Treatment in Lesioned Rats, Effects on Cholinergic Function and Weight Gain. NeuroReport. 1992;3:405-8.
14. Marosi K, Mattson MP. BDNF Mediates Adaptive Brain and Body Responses to Energetic Challenges. Trends in Endocrinology & Metabolism. 2014;25(2):89-98.
15. van Praag H, Fleshner M, Schwartz MW, Mattson MP. Exercise, Energy Intake, Glucose Homeostasis, and the Brain. Journal of Neuroscience. 2014;34(46):15139-49.
16. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. British Medical Journal. 2009;339:b2535.
17. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, *et al.* Assessing the Quality of Report of Randomized Clinical Trials: Is Blinding Necessary? Controlled Clinical Trials. 1996;17:1-12.
18. Sanchez-Villegas A, Galbete C, Martinez-Gonzalez MA, Martinez JA, Razquin C, Salas-Salvado J, *et al.* The Effect of the Mediterranean Diet on Plasma Brain-Derived Neurotrophic Factor

(BDNF) Levels: The PREDIMED-NAVARRA Randomized Trial. Nutritional Neuroscience. 2011;14(5):195-201.
19. Sandberg JC, Bjorck IME, Nilsson AC. Increased Plasma Brain-Derived Neurotrophic Factor 10.5 h after Intake of Whole Grain Rye-Based Products in Healthy Subjects. Nutrients. 2018;10(8).
20. Suzuki T, Kojima N, Osuka Y, Tokui Y, Takasugi S, Kawashima A, *et al.* The Effects of Mold-Fermented Cheese on Brain-Derived Neurotrophic Factor in Community-Dwelling Older Japanese Women With Mild Cognitive Impairment: A Randomized, Controlled, Crossover Trial. Journal of the American Medical Directors Association. 2019;20(12):1509-14 e2.
21. Carlson O, Martin B, Stote KS, Golden E, Maudsley S, Najjar SS, *et al.* Impact of Reduced Meal Frequency without Caloric Restriction on Glucose Regulation in Healthy, Normal-Weight Middle-Aged Men and Women. Metabolism. 2007;56(12):1729-34.
22. Cherif A, Meeusen R, Farooq A, Briki W, Fenneni MA, Chamari K, *et al.* Repeated Sprints in Fasted State Impair Reaction Time Performance. Journal of the American College of Nutrition. 2017;36(3):210-7.
23. Khoshandam Ghashang S, Hamdan I, Lichtinghagen R, Gutenbrunner C, Nugraha B. Alterations of Brain-Derived Neurotrophic Factor and Creatinine During Ramadan Fasting: A Prospective, Controlled Clinical Trial. Iranian Red Crescent Medical Journal. 2019;In Press(In Press).
24. Schübel R, Nattenmüller J, Sookthai D, Nonnenmacher T, Graf ME, Riedl L, *et al.* Effects of Intermittent and Continuous Calorie Restriction on Body Weight and Metabolism over 50 wk: A Randomized Controlled Trial. American Journal of Clinical Nutrition. 2018;108:933-45.
25. Catenacci VA, Pan Z, Ostendorf D, Brannon S, Gozansky WS, Mattson MP, *et al.* A Randomized Pilot Study Comparing Zero-Calorie Alternate-Day Fasting to Daily Caloric Restriction in Adults with Obesity. Obesity (Silver Spring). 2016;24(9):1874-83.
26. Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, *et al.* The Effects of Intermittent or Continuous Energy Restriction on Weight Loss and Metabolic Disease Risk Markers: A Randomized Trial in Young Overweight Women. International Journal of Obesity (Lond). 2011;35(5):714-27.
27. Guimaraes LR, Jacka FN, Gama CS, Berk M, Leitao-Azevedo CL, Belmonte de Abreu MG, *et al.* Serum Levels of Brain-Derived Neurotrophic Factor in Schizophrenia on a Hypocaloric Diet. Progress in Neuropsychopharmacology & Biological Psychiatry. 2008;32(6):1595-8.
28. Jafari F, Amani R, Tarrahi MJ. Effect of Zinc Supplementation on Physical and Psychological Symptoms, Biomarkers of Inflammation, Oxidative Stress, and Brain-Derived Neurotrophic Factor in Young Women with Premenstrual Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. Biological Trace Element Research. 2020;194(1):89-95.
29. Kheirouri S, Naghizadeh S, Alizadeh M. Zinc Supplementation does not Influence Serum Levels of VEGF, BDNF, and NGF in Diabetic Retinopathy Patients: A Randomized Controlled Clinical Trial. Nutritional Neuroscience. 2019;22(10):718-24.
30. Ranjbar E, Shams J, Sabetkasaei M, M MS, Rashidkhani B, Mostafavi A, *et al.* Effects of Zinc Supplementation on Efficacy of Antidepressant Therapy, Inflammatory Cytokines, and Brain-Derived Neurotrophic Factor in Patients with Major Depression. Nutritional Neuroscience. 2014;17(2):65-71.
31. Solati Z, Jazayeri S, Tehrani-Doost M, Mahmoodianfard S, Gohari MR. Zinc Monotherapy Increases Serum Brain-Derived Neurotrophic Factor (BDNF) Levels and Decreases Depressive Symptoms in Overweight or Obese Subjects: A Double-Blind, Randomized, Placebo-Controlled Trial. Nutritional Neuroscience. 2015;18(4):162-8.
32. Pirotta S, Kidgell DJ, Daly RM. Effects of Vitamin D Supplementation on Neuroplasticity in Older Adults: A Double-Blinded, Placebo-Controlled Randomised Trial. Osteoporosis International. 2015;26(1):131-40.

33. Walentukiewicz A, Lysak-Radomska A, Jaworska J, Prusik K, Prusik K, Kortas JA, *et al.* Vitamin D Supplementation and Nordic Walking Training Decreases Serum Homocysteine and Ferritin in Elderly Women. International Journal of Environmental Research and Public Health. 2018;15(10).
34. Yosaee S, Soltani S, Esteghamati A, Motevalian SA, Tehrani-Doost M, Clark CCT, *et al.* Effects of Zinc, Vitamin D, and their Co-Supplementation on Mood, Serum Cortisol, and Brain-Derived Neurotrophic Factor in Patients with Obesity and Mild to Moderate Depressive Symptoms: A Phase II, 12-wk, 2x2 Factorial Design, Double-Blind, Randomized, Placebo-Controlled Trial. Nutrition. 2020;71:110601.
35. Choi E-K, Ko M-H, Park S-H, Ha K-C, Baek H-I, Kim Y-J, *et al.* Eriobotrya Japonica Improves Cognitive Function in Healthy Adolescents: A 12-Week, Randomized Double-Blind, Placebo-Controlled Clinical Trial. International Journal of Pharmacology. 2016;12(4):370-8.
36. Choi W-c, Reid SNS, Ryu J-k, Kim Y, Jo Y-H, Jeon BH. Effects of γ-Aminobutyric Acid-Enriched Fermented Sea Tangle (Laminaria Japonica) on Brain Derived Neurotrophic Factor-Related Muscle Growth and Lipolysis in Middle Aged Women. Algae. 2016;31(2):175-87.
37. Reid SNS, Ryu JK, Kim Y, Jeon BH. The Effects of Fermented Laminaria Japonica on Short-Term Working Memory and Physical Fitness in the Elderly. Evidence-Based Complementary and Alternative Medicine. 2018;8109621.
38. Huhn S, Beyer F, Zhang R, Lampe L, Grothe J, Kratzsch J, *et al.* Effects of Resveratrol on Memory Performance, Hippocampus Connectivity and Microstructure in Older Adults - A Randomized Controlled Trial. Neuroimage. 2018;174:177-90.
39. Witte AV, Kerti L, Margulies DS, Floel A. Effects of Resveratrol on Memory Performance, Hippocampal Functional Connectivity, and Glucose Metabolism in Healthy Older Adults. The Journal of Neuroscience. 2014;34(23):7862-70.
40. Liu Y, Yu S, Wang F, Yu H, Li X, Dong W, *et al.* Chronic Administration of Ellagic Acid Improved the Cognition in Middle-Aged Overweight Men. Applied Physiology, Nutrition, and Metabolism. 2018;43(3):266-73.
41. Osali A. Aerobic Exercise and Nano-Curcumin Supplementation Improve Inflammation in Elderly Females with Metabolic Syndrome. Diabetology & Metabolic Syndrome. 2020;12:26.
42. Yu JJ, Pei LB, Zhang Y, Wen ZY, Yang JL. Chronic Supplementation of Curcumin Enhances the Efficacy of Antidepressants in Major Depressive Disorder: A Randomized, Double-Blind, Placebo-Controlled Pilot Study. Journal of Clinical Psychopharmacology. 2015;35(4):406-10.
43. Sadowska-Krepa E, Klapcinska B, Pokora I, Domaszewski P, Kempa K, Podgorski T. Effects of Six-Week Ginkgo Biloba Supplementation on Aerobic Performance, Blood Pro/Antioxidant Balance, and Serum Brain-Derived Neurotrophic Factor in Physically Active Men. Nutrients. 2017;9(8).
44. Sadowska-Krepa E, Domaszewski P, Pokora I, Zebrowska A, Gdanska A, Podgorski T. Effects of Medium-Term Green Tea Extract Supplementation Combined with CrossFit Workout on Blood Antioxidant Status and Serum Brain-Derived Neurotrophic Factor in Young Men: A Pilot Study. Journal of the International Society of Sports Nutrition. 2019;16(1):13.
45. Sumiyoshi E, Matsuzaki K, Sugimoto N, Tanabe Y, Hara T, Katakura M, *et al.* Sub-Chronic Consumption of Dark Chocolate Enhances Cognitive Function and Releases Nerve Growth Factors: A Parallel-Group Randomized Trial. Nutrients. 2019;11(11).
46. Bot M, Pouwer F, Assies J, Jansen EH, Beekman AT, de Jonge P. Supplementation with Eicosapentaenoic Omega-3 Fatty Acid does not Influence Serum Brain-Derived Neurotrophic Factor in Diabetes Mellitus Patients with Major Depression: A Randomized Controlled Pilot Study. Neuropsychobiology. 2011;63(4):219-23.
47. van der Burg KP, Cribb L, Firth J, Karmacoska D, Mischoulon D, Byrne GJ, *et al.* EPA and DHA as Markers of Nutraceutical Treatment Response in Major Depressive Disorder. European Journal of Nutrition. 2019.

48. Matsuoka Y, Nishi D, Tanima Y, Itakura M, Kojima M, Hamazaki K, *et al.* Serum Pro-BDNF/BDNF as a Treatment Biomarker for Response to Docosahexaenoic Acid in Traumatized People Vulnerable to Developing Psychological Distress: A Randomized Controlled Trial. Translational Psychiatry. 2015;5:e596.
49. Sedláček P, Plavinova I, Langmajerova J, Dvorakova J, Novak J, Trefil L, *et al.* Effect of N-3 Fatty Acids Supplementation During Life Style Modification in Women with Overweight. Central European Journal of Public Health. 2018;26(4):265-71.
50. Witte AV, Kerti L, Hermannstadter HM, Fiebach JB, Schreiber SJ, Schuchardt JP, *et al.* Long-Chain Omega-3 Fatty Acids Improve Brain Function and Structure in Older Adults. Cerebral Cortex. 2014;24(11):3059-68.
51. Pinto-Sanchez MI, Hall GB, Ghajar K, Nardelli A, Bolino C, Lau JT, *et al.* Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. Gastroenterology. 2017;153(2):448-59 e8.
52. Riezzo G, Chimienti G, Orlando A, D'Attoma B, Clemente C, Russo F. Effects of Long-term Administration of Lactobacillus Reuteri DSM-17938 on Circulating Levels of 5-HT and BDNF in Adults with Functional Constipation. Beneficial Microbes. 2019;10(2):137-47.
53. West NP, Horn PL, Barrett S, Warren HS, Lehtinen MJ, Koerbin G, *et al.* Supplementation with a Single and Double Strain Probiotic on the Innate Immune System for Respiratory Illness. European e-Journal Society for Clinical Nutrition and Metabolism. 2014;9(5):e178-e84.
54. Haghighat N, Rajabi S, Mohammadshahi M. Effect of Synbiotic and Probiotic Supplementation on Serum Brain-Derived Neurotrophic Factor Level, Depression and Anxiety Symptoms in Hemodialysis Patients: A Randomized, Double-Blinded, Clinical Trial. Nutritional Neuroscience. 2019:1-10.
55. Hwang YH, Park S, Paik JW, Chae SW, Kim DH, Jeong DG, *et al.* Efficacy and Safety of Lactobacillus Plantarum C29-Fermented Soybean (DW2009) in Individuals with Mild Cognitive Impairment: A 12-Week, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Nutrients. 2019;11(2).
56. Chung Y-C, Jin H-M, Cui Y, Kim DS, Jung JM, Park J-I, *et al.* Fermented Milk of Lactobacillus Helveticus IDCC3801 Improves Cognitive Functioning during Cognitive Fatigue Tests in Healthy Older Adults. Journal of Functional Foods. 2014;10:465-74.
57. Kim CS, Cha L, Sim M, Jung S, Chun WY, Baik HW, *et al.* Probiotic Supplementation Improves Cognitive Function and Mood with Changes in Gut Microbiota in Community-Dwelling Elderly: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial. The Journals of Gerontology: Series A, Biological Sciences and Medical Sciences. 2020.
58. Romijn AR, Rucklidge JJ, Kuijer RG, Frampton C. A Double-Blind, Randomized, Placebo-Controlled Trial of Lactobacillus Helveticus and Bifidobacterium Longum for the Symptoms of Depression. Australian & New Zealand Journal of Psychiatry. 2017;51(8):810-21.
59. de Almeida RN, Galvao ACM, da Silva FS, Silva E, Palhano-Fontes F, Maia-de-Oliveira JP, *et al.* Modulation of Serum Brain-Derived Neurotrophic Factor by a Single Dose of Ayahuasca: Observation From a Randomized Controlled Trial. Frontiers in Psychology. 2019;10:1234.
60. Chui DH, Marcellino M, Marotta F, Sweed H, Solimene U, Vignali AI, *et al.* A Double-Blind, RCT Testing Beneficial Modulation of BDNF in Middle-Aged, Life Style-Stressed Subjects: A Clue to Brain Protection? Journal of Clinical and Diagnostic Research. 2014;8(11):MC01-6.
61. Kim H, Suzuki T, Kim M, Kojima N, Ota N, Shimotoyodome A, *et al.* Effects of Exercise and Milk Fat Globule Membrane (MFGM) Supplementation on Body Composition, Physical Function, and Hematological Parameters in Community-Dwelling Frail Japanese Women: A Randomized Double Blind, Placebo-Controlled, Follow-Up Trial. Public Library of Science One. 2015;10(2):e0116256.

62. Petelin A, Kenig S, Kopinc R, Dezelak M, Cernelic Bizjak M, Jenko Praznikar Z. Effects of Royal Jelly Administration on Lipid Profile, Satiety, Inflammation, and Antioxidant Capacity in Asymptomatic Overweight Adults. Evidence-Based Complementary Alternative Medicine. 2019;2019:4969720.
63. Sarris J, Byrne GJ, Stough C, Bousman C, Mischoulon D, Murphy J, *et al.* Nutraceuticals for Major Depressive Disorder- More is not Merrier: An 8-Week Double-Blind, Randomised, Controlled Trial. Journal of Affective Disorders. 2019;245:1007-15.
64. Stringham NT, Holmes PV, Stringham JM. Effects of Macular Xanthophyll Supplementation on Brain-Derived Neurotrophic Factor, Pro-Inflammatory Cytokines, and Cognitive Performance. Physiology & Behavior. 2019;211:112650.
65. Varanoske AN, Wells AJ, Boffey D, Harat I, Frosti CL, Kozlowski GJ, *et al.* Effects of High-Dose, Short-Duration beta-Alanine Supplementation on Cognitive Function, Mood, and Circulating Brain-Derived Neurotropic Factor (BDNF) in Recreationally-Active Males Before Simulated Military Operational Stress. Journal of Dietary Supplements. 2020:1-22.
66. Cox KHM, Scholey A. Polyphenols for brain and cognitive health. 2016.
67. Gomez-Guzman M, Rodriguez-Nogales A, Algieri F, Galvez J. Potential Role of Seaweed Polyphenols in Cardiovascular-Associated Disorders. Marine Drugs. 2018;16(8).
68. Rendeiro C, Vauzour D, Rattray M, Waffo-Teguo P, Merillon JM, Butler LT, *et al.* Dietary Levels of Pure Flavonoids Improve Spatial Memory Performance and Increase Hippocampal Brain-Derived Neurotrophic Factor. Public Library of Science One. 2013;8(5):e63535.
69. Williams CM, El Mohsen MA, Vauzour D, Rendeiro C, Butler LT, Ellis JA, *et al.* Blueberry-Induced Changes in Spatial Working Memory Correlate with Changes in Hippocampal CREB Phosphorylation and Brain-Derived Neurotrophic Factor (BDNF) Levels. Free Radic Biology & Medicine. 2008;45(3):295-305.
70. Scalbert A, Williamson G. Dietary Intake and Bioavailability of Polyphenols. American Society for Nutritional Science. 2000;130(8S Suppl):2073S-85S.
71. Moosavi F, Hosseini R, Saso L, Firuzi O. Modulation of Neurotrophic Signaling Pathways by Polyphenols. Drug Design, Development and Therapy. 2016;10:23-42.
72. Sarraf P, Parohan M, Javanbakht MH, Ranji-Burachaloo S, Djalali M. Short-Term Curcumin Supplementation Enhances Serum Brain-Derived Neurotrophic Factor in Adult Men and Women: A Systematic Review and Dose-Response Meta-Analysis of Randomized Controlled Trials. Nutrition Research. 2019;69:1-8.
73. Cicero AFG, Ruscica M, Banach M. Resveratrol and Cognitive Decline: A Clinician Perspective. Archives of Medical Science. 2019;15(4):936-43.
74. Um MY, Lim DW, Son HJ, Cho S, Lee C. Phlorotannin-Rich Fraction from Ishige Foliacea Brown Seaweed Prevents the Scopolamine-Induced Memory Impairment via Regulation of ERK-CREB-BDNF Pathway. Journal of Functional Foods. 2018;40:110-6.
75. Lee B-J, Kim J-S, Kang YM, Lim J-H, Kim Y-M, Lee M-S, *et al.* Antioxidant Activity and γ-Aminobutyric Acid (GABA) Content in Sea Tangle Fermented by Lactobacillus Brevis BJ20 Isolated from Traditional Fermented Foods. Food Chemistry. 2010;122(1):271-6.
76. Obrietan K, Gao X, Van den Pol AN. Excitatory Actions of GABA Increase BDNF Expression via a MAPK-CREB-Dependent Mechanism - A Positive Feedback Circuit in Developing Neurons. Journal of Neurophysiology. 2002;88(2):1005-15.
77. Kim JY, Kwon YM, Kim IS, Kim JA, Yu DY, Adhikari B, *et al.* Effects of the Brown Seaweed Laminaria japonica Supplementation on Serum Concentrations of IgG, Triglycerides, and Cholesterol, and Intestinal Microbiota Composition in Rats. Frontiers in Nutrition. 2018;5:23.
78. Poulose SM, Miller MG, Scott T, Shukitt-Hale B. Nutritional Factors Affecting Adult Neurogenesis and Cognitive Function. Advances in Nutrition. 2017;8(6):804-11.

79. Zamora-Ros R, Knaze V, Rothwell JA, Hemon B, Moskal A, Overvad K, *et al.* Dietary Polyphenol Intake in Europe: The European Prospective Investigation into Cancer and Nutrition (EPIC) study. European Journal of Nutrition. 2016;55(4):1359-75.
80. Prykhodko O, Sandberg J, Burleigh S, Bjorck I, Nilsson A, Fak Hallenius F. Impact of Rye Kernel-Based Evening Meal on Microbiota Composition of Young Healthy Lean Volunteers With an Emphasis on Their Hormonal and Appetite Regulations, and Blood Levels of Brain-Derived Neurotrophic Factor. Frontiers in Nutrition. 2018;5:45.
81. Sandberg JC, Bjorck IM, Nilsson AC. Rye-Based Evening Meals Favorably Affected Glucose Regulation and Appetite Variables at the Following Breakfast; A Randomized Controlled Study in Healthy Subjects. Public Library of Science One. 2016;11(3):e0151985.
82. Khan NA, Raine LB, Drollette ES, Scudder MR, Kramer AF, Hillman CH. Dietary Fiber is Positively Associated with Cognitive Control among Prepubertal Children. Journal of Nutrition. 2015;145(1):143-9.
83. Chung YC, Park CH, Kwon HK, Park YM, Kim YS, Doo JK, *et al.* Improved Cognitive Performance Following Supplementation with a Mixed-Grain Diet in High School Students: A Randomized Controlled Trial. Nutrition. 2012;28(2):165-72.
84. Breugelmans T, De Winter BY, Smet A. The Microbiota-Gut-Brain Axis in Gastrointestinal Inflammation and Neurological Comorbidities. Microbiota in Health and Disease. 2019;1(e201).
85. Proctor C, Thiennimitr P, Chattipakorn N, Chattipakorn SC. Diet, Gut Microbiota and Cognition. Metabolic Brain Disease. 2017;32(1):1-17.
86. Radka SF, Holst PA, Fritsche M, Altar CA. Presence of Brain-Derived Neurotrophic Factor in Brain and Human and Rat but Not Mouse Serum Detected by a Sensitive and Specific Immunoassay. Brain Research. 1996;709:122-30.
87. Gejl AK, Enevold C, Bugge A, Andersen MS, Nielsen CH, Andersen LB. Associations between Serum and Plasma Brain-Derived Neurotrophic Factor and Influence of Storage Time and Centrifugation Strategy. Scientific Reports. 2019;9(1):9655.
88. Dyall SC. Long-Chain Omega-3 Fatty Acids and the Brain: A Review of the Independent and Shared Effects of EPA, DPA and DHA. Frontiers in Aging Neuroscience. 2015;7:52.
89. Wu A, Ying Z, Gomez-Pinilla F. Dietary Omega-3 Fatty Acids Normalize BDNF Levels, Reduce Oxidative Damage, and Counteract Learning Disability after Traumatic Brain Injury in Rats. Journal of Neurotrauma. 2004;21(10):1457-67.
90. Pawelczyk T, Grancow-Grabka M, Trafalska E, Szemraj J, Zurner N, Pawelczyk A. An Increase in Plasma Brain-Derived Neurotrophic Factor Levels is Related to N-3 Polyunsaturated Fatty Acid Efficacy in First Episode Schizophrenia: Secondary Outcome Analysis of the OFFER Randomized Clinical Trial. Psychopharmacology (Berl). 2019;236(9):2811-22.
91. Kristensen SD, Schmidt EB, Dyerberg J. Dietary Supplementation with N-3 Polyunsaturated Fatty Acids and Human Platelet Function: A Review with Particular Emphasis on Implications for Cardiovascular Disease. Journal of Internal Medicine. 1989;225:141-50.
92. Stoll P, Plessow A, Bratke K, Virchow JC, Lommatzsch M. Differential Effect of Clopidogrel and Aspirin on the Release of BDNF from Platelets. Journal of Neuroimmunology. 2011;238(1-2):104-6.
93. Mazereeuw G, Lanctot KL, Chau SA, Swardfager W, Herrmann N. Effects of Omega-3 Fatty Acids on Cognitive Performance: A Meta-Analysis. Neurobiology of Aging. 2012;33:1482.e17-.e29.
94. Lim Y, Zhong JH, Zhou XF. Development of Mature BDNF-Specific Sandwich ELISA. Journal of Neurochemistry. 2015;134(1):75-85.
95. Wu A, Ying Z, Gomez-Pinilla F. Docosahexaenoic Acid Dietary Supplementation Enhances the Effects of Exercise on Synaptic Plasticity and Cognition. Neuroscience. 2008;155(3):751-9.

96. Hwang JJ, Park MH, Choi SY, Koh JY. Activation of the Trk Signaling Pathway by Extracellular Zinc. Role of metalloproteinases. Journal of Biological Chemistry. 2005;280(12):11995-2001.
97. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, *et al.* Blood BDNF Concentrations Reflect Brain-Tissue BDNF Levels Across Species. International Journal of Neuropsychopharmacology. 2011;14(3):347-53.
98. Dinoff A, Herrmann N, Swardfager W, Liu CS, Sherman C, Chan S, *et al.* The Effect of Exercise Training on Resting Concentrations of Peripheral Brain-Derived Neurotrophic Factor (BDNF): A Meta-Analysis. Public Library of Science One. 2016;11(9):e0163037.
99. Pillai A, Kale A, Joshi S, Naphade N, Raju MS, Nasrallah H, *et al.* Decreased BDNF Levels in CSF of Drug-Naive First-Episode Psychotic Subjects: Correlation with Plasma BDNF and Psychopathology. International Journal of Neuropsychopharmacology. 2010;13(4):535-9.

Chapter 3

Effects of the egg protein hydrolysate NWT-03 on cognitive function in men and women with the metabolic syndrome: A randomized, double-blind, placebo-controlled study

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Submitted

Chapter 4

Dietary macronutrients do not differently influence postprandial serum and plasma brain-derived neurotrophic factor concentrations: A randomized, double-blind, controlled cross-over trial

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) plays a role in cognition and metabolism. Specific nutrients can affect fasting BDNF concentrations, which are potentially mediated by insulin and/or glucose. Since macronutrients trigger each a different insulin and glucose response, we examined postprandial effects of meals rich in fat, carbohydrates, or protein on BDNF concentrations. BDNF was analyzed in serum and plasma, since concentration differences can be found between matrices.

Methods: Healthy overweight/obese male participants (n = 18) participated in this randomized, double-blind, cross-over trial consisting of three test days with 1 week wash-out periods. Either a high-fat (En% fat, carbohydrates, protein: 52.3, 39.2, 8.0), high-carbohydrate (En% 9.6, 81.5, 8.6) or high-protein meal (En% 10.6, 51.5, 36.9) was consumed on each test day. BDNF concentrations were measured after 0, 60, and 240 min. Glucose and insulin concentrations were measured after 0, 15, 30, 45, 60, 90, 120, and 240 min.

Results: BDNF concentrations were higher in serum compared with plasma ($P < 0.001$). Postprandial BDNF concentrations in serum decreased significantly after the high-fat ($P = 0.013$) and high-carbohydrate meals ($P = 0.040$), and showed a trend after the high-protein meal ($P = 0.076$). No differences were found between meals ($P = 0.66$). Postprandial BDNF concentrations measured in plasma did not significantly change after the different meals ($P = 0.47$). As total area under the curve (AUC) for glucose was significantly higher after the high-carbohydrate meal compared with the high-fat ($P = 0.003$) and high-protein meals ($P < 0.001$), and the total AUC for insulin was higher after the high-carbohydrate ($P < 0.001$) and high-protein meals ($P < 0.001$) compared with the high-fat meal, it seems that acute changes in glucose and insulin do not affect postprandial BDNF concentrations. However, after the high-protein meal, the higher total AUC for glucose correlated with lower serum BDNF concentrations, and a higher maximal increase in glucose correlated with a lower maximal increase in plasma BDNF concentrations. There were no correlations with insulin concentrations after either meal.

Conclusion: Serum BDNF concentrations were higher than plasma concentrations. Since postprandial BDNF responses were not different between the meals, we conclude that there is no role for insulin or glucose in regulating postprandial BDNF concentrations.

Clinical Trial Registration: www.ClinicalTrials.gov, NCT03139890.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a protein that contributes to brain development in childhood, and positively affects neuronal functioning and cognition later in life [1]. The relevance of understanding the physiology of BDNF becomes even more relevant as it has been discovered that BDNF also plays a role in metabolism [2]. Overall, higher circulating BDNF concentrations are associated with improved health [3].

We have recently concluded in a systematic literature review that specific dietary components can influence circulating fasting BDNF concentrations [4]. Amongst others, we concluded that a link could be possible between dietary macronutrient composition and fasting BDNF concentrations, which is potentially mediated by insulin. Moreover, Lee *et al.* showed a decrease in serum BDNF concentrations in older participants after an oral glucose tolerance test (OGTT; [5, 6]). In addition, diabetic patients are characterized by lower BDNF concentrations as compared to healthy controls [7, 8]. In other words, there is an apparent link between insulin and/or glucose concentrations and BDNF concentrations. So far, it remains unanswered whether BDNF relates to plasma insulin or plasma glucose concentrations, or both.

Next to dietary interventions evaluating changes in fasting BDNF concentrations, a number of studies also evaluated postprandial changes in BDNF concentrations. This can be of interest since it provides the possibility to differentiate between the role of insulin and glucose in BDNF changes, as insulin and glucose responses are different for each macronutrient: carbohydrates elevate both insulin and glucose, proteins also trigger the release of insulin but do not elevate glucose as pronounced as carbohydrates, and fats do not give a clear insulin or glucose response, but mostly elevate triacylglycerol (TAG) concentrations [9]. In this context, high protein diets induced BDNF expression in C57Bl/6 mice brain [10] and increased plasma BDNF levels in Wistar rats [11]. However, the effect of protein intake on circulating BDNF concentrations in humans has not been evaluated. In contrast to protein, effects of a high-fat diet have already been examined in humans. More specific, a high fat intake showed a decrease in plasma BDNF concentrations in healthy young male adults [12]. Finally, the earlier mentioned OGTT studies by Lee *et al.* [5, 6] showed a decrease in BDNF. Altogether, there are clear indications for differences in postprandial BDNF responses after intake of the three macronutrients, but designs, populations, and details of the meals in the different studies are too distinct to draw conclusions.

Therefore, the aim of this study was to investigate the influence of three different meals high in either one of the three macronutrients fat, carbohydrates, and protein on circulating postprandial BDNF concentrations in a systematic, side by side manner. Since peripheral BDNF is mainly stored in platelets [13], BDNF concentrations in the circulation highly vary depending on the matrix in which it is analyzed [4]. It is, therefore, interesting to evaluate these postprandial changes after the three meals both in serum and plasma.

METHODS

Participants

For this study, 20 apparently healthy overweight and obese male participants were recruited for their increased glucose and insulin postprandial responses from Maastricht and surrounding area. Inclusion criteria to evaluate eligibility for participation were: male gender, aged 18-70 y, BMI 25-35 kg/m^2, stable body weight defined as weight gain or loss < 3 kg in the past 3 months, nonsmoker or smoking cessation > 1 year, no medication targeting blood pressure, lipid or glucose metabolism, no diabetic patient, and no active cardiovascular disease. All participants gave their written consent before the start of the study. Study procedures were in accordance with the Declaration of Helsinki, and approved by the Medical Ethical Committee of the University Hospital Maastricht/Maastricht University (METC173012). The study was registered in May 2017 at ClinicalTrials.gov (NCT03139890).

Study design

The study had a randomized, double-blind, controlled cross-over design. In total, 20 participants participated in three postprandial test days, each separated by a minimum of 1-week wash-out period. The order of the three test meals was equally randomly assigned to participants by an independent researcher. Participants were instructed to refrain from strenuous physical activity 48 hours before each test day, and from alcohol consumption 24 hours before each test day. In addition, they were asked to keep their habitual food intake and physical activity levels constant during the entire study period.

Postprandial test day

Participants arrived in the morning of the test days after an overnight fast (from 8PM) to the Metabolic Research Unit Maastricht. To standardize the measurements, men

were instructed to travel either by car or public transport, but identical on all three occasions. Upon arrival, first, an intravenous cannula was placed and a fasting blood sample (T0) was taken. Subsequently, participants consumed either the high-fat, high-carbohydrate or high-protein mixed meal. The meals had to be consumed in three stages: one minute was provided to consume 1/3rd of the meal, which was followed by a 2-minute break. This was repeated until the meal was consumed completely. When participants did not manage to consume the meal according to this protocol, the breaks were shortened to provide participants more time for the consumption periods.

The mixed meals were prepared on the morning of the test days by an independent researcher. All three meals contained 953 kilocalories each. The composition of the high-fat meal comprised of 52.3 En% from fat, 39.2 En% from carbohydrates, and 8.0 En% from protein, the high-carbohydrate meal of 9.6 En%, 81.5 En%, and 8.6 En%, respectively, and the high-protein meal of 10.6 En%, 51.5 En%, and 36.9 En%. The meal compositions are shown in Table 4.1. Depending on the meal's volume participants received a certain amount of water separately to assure that the total volume of each meal was 730 mL. Subsequent postprandial blood samples were collected at the following time points after meal consumption: 15 min (T15), 30 min (T30), 45 min (T45), 60 min (T60), 90 min (T90), 120 min (T120), and 240 min (T240).

Biochemical analyses

Blood was collected in serum STT-II advance tubes (Becton Dickinson) for the analyses of TAG (GPO Trinder, Sigma-Aldrich Corp., St. Louis, MO, United States) at T0, T30, T60, T120, T180, and T240, with correction for free glycerol, and insulin at all time points (human insulin specific RIA kit, Millipore, Billerica, MA, United States). The minimum clotting time was 30 min at room temperature. To obtain serum, the tubes were centrifuged at 1300xg for 15 min at 21 °C. In addition, blood was collected in EDTA, heparin, and NaF tubes. NaF samples were used for the analyses of glucose (Glucose HK CP, Horiba ABX, Montpellier, France), and free fatty acids (FFA; NEFA-HR, HUIJFILM Wako Diagnostics U.S.A. Corp. Mountain View, CA, United States) at all time points. To obtain NaF plasma, the tubes were immediately centrifuged at 1300xg for 15 min at 4 °C. Finally, EDTA and heparin tubes were processed based on the same protocol as the NaF tubes. After centrifugation, all serum and plasma samples were portioned into aliquots, snap-frozen in liquid nitrogen, and stored at -80 °C until biochemical analyses after the study was completed. Both serum and plasma (EDTA and heparin) samples were used for the analyses of BDNF at T0, T60, and T240 by an enzyme-linked immunosorbent assay (Duo Kit ELISA, R & D Systems, Minneapolis, MN, United States)

according instructions by manufacturer. The ELISA cannot distinguish between precursor BDNF (proBDNF) and mature BDNF (mBDNF). Therefore, BDNF concentrations reported here are the sum of proBDNF and mBDNF.

Table 4.1 | Meal composition of the high-fat, high-carbohydrate, and high-protein meals

Nutritional value	High-fat meal	High-carbohydrate meal	High-protein meal
Energy (kcal)	953	953	953
Cholesterol (mg)	331.2	334.1	334.1
Fat (g)	55.4	10.2	11.3
- SFA (g)	33.1	3.4	4.0
- MUFA (g)	16.0	4.0	4.1
- PUFA (g)	5.0	0.9	0.9
Total fat (En%)	**52.3**	**9.6**	**10.6**
Carbohydrates (g)	93.5	194.3	122.7
- Glucose (g)	0.3	0.3	1.2
- Sucrose (g)	45.6	145.6	59.1
- Lactose (g)	0.5	0.0	1.3
- Polysaccharide (g)	45.0	45.0	58.1
Total carbohydrates (En%)	**39.2**	**81.5**	**51.5**
Protein (g)	19.2	20.4	87.9
Total protein (En%)	**8.0**	**8.6**	**36.9**
Total volume (g)	**433**	**468**	**615**
Water (mL)	297	262	115

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Statistical analyses

Data are presented as means ± standard deviation (SD), unless indicated otherwise. Despite the research objective is not part of the primary outcome measure, the initial statistical plan was followed to evaluate the postprandial effects of the macronutrients fat, carbohydrates, or protein on BDNF concentrations and other biomarkers. Fasting values between the three test days were compared using repeated-measures ANOVA. In order to test the pattern of the dependent variable after each of the three meals over time, postprandial curves were analyzed with linear mixed models. For this, the absolute BDNF concentration was used as dependent variable with time as fixed factor. If statistically significant, time points T60 and T240 were compared to fasting values with Bonferroni adjustment for multiple comparisons. Next, to compare the patterns of BDNF after the three meals side-by-side, again a linear mixed model analysis was performed,

now with the changes in BDNF concentrations as dependent variable and with period, time, diet, and interaction [time*diet] as fixed factors. When the interaction term was not statistically significant, it was excluded from the model. Next, total area under the curve (total AUC) was calculated for all parameters. Incremental area under the curve (iAUC) was calculated for glucose, TAG, insulin, and plasma BDNF. Decremental area under the curve (dAUC) was calculated for FFA, and serum BDNF. Maximal increases or decreases were calculated by comparing the changes between the time points relative to fasting values. In order to compare the AUC data between the three meals a Friedman test was used. Bonferroni corrections were applied to correct for multiple comparisons. Relations between the AUC data for BDNF and glucose, TAG, insulin, and FFA were analyzed with Spearman correlation coefficients. Results were considered statistically significant at $P < 0.05$. Statistical analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, version 26.0, Armonk, NY, United States).

RESULTS

Study participants

During the study, two participants dropped out due to personal reasons, which implies that a total of 18 men with a median (interquartile range) age of 65 (51-67) years and a BMI of 30.5 ± 2.9 kg/m^2 completed the study. All baseline characteristics are presented in **Supplemental Table 4.1**.

Postprandial BDNF concentrations

As shown in **Table 4.2** and **Supplemental Figure 4.1**, fasting BDNF concentrations were not significantly different between the three test days, in both serum (P=0.769) and EDTA plasma (P=0.858) samples. Interestingly, fasting BDNF concentrations in serum samples were 4- to 5-fold higher as compared to fasting BDNF concentrations in EDTA plasma samples (P<0.001). When visually inspecting the data, the overall pattern indicated that serum BDNF concentrations decreased till 240 minutes postprandial after all three meals, whereas plasma BDNF concentrations increased during the same postprandial interval after all three meals. Fasting BDNF concentrations in heparin plasma were even lower as compared to EDTA plasma (P<0.001), but showed the same time pattern as compared to EDTA plasma (**Supplemental Table 4.2** and **Supplemental Figure 4.1C**).

Table 4.2 | BDNF concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meals in serum and plasma (EDTA)

Medium	Meal	BDNF (pg/ml)		
		T0	T60	T240
Serum	Fat*	24290 ± 8842	19120 ± 7723	16366 ± 6793[b]
	Carbohydrates*	24073 ± 9227	18502 ± 8733	16535 ± 8869[b]
	Protein[a]	22559 ± 10077	21229 ± 8164	16199 ± 7495
EDTA	Fat	5154 ± 2002	5444 ± 2440	5977 ± 1948
	Carbohydrates	5072 ± 1549	5084 ± 1879	5833 ± 1947
	Protein	5335 ± 1886	5460 ± 1931	5256 ± 1876

*Values are presented as mean ± SD; * significant time effect after the high-fat (P<0.05) and high-carbohydrate meal (P<0.05); a trend after the high-protein meal (P=0.076); b significant time effect at time point T240 compared to T0 (P<0.05) after Bonferroni correction*

Within the serum samples, the postprandial changes in BDNF concentrations showed a significant time effect after the high-fat meal (P=0.013; **Table 4.2**, **Figure 4.1A** and **Supplemental Figure 4.1A**) and the high-carbohydrate meal (P=0.040), whereas the response after the high-protein meal showed a trend (P=0.076). More specific, the time effects after the high-fat and high-carbohydrate meals were significant at time point T240 as compared to T0 (P=0.008 and P=0.029, respectively). Comparing the postprandial responses relative to fasting concentrations side-by-side indicated no significant difference between the three meals (P=0.662).

In contrast to the patterns shown in serum, the postprandial changes in BDNF concentrations within EDTA plasma samples did not show a time effect after any of the three meals; after the high-fat meal (P=0.509; **Figure 4.1B** and **Supplemental Figure 4.1B**), after the high-carbohydrate meal (P=0.355), and after the high-protein meal (P=0.949). Again, no significant differences in postprandial plasma BDNF concentrations were found between meals (P=0.473). In line with EDTA plasma samples, also the postprandial changes in BDNF concentrations within heparin plasma did not show a time effect after the high-fat (P=0.224), high-carbohydrate (P=0.083), or high-protein meal (P=0.909), and no significant differences were found between meals (P=0.499).

When the postprandial concentrations and changes in BDNF concentrations in serum (**Table 4.3**) and EDTA plasma (**Table 4.4**) were used to calculate the total AUC, dAUC, iAUC, and maximal increases or decreases, it appeared that the total AUC was not significantly different between the three meals (P=0.607 and P=0.486, for serum and EDTA plasma, respectively). No significant differences were found for the dAUC in serum

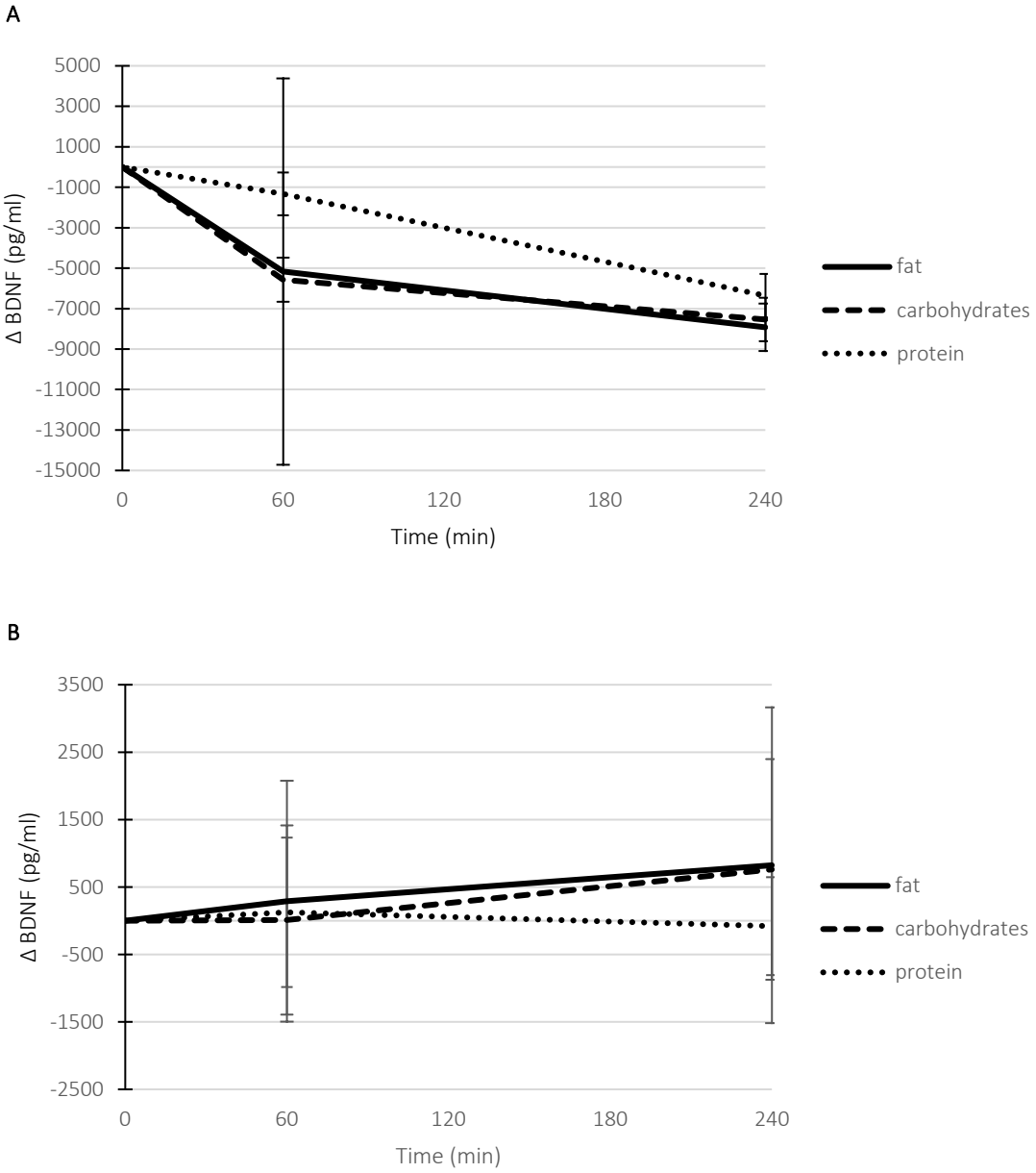


Figure 4.1 | Postprandial mean changes (± SD) in BDNF concentrations (pg/ml) after consumption of the high-fat (solid line), high-carbohydrate (dashed line) and high-protein (dotted line) meal in serum (A) and EDTA plasma (B)

(P=0.642) or the iAUC in EDTA plasma (P=0.073), neither for the maximal decreases (P=0.958) or the maximal increases (P=0.146), respectively. These postprandial BDNF parameters in heparin plasma showed the same pattern as observed in EDTA plasma, but were even lower (Supplemental Table 4.3).

Table 4.3 | Postprandial responses (dAUCs) and maximal decreases of BDNF concentrations (pg/ml) after consumption of the high-fat, high-carbohydrate or high-protein meal in serum

Meal	Total AUC (concentration/240 min)	dAUC (concentration/240 min)	Max decrease
Fat	4285485 (3369833 – 5667180)	795871 (109779 – 2888708)	9110 (2817 – 19988)
Carbohydrates	4125105 (3166148 – 5197710)	1887510 (146304 – 2683538)	11548 (2860 – 17534)
Protein	4591665 (4168493 – 5487653)	863347 (125501 – 1656218)	9065 (4456– 14669)

Values are presented as median with ranges (25-75th percentiles)

Table 4.4 | Postprandial responses (iAUCs) and maximal increases of BDNF concentrations (pg/ml) after consumption of the high-fat, high-carbohydrate or high-protein meal in EDTA plasma

Meal	Total AUC (concentration/240 min)	iAUC (concentration/240 min)	Max increase
Fat	1296285 (1016633 – 1770825)	142899 (5164 – 324556)	1092 (308 – 2408)
Carbohydrates	1380855 (1033763 – 1549095)	87512 (23925 – 235238)	1037 (255 – 1763)
Protein	1238220 (1004618 – 1556190)	38363 (0 – 174233)	439 (0 – 962)

Values are presented as median with ranges (25-75th percentiles)

Postprandial lipemia and glycemia

Fasting glucose, TAG, and FFA concentrations at the start of the three test days were comparable, but fasting insulin concentrations were significantly higher at the start of the day of the high-protein meal compared to the high-fat meal (P=0.003) and the high-carbohydrate meal (P=0.046; Table 4.5 and Supplemental Figure 4.2). The total AUC based on the postprandial glucose concentrations was higher after the high-carbohydrate meal compared to the high-fat meal (P=0.003) and the high-protein meal (P<0.001). Consequently, also the iAUC and maximal increases in glucose concentrations were higher after the high-carbohydrate meal compared to the high-fat

Table 4.5 | Postprandial responses (iAUCs) and maximal increases of glucose, TAG, and insulin, and postprandial responses (dAUCs) and maximal decreases of FFA after consumption of the high-fat, high-carbohydrate or high-protein meal

Blood marker	Meal	Fasting	Total AUC (concentration/240 min)	iAUC/dAUC (concentration/240 min)	Max increase/decrease
Glucose (mmol/L)	Fat	5.35 (5.11 – 5.73)	1343 (1231 – 1422)	67 (43 – 108)	1.33 (1.14 – 1.89)
	Carbohydrates	5.53 (5.26 – 5.66)	1428 (1267 – 1589)[b]	106 (59 – 263)[b]	2.31 (1.92 – 3.38)[b]
	Protein	5.42 (5.26 – 5.65)	1328 (1202 – 1456)[c]	49 (19 – 97)[c]	1.17 (0.85 – 1.47)[c]
TAG (mmol/L)	Fat	1.16 (0.89 – 1.36)	382 (280 – 441)	82 (55 – 119)	0.76 (0.60 – 1.04)
	Carbohydrates	1.17 (0.76 – 1.87)	323 (226 – 565)	63 (38 – 108)	0.51 (0.26 – 0.92)[b]
	Protein	1.10 (0.78 – 1.51)	297 (244 – 453)	56 (32 – 114)	0.57 (0.30 – 1.01)
Insulin (µU/mL)	Fat	9.88 (7.90 – 0.57)[a]	7767 (5895 – 12318)[a]	5746 (4126 – 9376)[a]	59.06 (41.39 – 94.98)[a]
	Carbohydrates	10.52 (8.55 – 11.57)	12905 (10323 – 21478)[b]	10851 (7808 – 18549)[b]	106.79 (73.96 – 157.58)[b]
	Protein	11.11 (9.08 – 17.29)[c]	11824 (8594 – 17281)	8692.43 (6198.06 – 14350.26)	98.70 (58.01 – 134.19)
FFA (µmol/L)	Fat	254.99 (147.66 – 328.30)	67465 (32598 – 93810)	11533 (4432 – 27695)[a]	112.53 (51.09 – 188.87)[a]
	Carbohydrates	288.98 (161.38 – 371.62)	112788 (52729 – 140852)	43671 (17951 – 54575)[b]	237.67 (118.36 – 306.21)[b]
	Protein	260.07 (171.02 – 310.22)	85939 (44926 – 109584)	26574 (13026 – 39299)	189.20 (101.61 – 225.13)

Values are presented as median with ranges (25-75th percentiles); TAG: triacylglycerol; FFA: free fatty acids; [a] significant difference between high-fat and high-protein meal; [b] significant difference between high-fat and high-carbohydrate meal; [c] significant difference between high-carbohydrate and high-protein meal

meal (P<0.001 and P<0.001, respectively) and the high-protein meal (P<0.001 and P<0.001, respectively). The total AUC based on the postprandial insulin concentrations was lower after the high-fat meal compared to the high-carbohydrate meal (P<0.001) and the high-protein meal (P<0.001), but no significant differences were found between the high-carbohydrate and high-protein meal (P=0.134). Moreover, the iAUC and maximal increases for insulin were also lower after the high-fat meal compared to the high-carbohydrate meal (P<0.001 and P<0.001, respectively) and the high-protein meal (P=0.002 and P=0.001, respectively), but again no significant differences were found between the high-carbohydrate and high-protein meal (P=0.096 and P=0.739, respectively). The total AUC based on the postprandial TAG concentrations were not different between the meals (P=0.311). However, the iAUC for TAG tended to be different between the meals (P=0.092). The difference in maximal increases did reach significance between the high-fat and high-carbohydrate meal (P=0.008). The total AUC based on the postprandial FFA concentrations tended to be different between the meals (P=0.092). The dAUC and maximal decreases for FFA were lower after the high-fat meal compared to the high-carbohydrate meal (P<0.001 and P<0.001, respectively) and the high-protein meal (P=0.003 and P=0.005, respectively).

Correlations between BDNF, lipemia and glycemia

The total AUC for BDNF in serum samples correlated with the total AUC for the glucose response after the high-protein meal (r=0.486; P=0.041), which indicates that a higher total AUC for glucose is associated with lower BDNF responses. No correlations were found between the total AUC of BDNF in serum samples and TAG, insulin, and FFA after one of the meals. Moreover, there were no correlations between the dAUC or the maximal decrease of BDNF in serum and glucose, TAG, insulin, and FFA after one of the meals. For the EDTA plasma samples, a correlation was found between the total AUC of BDNF and the total AUC of FFA after the high-carbohydrate meal (r=-0.478; P=0.045), which indicates that higher FFA responses are associated with lower BDNF responses. No correlations were found between the total AUC of BDNF in EDTA samples and glucose, TAG, and insulin after one of the meals. A correlation was found between the maximal increase of BDNF in plasma samples and glucose after the high-protein meal (r=-0.701; P=0.001), which indicates that higher maximal increases of glucose are associated with lower maximal increases of BDNF. No correlations were found between the iAUC or the maximal increase of BDNF in plasma samples and glucose, TAG, insulin, and FFA after one of the meals.

DISCUSSION

Based on the literature, we hypothesized that postprandial BDNF responses after intake of fat, carbohydrate and protein rich meals were potentially different because of the suggested role for insulin and/or glucose in BDNF regulation. Variations in designs, populations and meal composition between those studies made it difficult to be conclusive. Therefore, we here evaluated the influence of the three macronutrients on postprandial BDNF concentrations side by side. We found that BDNF concentrations significantly decreased postprandially in serum, but not in plasma. However, no differences were found between the three meals rich in either one of the macronutrients, in serum or plasma. Since postprandial plasma insulin and glucose concentrations were clearly distinct between the three meals, as expected, this also implies that both insulin and glucose, at least in the acute situation, are not involved in regulating circulating BDNF concentrations. However, it should be considered that measuring postprandial BDNF was not the primary end point for which this study was initially designed and powered.

Although we hypothesized that insulin and/or glucose regulates circulating BDNF concentrations, there are also arguments to suggest the opposite, *i.e.*, that BDNF regulates metabolic control, thereby affecting insulin sensitivity [14]. In more detail, in obese diabetic C57BL/KsJ mice BDNF administration increased pancreatic beta cells sensitization [15]. Furthermore, in Zucker diabetic fatty rats BDNF administration lowered hepatic gluconeogenesis [16]. Finally, De Luis *et al.* [17] showed that specific BDNF gene variants are associated with insulin resistance in humans. Interestingly, plasma BDNF concentrations were negatively correlated with insulin resistance using the homeostatic model assessment version 2 (HOMA2-IR), but not with insulin concentrations [18]. More mechanistically, BDNF and insulin are involved in overlapping signaling pathways via activation of protein kinase B [19]. Therefore, we postulated that it could also be that insulin affects circulating BDNF concentrations instead of the other way around as others postulated. However, our data could not confirm this assumption, since changes in insulin concentrations were not aligned with changes in BDNF concentrations.

Instead of the potential direct effect of insulin, as suggested from the above-mentioned overlapping signaling pathways, such an effect of insulin on BDNF concentrations could also be indirect via changing postprandial plasma glucose or FFA concentrations. Notwithstanding the differences in postprandial glucose concentrations between meals, we were not able to show a difference in BDNF concentrations after the

high-carbohydrate meal compared to the high-protein meal. However, there was a correlation between the total AUC for serum BDNF and the total AUC for glucose, and between the maximal increase of plasma BDNF and the maximal increase of glucose after the high-protein meal, indicating a possible indirect effect of insulin on BDNF concentrations via plasma glucose concentrations.

Besides the glucose axis, insulin could also have an indirect effect on BDNF concentrations via changes in plasma FFA concentrations. Indeed, we found a correlation between the total AUC for plasma BDNF and the total AUC for FFA after the high-carbohydrate meal. Insulin inhibits lipolysis, thereby decreasing FFA concentrations. Conversely, elevated FFA concentrations induce insulin resistance [20]. The results of a postprandial analysis by Karczewska-Kupczewska *et al.* [12] showed indeed that an elevation of FFA by a high-fat meal decreased plasma BDNF concentrations in healthy young male adults. In this same study, a euglycemic hyperinsulinemic clamp with intralipid infusion decreased both insulin sensitivity and BDNF concentrations as compared to a clamp without intralipid infusion. However, a high-fat meal does not substantially affect insulin concentrations as a high-carbohydrate or high-protein meal do. This could mean that the changes in BDNF concentrations seen in the study by Karczewska-Kupczewska *et al.* [12] could be attributed to the direct effect of FFA but not to the indirect effect of insulin via FFA.

Finally, it could also be possible that postprandial TAG concentrations affect BDNF concentrations. However, to the best of our knowledge, there is lack of evidence connecting postprandial changes in TAG and BDNF concentrations. Also in the current study, despite the increased TAG response after the high-fat meal no difference in changes in BDNF concentrations were found between the meals, which excludes a possible role for TAG.

Based on our findings presented here, it seems that there is no role for insulin and/or glucose in changing postprandial BDNF. Therefore, the question now is how to explain the earlier findings showing that insulin resistance or higher glucose levels in diabetic patients is associated with lower circulating BDNF concentrations in both serum [7, 8] and plasma [18]. The most likely explanation is that the acute effects of increased postprandial insulin or glucose in this postprandial situation, are not strong enough to induce changes in BDNF concentrations, whereas long term exposure to chronically elevated glucose and/or insulin, as seen in diabetic patients, might have such an effect. However, it seems a bit more complex since prediabetes is associated with higher BDNF concentrations [21]. Moreover, higher BDNF concentrations were also found in untreated newly diagnosed diabetic patients [22], but as well as in previous diagnosed

type 2 diabetic patients [23, 24]. In the latter two studies, though, it should be considered that the control group had a lower weight which could indicate that BDNF concentrations were influenced by BMI [8], albeit evidence is controversial. Finally, these apparent discrepancies could also relate to ethnic differences between study populations [7, 23]. Altogether, this at least indicates that the diabetic stage influences BDNF concentrations [14]. However, it is difficult to conclude how this is regulated. Moreover, it also implies that in our study population of healthy overweight or obese participants, postprandial effects of insulin and glucose on BDNF might be compensated. After all, it is also still a possibility that the relation between insulin and BDNF is actually in the other direction, *i.e.*, BDNF concentrations affecting insulin, as suggested from animal studies, which might not be found in healthy insulin sensitive participants.

Interestingly, both fasting and postprandial BDNF concentrations were higher in serum than plasma, which can be explained by the fact that BDNF is mainly stored in platelets and released during clotting [25]. However, the interesting observation was that serum BDNF concentrations showed a significant postprandial decrease, whereas plasma BDNF concentrations did not show a significant postprandial change. Therefore, it could be that either there may be less platelets in the postprandial state or the clotting process, in which BDNF is released from platelets, is less efficient in the samples collected during the postprandial state. In this context, it was found that a high-fat meal elevated platelet count [26], but a high-carbohydrate meal did not [27]. Regarding the clotting process, it has been shown that meals rich in fats seem to activate coagulation factor VII which stimulates the clotting process [28]. Hyperglycemia also activates the coagulation process [29], in contrast to meals rich in proteins [28]. In other words, when the postprandial clotting process is indeed activated more after the high-fat and high-carbohydrate meals, higher postprandial serum BDNF concentrations would have been expected as compared to the high-protein meal. Moreover, since postprandial insulin also stimulates platelet activation, platelet activation upon hyperglycemia seems to be actually triggered by hyperinsulinemia [30]. As proteins trigger the release of insulin, higher postprandial serum BDNF concentrations could be expected after the high-protein meal as well. Therefore, this does not seem to be a likely explanation for the observed decrease in serum BDNF concentrations after all three macronutrient rich meals. More important, however, if we assume that the clotting process in serum samples was complete, this means that the previously mentioned postprandial changes in clotting factors are unlikely to affect serum BDNF concentrations, but would rather have affected plasma BDNF concentrations.

In conclusion, we have demonstrated that macronutrients do not differently affect BDNF concentrations in the postprandial state. We found that postprandial BDNF concentrations decreased and were higher in serum compared to plasma. Based on these differences, we postulate to analyze BDNF concentrations in future studies at least in serum, and preferable in both serum and plasma to enlarge our understanding of BDNF regulation. Although it seems there is no direct role for insulin or glucose on BDNF, at least not in the acute situation, it would be valuable that future studies focus on the role of insulin on BDNF in the long-term situation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

CONFLICT OF INTEREST

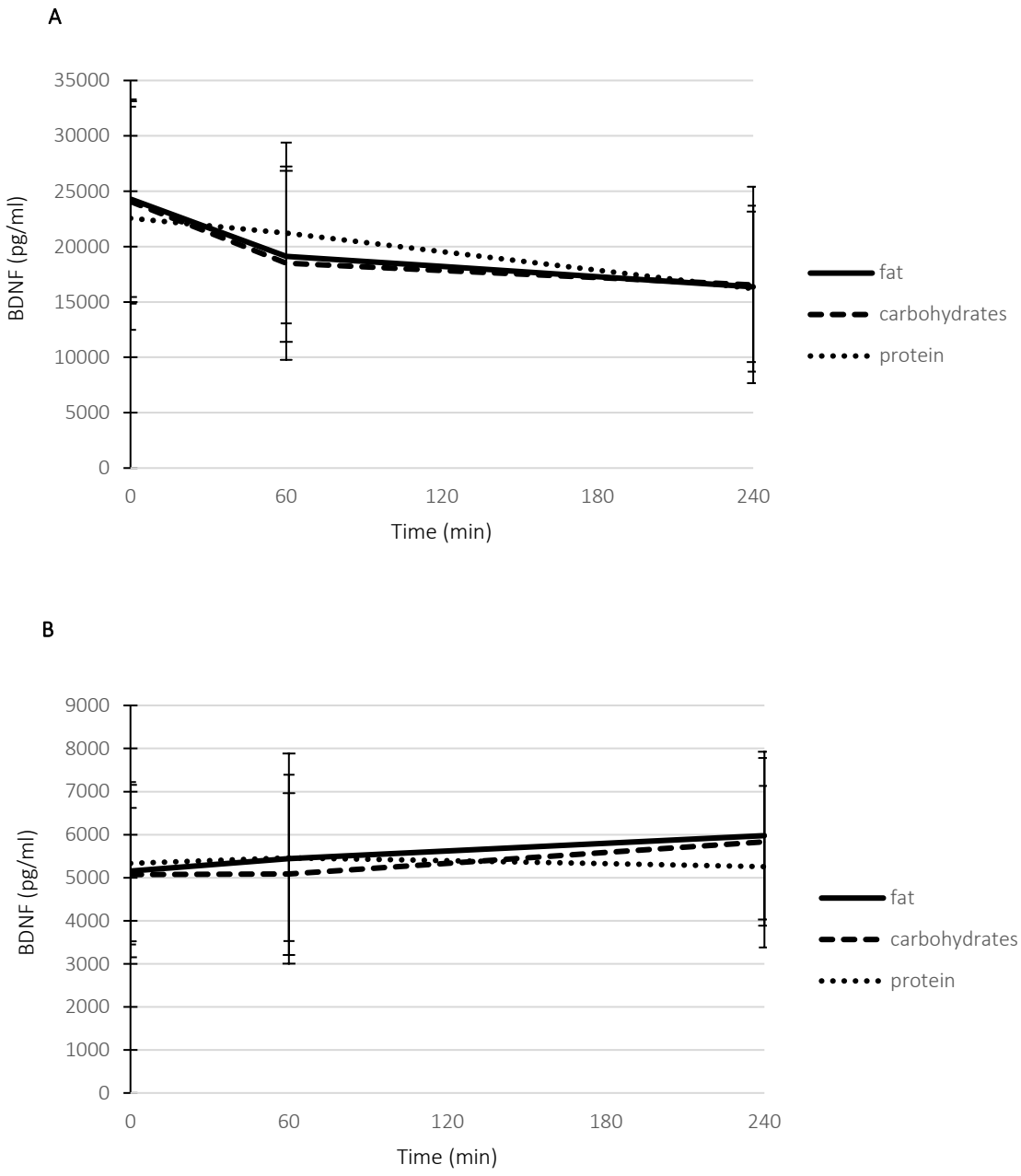
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTAL MATERIAL

Supplemental Table 4.1 | Baseline participant characteristics

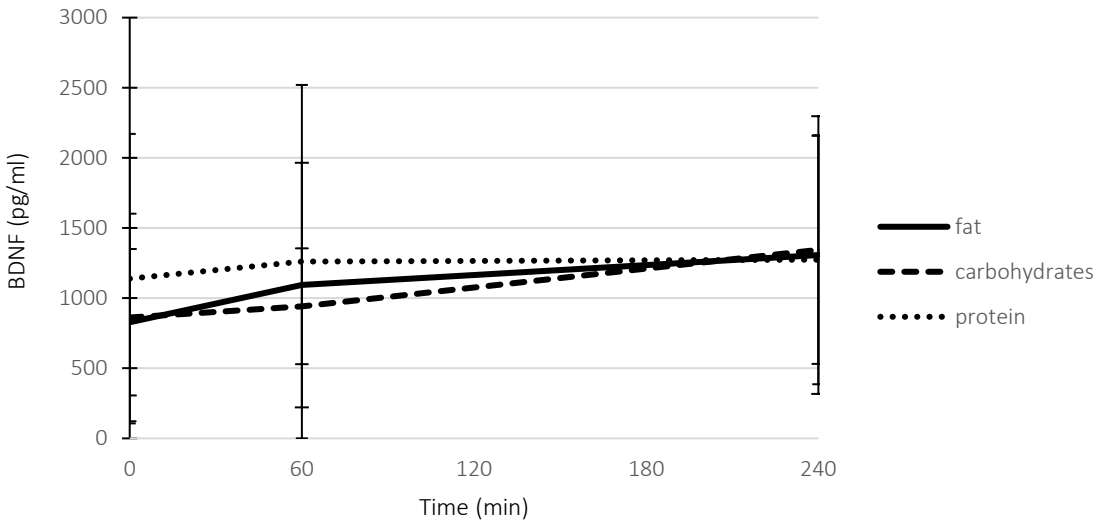
	Participants (n=18)
Age (years)	65 (51 – 67)*
BMI (kg/m^2)	30.5 ± 2.9
Fasting glucose (mmol/L)	5.67 ± 0.49
Fasting TAG (mmol/L)	1.27 ± 0.47
Fasting insulin (µU/mL)	12.19 ± 6.13
Fasting FFA (µmol/L)	308.12 ± 133.21

*Values are presented as mean ± SD; BMI: body mass index; TAG: triacylglycerol; FFA: free fatty acids; * median (interquartile range)*



Supplemental Figure 4.1 | BDNF concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meal in serum (A), EDTA plasma (B), and heparin plasma (C)

C



Supplemental Figure 4.1 (continued) | BDNF concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meal in serum (A), EDTA plasma (B), and heparin plasma (C)

Supplemental Table 4.2 | BDNF concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meal in heparin plasma

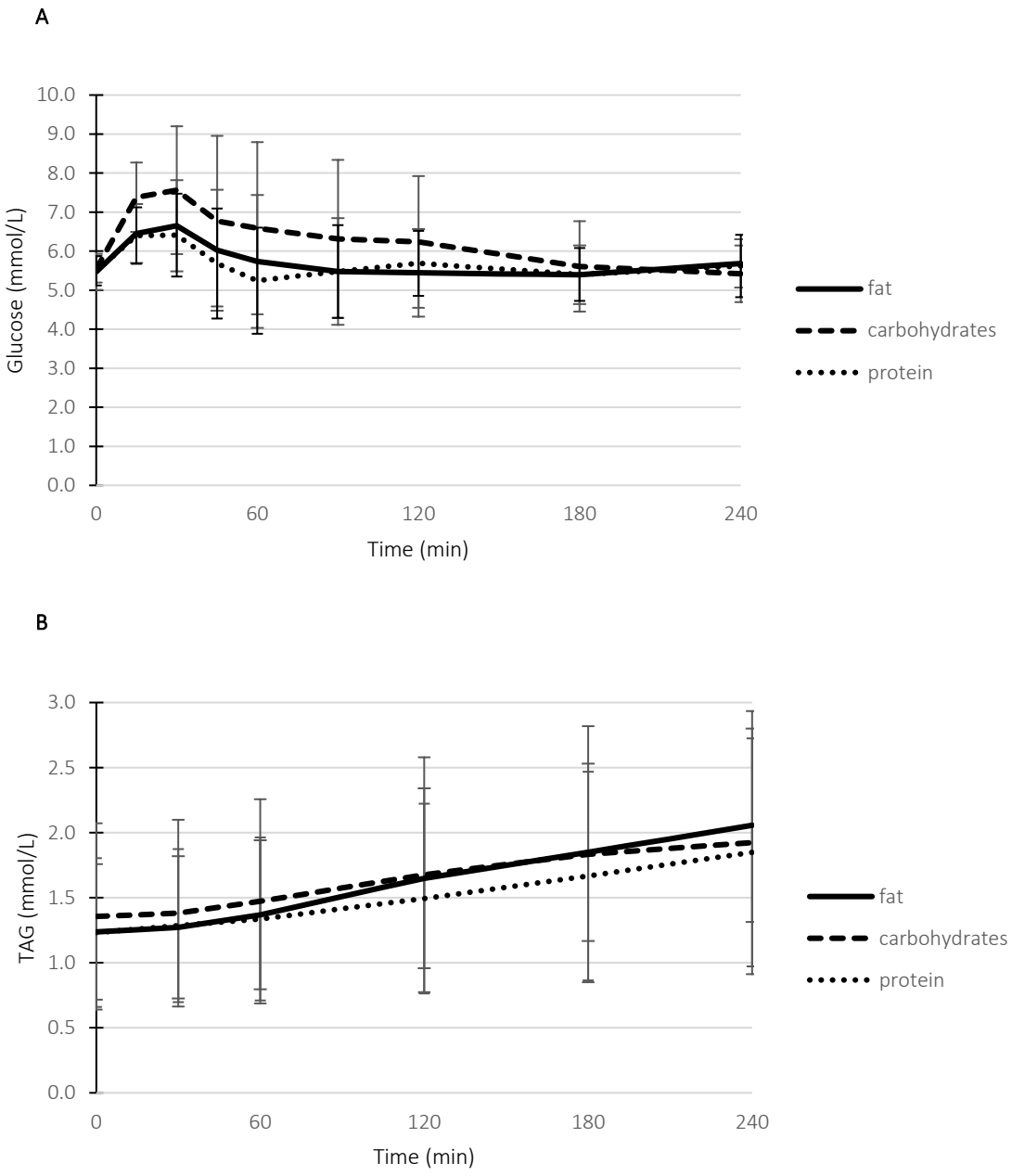
Medium	Meal	BDNF (pg/ml)		
		T0	T60	T240
Heparin	Fat	828 ± 522	1093 ± 872	1307 ± 990
	Carbohydrates	862 ± 740	942 ± 413	1344 ± 813
	Protein	1139 ± 1032	1260 ± 1140	1274 ± 888

Values are presented as mean ± SD

Supplemental Table 4.3 | Postprandial responses (iAUCs) and maximal increases of BDNF concentrations (pg/ml) after consumption of the high-fat, high-carbohydrate or high-protein meal in heparin plasma

Meal	Total AUC (concentration/240 min)	iAUC (concentration/240 min)	Max increase
Fat	243555 (157088 – 368423)	37087 (591 – 140250)	271 (24 – 929)
Carbohydrates	247065 (176288 – 313260)	71880 (26480 – 153623)	533 (176 – 1060)
Protein	236445 (163193 – 324045)	42360 (1417 – 71562)	226 (26 – 464)

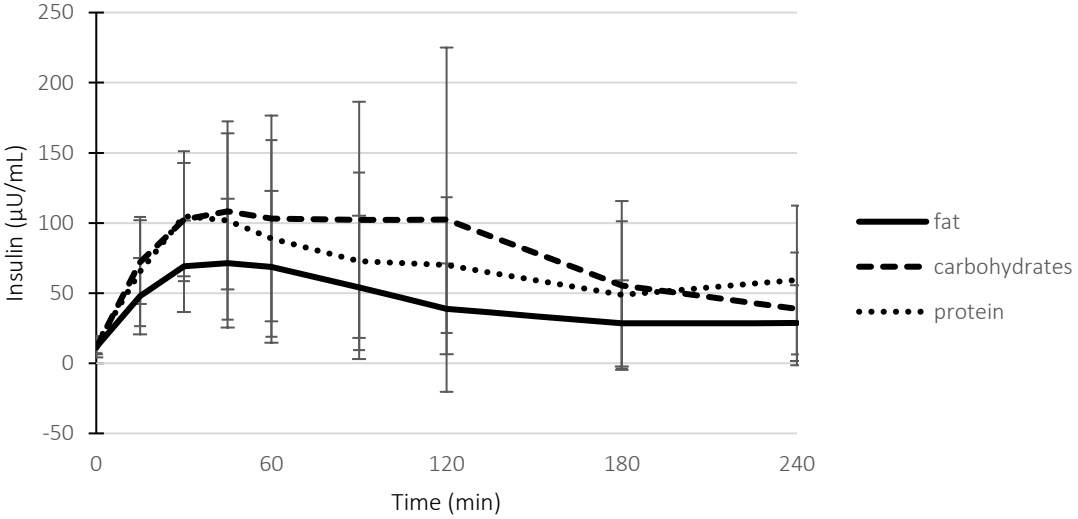
Values are presented as median with ranges (25-75th percentiles)



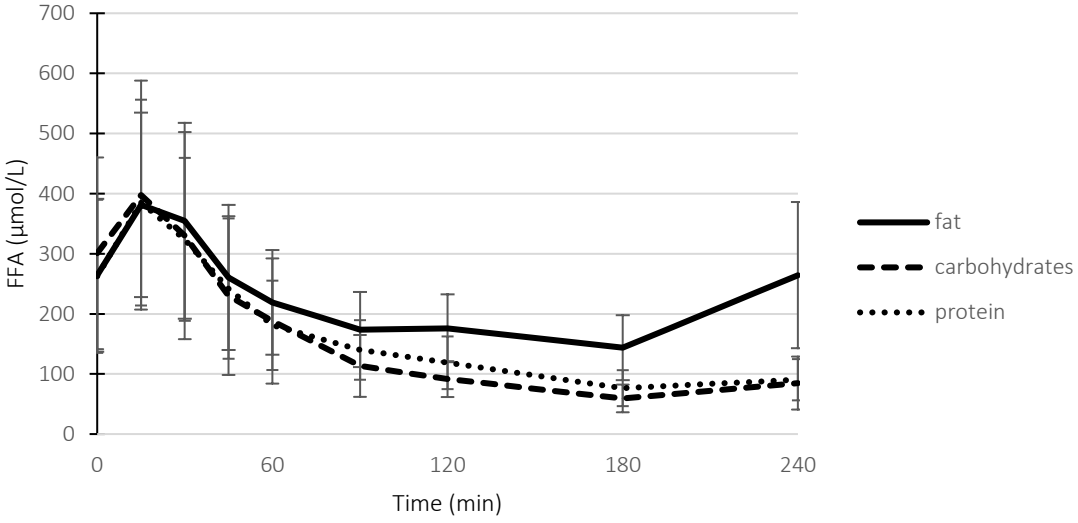
Supplemental Figure 4.2 | Glucose (A), TAG (B), insulin (C), and FFA (D) concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meal

TAG: triacylglycerol; FFA: free fatty acids

C



D



Supplemental Figure 4.2 (continued) | Glucose (A), TAG (B), insulin (C), and FFA (D) concentrations before and after consumption of the high-fat, high-carbohydrate or high-protein meal

TAG: triacylglycerol; FFA: free fatty acids

REFERENCES

1. McAllister AK, Katz LC, Lo DC. Neurotrophins and Synaptic Plasticity. Annu Rev Neurosci. 1999;22:295-318.
2. Marosi K, Mattson MP. BDNF Mediates Adaptive Brain and Body Responses to Energetic Challenges. Trends Endocrinol Metab. 2014;25(2):89-98.
3. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. Front Cell Neurosci. 2019;13:363.
4. Gravesteijn E, Mensink RP, Plat J. Effects of Nutritional Interventions on BDNF Concentrations in Humans: A Systematic Review. Nutr Neurosci. 2021:1-12.
5. Lee IT, Chen CH, Wang JS, Fu CP, Lee WJ, Liang KW, *et al*. The Association between Brain-Derived Neurotrophic Factor and Central Pulse Pressure after an Oral Glucose Tolerance Test. Clin Chim Acta. 2018;476:1-8.
6. Lee IT, Li YH, Sheu WH. Brain-Derived Neurotrophic Factor during Oral Glucose Tolerance Test Predicts Cardiovascular Outcomes. Int J Mol Sci. 2020;21(14).
7. Fujinami A, Ohta K, Obayashi H, Fukui M, Hasegawa G, Nakamura N, *et al*. Serum Brain-Derived Neurotrophic Factor in Patients with Type 2 Diabetes Mellitus: Relationship to Glucose Metabolism and Biomarkers of Insulin Resistance. Clin Biochem. 2008;41(10-11):812-7.
8. Li B, Lang N, Cheng ZF. Serum Levels of Brain-Derived Neurotrophic Factor Are Associated with Diabetes Risk, Complications, and Obesity: A Cohort Study from Chinese Patients with Type 2 Diabetes. Mol Neurobiol. 2016;53(8):5492-9.
9. Nuttall FQ, Gannon MC. Plasma Glucose and Insulin Response to Macronutrients in Nondiabetic and NIDDM Subjects. Diabetes Care. 1991;14(9):824-38.
10. Zeeni N, Haidar EA, Azar A, Ghanem A, Bassil K, Bassil M, *et al*. The Combinatory Effects of Diet and Exercise on BDNF Gene Expression. FASEB J. 2018;31(S1):150.8.
11. Shafie A, Rahimi AM, Ahmadi I, Nabavizadeh F, Ranjbaran M, Ashabi G. High-Protein and Low-Calorie Diets Improved the Anti-Aging Klotho Protein in the Rats' Brain: The Toxic Role of High-Fat Diet. Nutr Metab (Lond). 2020;17:86.
12. Karczewska-Kupczewska M, Kowalska I, Nikolajuk A, Adamska A, Zielinska M, Kaminska N, *et al*. Circulating Brain-Derived Neurotrophic Factor Concentration is Downregulated by Intralipid Heparin Infusion or High-Fat Meal in Young Healthy Male Subjects. Diabetes Care. 2012;35(2):358-62.
13. Eyileten C, Kaplon-Cieslicka A, Mirowska-Guzel D, Malek L, Postula M. Antidiabetic Effect of Brain-Derived Neurotrophic Factor and Its Association with Inflammation in Type 2 Diabetes Mellitus. J Diabetes Res. 2017;2017:2823671.
14. Rozanska O, Uruska A, Zozulinska-Ziolkiewicz D. Brain-Derived Neurotrophic Factor and Diabetes. Int J Mol Sci. 2020;21(3).
15. Yamanaka M, Itakura Y, Inoue T, Tsuchida A, Nakagawa T, Noguchi H, *et al*. Protective Effect of Brain-Derived Neurotrophic Factor on Pancreatic Islets in Obese Diabetic Mice. Metabolism. 2006;55(10):1286-92.
16. Kuroda A, Yamasaki Y, Matsuhisa M, Kubota M, Nakahara I, Nakatani Y, *et al*. Brain-Derived Neurotrophic Factor Ameliorates Hepatic Insulin Resistance in Zucker Fatty Rats. Metabolism. 2003;52(2):203-8.
17. de Luis DA, Fernandez Ovalle H, Izaola O, Primo D, Aller R. RS 10767664 Gene Variant in Brain Derived Neurotrophic Factor (BDNF) Affect Metabolic Changes and Insulin Resistance after a Standard Hypocaloric Diet. J Diabetes Complications. 2018;32(2):216-20.

18. Krabbe KS, Nielsen AR, Krogh-Madsen R, Plomgaard P, Rasmussen P, Erikstrup C, *et al*. Brain-Derived Neurotrophic Factor (BDNF) and Type 2 Diabetes. Diabetologia. 2007;50(2):431-8.
19. Foulstone EJ, Tavaré JM, Gunn-Moore FJ. Sustained Phosphorylation and Activation of Protein Kinase B Correlates with Brain-Derived Neurotrophic Factor and Insulin Stimulated Survival of Cerebellar Granule Cells. Neuroscience Letters. 1999;264:125-8.
20. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, *et al*. Effects of Free Fatty Acids on Glucose Transport and IRS-1–Associated Phosphatidylinositol 3-Kinase Activity. J Clin Invest. 1999;103(2):253-9.
21. Liu W, Han X, Zhou X, Zhang S, Cai X, Zhang L, *et al*. Brain Derived Neurotropic Factor in Newly Diagnosed Diabetes and Prediabetes. Mol Cell Endocrinol. 2016;429:106-13.
22. Suwa M, Kishimoto H, Nofuji Y, Nakano H, Sasaki H, Radak Z, *et al*. Serum Brain-Derived Neurotrophic Factor Level is Increased and Associated with Obesity in Newly Diagnosed Female Patients with Type 2 Diabetes Mellitus. Metabolism. 2006;55(7):852-7.
23. Boyuk B, Degirmencioglu S, Atalay H, Guzel S, Acar A, Celebi A, *et al*. Relationship between Levels of Brain-Derived Neurotrophic Factor and Metabolic Parameters in Patients with Type 2 Diabetes Mellitus. J Diabetes Res. 2014;2014:978143.
24. Civelek S, Konukoglu D, Erdenen F, Uzun H. Serum Neurotrophic Factor Levels in Patients with Type 2 Diabetes Mellitus: Relationship to Metabolic Syndrome Components. Clin Lab. 2013;59(3-4):369-74.
25. Rosenfeld RD, Zeni L, Haniu M, Talvenheimo J, Radka SF, Bennett L, *et al*. Purification and Identification of Brain-Derived Neurotrophic Factor from Human Serum. Protein Expression and Purification. 1995;6(4):465-71.
26. Sinzinger H, Berent R. Platelet Function in the Postprandial Period. Thromb J. 2012;10(1):19.
27. Ahuja KD, Adams MJ, Robertson IK, Ball MJ. Acute Effect of a High Carbohydrate Low Fat Meal on Platelet Aggregation. Platelets. 2009;20(8):606-9.
28. Silveira A. Postprandial Triglycerides and Blood Coagulation. Exp Clin Endocrinol Diabetes. 2001;109(4):S527-32.
29. Onbaşi K, Efe B, Çeler Ö. Postprandial Phase Fluctuations can Trigger the Coagulation Cascade. Int J Clin Exp Med. 2016;9(3):5891-901.
30. Spectre G, Ostenson CG, Li N, Hjemdahl P. Postprandial Platelet Activation Is Related to Postprandial Plasma Insulin Rather Than Glucose in Patients With Type 2 Diabetes. Diabetes. 2012;61(9):2380-4

Chapter 5

The associations between whole-body insulin sensitivity, peripheral and brain vascular function, and cognitive performance in prediabetic men and women

Elske Gravesteijn, Ronald P. Mensink, Peter J. Joris and Jogchum Plat

In preparation

Chapter 6

The effects of long-term almond consumption on whole-body insulin sensitivity, postprandial glucose responses, and free-living glucose patterns in men and women with prediabetes: A randomized controlled trial

Elske Gravesteijn, Ronald P. Mensink and Jogchum Plat

In preparation

Chapter 7

General discussion

Appendices

IMPACT

The main objective of this thesis was to examine the effects of diet on metabolic health, vascular function, and cognition in subjects at risk for type 2 diabetes and cardiovascular disease development. We systematically reviewed that dietary interventions can elevate circulating BDNF concentrations, specifically whole foods and polyphenols. We concluded that these effects could be mediated by insulin. However, in our controlled intervention study we showed that macronutrients did not differently affect postprandial BDNF concentrations, regardless of acute changes in glucose and insulin concentrations. The egg protein hydrolysate NWT-03 also did not change serum BDNF concentrations. However, it did have the potential to improve cognitive function within the executive function domain. The impact of NWT-03 supplementation might be of great interest as it appeared to improve not only peripheral cardiovascular risk factors [1-3], but also central risk factors.

In contrast to the well-known lipid lowering effects of almonds, we found that long-term almond consumption did not improve glucose metabolism. This unexpected adverse effect can partly be due to the observed increase in body weight during the almond intervention period, which affects insulin sensitivity. Regarding this risk of weight gain, it should be taking into consideration to extend the dietary guidelines with instructions on how to incorporate nuts in the healthy diet. Overall, lifestyle modifications are key to attenuate or prevent disease development, in particular, disturbances in peripheral vascular function which can already be observed in prediabetes. The potential impact of the research in this thesis will be discussed here from a societal and economic perspective.

Societal relevance

The metabolic syndrome, covering abdominal obesity, insulin resistance, hypertension, and hyperlipidemia, has become a global epidemic [4]. Besides these well-known characteristics, the metabolic syndrome is also associated with cognitive decline and dementia [5] The metabolic syndrome often resembles the prevalence of obesity, and increases the risk of cardiovascular disease and type 2 diabetes. Cardiovascular disease is the number one cause of morbidity and mortality worldwide, with 17.9 million deaths in 2019 [6], whereas type 2 diabetes has prevalence estimates of 463 million in the same year [7]. As with both metabolic syndrome and prediabetes there are no clear signs or symptoms [8, 9], the prevalence is probably even higher than type 2 diabetes. Furthermore, estimates vary based on the criteria used [4]. For clinical diagnosis and

treatment, it is relevant to define pathology thresholds, for example, for prediabetes in terms of glucose concentrations [10]. In contrast to genetic predisposition for disease development, there are modifiable lifestyle risk factors such as obesity, physical inactivity, and an unhealthy diet [11]. Therefore, it is of great importance to examine the health effects of diet to decrease the negative social impact of these diseases.

Based on our systematic review, we posed that dietary interventions can elevate circulating BDNF concentrations. BDNF is a protein involved in neuronal survival and growth [12], but also acts as a metabolic modulator [13]. This makes dietary interventions a potential strategy to improve metabolic and cognitive health. Unfortunately, we did not find changes in BDNF concentrations upon consuming diets enriched in either one of the different macronutrients. However, dietary macronutrient composition is still important to reduce the risk of cardiovascular disease [14]. NWT-03 supplementation also contributes to an improved cardiovascular and metabolic health [3], and in addition to that, we here provided evidence for its contribution to improve cognitive health. The dietary guidelines advise to consume nuts as part of a healthy diet to support cardiovascular risk reduction. Almonds are indeed associated with ameliorations in various factors related to lipid metabolism [15]. However, we found no beneficial effects of almonds on glucose metabolism. This lack of effect could only partly be attributed to the observed weight gain. When body weight is maintained, not just dietary factors, but a balanced diet is still key to health effects. Overall, dietary interventions have strong implications for public health. These implications might be even bigger as dietary effects might be passed on to the next generation via epigenetic changes [16].

Economic relevance

The economic burden of the metabolic syndrome is in trillions, including health care costs and loss of potential economic activity [4]. The major components of costs are disease management and particularly the consequent cardiovascular events [17]. Costs increase when more conditions of the metabolic syndrome are met. In 2020, 210 billion euros were spent in Europe on cardiovascular disease [18], and in 2021, 173 billion euros on type 2 diabetes [19]. Since the prevalence of the metabolic syndrome increases with age [20], this economic burden will rise in the future because of the continuously ageing population. Therefore, it is of importance to implement treatment and management strategies, but also preventive strategies to lower the number of individuals with the metabolic syndrome. Cost-effective lifestyle interventions can be applied to attenuate or prevent disease development. Here we showed that dietary factors such as NWT-03

and dietary interventions with whole foods or polyphenols targeting BDNF improved metabolic risk markers and markers of cognitive health, thereby lowering the health and economic burden of the metabolic syndrome and its consequences.

Target population

All human intervention studies presented in this thesis were performed in (older) adults. While the first study with NWT-03 included men and women with the metabolic syndrome, the study with dietary macronutrients included healthy overweight and obese men, and the study with almonds both overweight and obese men and women with prediabetes, but not yet diabetes. These study populations all have an increased risk for developing cardiovascular disease and type 2 diabetes, but hypothetically can benefit most of the dietary interventions aimed to reverse disease development. The study with dietary macronutrients focused only on men in order to eliminate possible sex differences. Targeting men was applied to the initial design of the study to examine postprandial vascular function [21], but this selection was also favorable in the study presented here as sex hormones or steroids seemingly modulate BDNF regulation [22]. Moreover, discrepancies exist in BDNF concentrations between different populations, *i.e.*, BDNF concentrations are influenced by the diabetic state [23]. Future research on BDNF should take into account these differences between target populations. Another focus should be on healthy individuals to examine BDNF reference concentrations and whether they can benefit of such dietary interventions as presented in this thesis by boosting BDNF.

Translation into practice

The research findings presented in this thesis have been published or are in process of being published in peer-reviewed scientific journals, which makes the obtained knowledge openly accessible for scientists, health professionals, and the general public. One of the goals is to stimulate more research on the effects of dietary interventions on metabolic health, vascular function, and cognition. Furthermore, the obtained information might be relevant to authorities, policy makers, and health agencies to be implemented in the dietary guidelines supporting a healthy diet. For example, nut consumption is already part of the generally advised dietary guidelines. However, we found no beneficial effects of almonds when they are added to the diet. It could be that more supporting guidelines are needed on how to incorporate them into the diet to result in its proclaimed health benefits. Feasibility of diet changes is important for its

success. Findings have also been presented at scientific conferences to share knowledge and increase awareness of the important role of a healthy diet in the treatment, management, and prevention of cardiovascular disease and type 2 diabetes.

REFERENCES

1. Wang Y, Landheer S, van Gilst WH, van Amerongen A, Hammes HP, Henning RH, et al. Attenuation of Renovascular Damage in Zucker Diabetic Fatty Rat by NWT-03, An Egg Protein Hydrolysate with ACE- and DPP4-Inhibitory Activity. PLoS One. 2012;7(10):e46781.
2. Plat J, Severins N, Morrison S, Mensink RP. Effects of NWT-03, an Egg-Protein Hydrolysate, on Blood Pressure in Normotensive, High-Normotensive and Mild-Hypertensive Men and Women: A Dose-Finding Study. Br J Nutr. 2017;117(7):942-50.
3. Plat J, Severins N, Mensink RP. Improvement of Pulse Wave Velocity and Metabolic Cardiovascular Risk Parameters Through Egg Protein Hydrolysate Intake: A Randomized Trial in Overweight or Obese Subjects with Impaired Glucose Tolerance or Type 2 Diabetes. Journal of Functional Foods. 2019;52:418-23.
4. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018;20(2):12.
5. Birdsill AC, Carlsson CM, Willette AA, Okonkwo OC, Johnson SC, Xu G, et al. Low Cerebral Blood Flow is Associated with Lower Memory Function in Metabolic Syndrome. Obesity (Silver Spring). 2013;21(7):1313-20.
6. World Health Organization. Cardiovascular diseases (CVDs) fact sheet. June 2021. [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). [Accessed on 04/04/2022].
7. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and Regional Diabetes Prevalence Estimates for 2019 and Projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th Edition. Diabetes Res Clin Pract. 2019;157.
8. Stern MP, Williams K, Gonzalez-Villalpando C, Hunt KJ, Haffner SM. Does the Metabolic Syndrome Improve Identification of Individuals at Risk of Type 2 Diabetes andor Cardiovascular Disease. Diabetes Care. 2004;27:2676-81.
9. Alvarez S, Coffey R, Algotar AM. Prediabetes. StatPearls Publishing; 2021.
10. Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of Prediabetes. Med Clin North Am. 2011;95(2):327-39, vii-viii.
11. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112(17):2735-52.
12. Kowianski P, Lietzau G, Czuba E, Waskow M, Steliga A, Morys J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. Cell Mol Neurobiol. 2018;38(3):579-93.
13. Marosi K, Mattson MP. BDNF Mediates Adaptive Brain and Body Responses to Energetic Challenges. Trends Endocrinol Metab. 2014;25(2):89-98.
14. Brandhorst S, Longo VD. Dietary Restrictions and Nutrition in the Prevention and Treatment of Cardiovascular Disease. Circ Res. 2019;124(6):952-65.
15. Musa-Veloso K, Paulionis L, Poon T, Lee HY. The Effects of Almond Consumption on Fasting Blood Lipid Levels: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. J Nutr Sci. 2016;5:e34.
16. Gómez-Pinilla F. Brain Foods: The Effects of Nutrients on Brain Function. Nat Rev Neurosci. 2008;9(7):568-78.
17. Scholze J, Alegria E, Ferri C, Langham S, Stevens W, Jeffries D, et al. Epidemiological and Economic Burden of Metabolic Syndrome and Its Consequences in Patients with Hypertension in Germany, Spain and Italy; A Prevelance-Based Model. BMC Public Health. 2010;10(529).

18. European Society of Cardiology. Fighting cardiovascular disease – a blueprint for EU action. June 2020. [Internet]. Available from: https://www.mepheartgroup.eu/wp-content/uploads/05748-CVD-plan_FINAL.pdf. [Accessed on 06/04/2022].
19. International Diabetes Federation. IDF Diabetes Atlas, 10th edition. Diabetes around the world in 2021. [Internet]. Available from: https://diabetesatlas.org/. [Accessed on 06/04/2022].
20. Hildrum B, Mykletun A, Hole T, Midthjell K, Dahl AA. Age-Specific Prevalence of the Metabolic Syndrome Defined by the International Diabetes Federation and the National Cholesterol Education Program: The Norwegian HUNT 2 Study. BMC Public Health. 2007;7:220.
21. Smeets E, Mensink RP, Joris PJ. Dietary Macronutrients Do Not Differently Affect Postprandial Vascular Endothelial Function in Apparently Healthy Overweight and Slightly Obese Men. Eur J Nutr. 2021;60(3):1443-51.
22. Chan CB, Ye K. Sex Differences in Brain-Derived Neurotrophic Factor Signaling and Functions. J Neurosci Res. 2017;95(1-2):328-35.
23. Rozanska O, Uruska A, Zozulinska-Ziolkiewicz D. Brain-Derived Neurotrophic Factor and Diabetes. Int J Mol Sci. 2020;21(3).

SUMMARY

The number of individuals suffering from type 2 diabetes is rapidly rising with more than 537 million cases worldwide in 2021. Prediabetes is an intermediate condition of insulin resistance resulting in temporary glucose excursions, but not yet as pronounced as in type 2 diabetes. Together with obesity, dyslipidemia, and hypertension, this condition is part of the metabolic syndrome. Individuals with the metabolic syndrome have a predisposition for development into type 2 diabetes, but also have an increased risk for cardiovascular diseases, and cognitive decline. This great health impact can be influenced by lifestyle. A healthy diet is a cornerstone in maintaining metabolic, vascular, and cognitive health, which is shown to be effective in delaying or even preventing the onset of non-communicable diseases such as type 2 diabetes, cardiovascular disease, and cognitive disorders. The research in this thesis aimed to investigate the effects of different dietary components or foods on metabolic health, vascular function, and cognition in humans.

Brain-derived neurotrophic factor (BDNF) is an important protein for neuronal plasticity as well as energy metabolism. Therefore, it seems to be an attractive target to improve both cognitive health and metabolic health. Various controlled human intervention studies have been conducted aiming at elevating BDNF concentrations. In Chapter 2, a systematic review provided an overview of the effects of these dietary interventions on BDNF concentrations in human adults. In total, 48 dietary interventions were selected after a systematic literature search and clustered based on dietary patterns or foods, diets based on energy intake, and supplements. The supplements cluster was further divided into vitamins and minerals, polyphenols, long-chain omega-3 polyunsaturated fatty acids, probiotics, and miscellaneous food supplements. In particular, interventions with whole foods and interventions with polyphenols showed the possibility to significantly increase peripheral BDNF concentrations, whereas for the other interventions no clear effect could be found. Regarding polyphenols, phenolic acids, and other phenolic compounds were responsible for the positive effect on BDNF. We concluded that dietary interventions have potential to elevate circulating BDNF concentrations.

Upon these review results, we speculated that the effects of dietary factors on fasting BDNF concentrations could be mediated by changes in insulin and/or glucose concentrations. In Chapter 4 the postprandial effects of the three dietary macronutrients fat, carbohydrates, or protein on BDNF concentrations were investigated side by side. This enabled us to differentiate between the role of insulin and

glucose in BDNF changes, since each macronutrient triggers a different insulin and glucose response. In total, 18 healthy, but overweight/obese men participated in this randomized, double-blind, controlled cross-over trial. Three postprandial tests were performed with either a high-fat, high-carbohydrate, or high-protein meal with a wash-out of one week in between. As expected, postprandial insulin and glucose concentrations changed upon consumption of the dietary macronutrients and were significantly different between meals. However, postprandial BDNF concentrations were not significantly different between meals. Chapter 2 already highlighted differences in BDNF concentrations when measured in serum compared to plasma due to the predominant storage of BDNF in platelets. Again, BDNF concentrations were higher in serum than in plasma. We concluded that macronutrients do not differently influence postprandial serum and plasma BDNF concentrations, indicating that there is no effect of acute changes in insulin and glucose on BDNF. More research is needed to investigate the role of insulin and glucose on BDNF in a long-term situation.

Previous research showed beneficial effects of the egg protein hydrolysate NWT-03 on peripheral cardiovascular risk factors. As the small peptide fragments might be able to transfer to the brain, we speculated that NWT-03 consumption might also have beneficial effects on central risk factors. In Chapter 3 we investigated the effects of a daily intake of 5 g NWT-03 for 4 weeks on parameters reflecting cognitive function, as well as serum BDNF concentrations. In total, 79 men and women with the metabolic syndrome participated in this randomized, double-blind, placebo-controlled study. Cognitive function was assessed by the anti-cue reaction time test measuring impulse control, and the psychomotor vigilance test measuring sustained attention three times each period with a wash-out of 2-8 weeks in between. We evaluated the change in cognitive performance between the start and end of each experimental period. NWT-03 consumption significantly decreased response times of the anti-cue reaction time test compared to placebo, but not of the psychomotor vigilance test. No effects were found on serum BDNF concentrations. We concluded that, regardless of stable BDNF concentrations, NWT-03 has potential to improve cognitive function within the executive domain.

In Chapter 5 our target population shifted from the metabolic syndrome to prediabetes. In total, data of 34 men and women with prediabetes was used in a cross-sectional correlation analysis. We investigated whether the insulin resistant condition in prediabetes was already linked to disturbed cognitive performance as seen with type 2 diabetes, and whether this was linked to disturbances in peripheral and brain vascular function. In more detail, we evaluated the associations between insulin sensitivity and

peripheral vascular function, brain vascular function, and cognitive performance. Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp and expressed as the M-value, or as HOMA-IR. Peripheral vascular function was evaluated with PWA or PWV as a measure of arterial stiffness, whereas brain vascular function was evaluated with gray matter CBF. Finally, cognitive performance was evaluated using a set of neuropsychological tests by CANTAB. Insulin sensitivity did not show a clear correlation with cognitive performance or brain vascular function, but significantly correlated with peripheral vascular function. In turn, brain vascular function significantly correlated with cognitive performance. The correlation between peripheral and brain vascular function remains speculative. We concluded that disturbances in peripheral vascular can already be observed with prediabetes. However, potential disturbances in the periphery and the brain are not clearly linked. Whether this is due to the fact that it is too early in the development of diabetes or that these processes are epiphenomena occurring side by side needs further investigation.

The cross-sectional data in Chapter 5 was obtained as part of a dietary intervention study evaluating the effects of almonds on glucose metabolism, which is described in Chapter 6. Nuts are part of the dietary guidelines for cardiovascular prevention because of their lipid lowering effects. In addition, improvement of insulin sensitivity and glucose metabolism could prevent the development of prediabetes into type 2 diabetes. However, findings on such health effects of almonds are inconclusive, which could be related to fluctuations in body weight. Therefore, we investigated the effects of long-term almond consumption on glucose metabolism under free living conditions, *i.e.*, without detailed dietary instructions on how to incorporate the almonds into the habitual diet. Glucose metabolism was assessed by a hyperinsulinemic euglycemic clamp to measure insulin sensitivity, a mixed meal test to measure postprandial glucose concentrations, and a continuous glucose monitor to measure free-living glucose profiles. In total, 34 prediabetic men and women consumed 50 g almonds daily for 5 months in this randomized controlled trial with a wash-out of 2 months. No detailed instructions were provided related to consumption of the almonds, mimicking real life conditions. At the end of each experimental period, the different methodologies to evaluate effects on glucose metabolism were assessed side by side. Almond consumption significantly decreased insulin sensitivity, and increased postprandial glucose concentrations, and fasting insulin concentrations, as compared to the control period. Continuous glucose profiles just did not reach statistical significance. In the almond period, BMI and waist circumference also increased significantly, as well as the energy intake as derived from the food frequency questionnaires. This suggests that without additional dietary guidelines, almonds were consumed on top of the habitual

diet, not fully replacing other energy-dense food items. We concluded that long-term almond consumption under free living conditions has adverse effects on glucose metabolism.

Based on a systematic review, a cross-sectional study, and three human intervention trials in this thesis, we conclude that dietary interventions are able to influence fasting BDNF concentrations in favor of metabolic and cognitive health, but not postprandial BDNF concentrations. NWT-03 consumption is an additional measure to improve cognitive health. Regarding prediabetes, correlations indicated that concomitant disturbances in peripheral vascular function can already be observed, but insulin sensitivity and glucose metabolism cannot be improved by adding almonds to the diet.

SAMENVATTING

Het aantal mensen met diabetes type 2 neemt snel toe met meer dan 537 miljoen gevallen wereldwijd in 2021. Prediabetes is een intermediaire conditie van insulineresistentie die leidt tot tijdelijke glucose-excursies, maar nog niet zo uitgesproken als bij type 2 diabetes. Samen met obesitas, dyslipidemie en hypertensie maakt deze aandoening deel uit van het metabool syndroom. Personen met het metabool syndroom hebben aanleg voor de ontwikkeling tot diabetes type 2, maar hebben ook een verhoogd risico op hart- en vaatziekten, en cognitieve achteruitgang. Deze grote impact op de gezondheid kan worden beïnvloed door de levensstijl. Een gezond dieet is essentieel in het behouden van metabole, vasculaire en cognitieve gezondheid, waarvan is aangetoond dat het effectief is in het uitstellen of zelfs voorkomen van het ontstaan van niet-overdraagbare aandoeningen zoals diabetes type 2, hart- en vaatziekten, en cognitieve stoornissen. Het onderzoek in dit proefschrift was gericht op de effecten van verschillende voedingscomponenten of voedingsmiddelen op de metabole gezondheid, vaatfunctie, en cognitie bij mensen.

Brain-derived neurotrophic factor (BDNF) is een belangrijk eiwit voor zowel neuronale plasticiteit als energiemetabolisme. Daarom lijkt het een aantrekkelijk doelwit te zijn om zowel de cognitieve als de metabole gezondheid te verbeteren. Verschillende gecontroleerde humane interventiestudies zijn uitgevoerd gericht op het verhogen van BDNF concentraties. In Hoofdstuk 2 is een systematisch overzicht gegeven van de effecten van deze voedingsinterventies op BDNF concentraties bij volwassenen. In totaal werden 48 voedingsinterventies geselecteerd na een systematische literatuuronderzoek en geclusterd op basis van voedingspatronen of voedingsmiddelen, diëten op basis van energie-inname, en supplementen. De supplementen cluster werd verder onderverdeeld in vitaminen en mineralen, polyfenolen, lange-keten omega-3 meervoudig onverzadigde vetzuren, probiotica, en diverse voedingssupplementen. Met name interventies met voedingsmiddelen en interventies met polyfenolen toonden de mogelijkheid om perifere BDNF concentraties significant te verhogen, terwijl voor de andere interventies geen duidelijk effect kon worden gevonden. Wat polyfenolen betreft, waren fenolzuren en andere fenolverbindingen verantwoordelijk voor het positieve effect op BDNF. We concludeerden dat voedingsinterventies potentie hebben om circulerende BDNF concentraties te verhogen.

Op basis van deze resultaten speculeerden we dat de effecten van voeding op nuchtere BDNF concentraties gemedieerd zouden kunnen worden door veranderingen in insuline- en/of glucoseconcentraties. In Hoofdstuk 4 werden de postprandiale effecten van de

drie macronutriënten vet, koolhydraten en eiwit op BDNF concentraties naast elkaar onderzocht. Dit stelde ons in staat onderscheid te maken tussen de rol van insuline en glucose in BDNF veranderingen, aangezien elk macronutriënt een andere insuline- en glucoserespons teweegbrengt. In totaal namen 18 gezonde mannen met overgewicht of obesitas deel aan deze gerandomiseerde, dubbelblinde, gecontroleerde cross-over studie. Er werden drie postprandiale tests uitgevoerd met ofwel een vetrijke, koolhydraatrijke of eiwitrijke maaltijd met een wash-out van een week ertussen. Zoals verwacht veranderden de postprandiale insuline- en glucoseconcentraties na consumptie van de macronutriënten en waren er significante verschillen tussen de maaltijden. De postprandiale BDNF concentraties waren echter niet significant verschillend tussen de maaltijden. In Hoofdstuk 2 werd al gewezen op verschillen in BDNF concentraties wanneer gemeten in serum vergeleken met plasma, omdat BDNF overwegend in bloedplaatjes wordt opgeslagen. Ook hier waren de BDNF concentraties hoger in serum dan in plasma. We concludeerden dat macronutriënten geen verschillende invloed hebben op postprandiale serum en plasma BDNF concentraties, wat erop wijst dat er geen effect is van acute veranderingen in insuline en glucose op BDNF. Meer onderzoek is nodig om de rol van insuline en glucose op BDNF op de lange termijn te onderzoeken.

Eerder onderzoek toonde gunstige effecten aan van het ei eiwithydrolysaat NWT-03 op perifere cardiovasculaire risicofactoren. Aangezien de kleine peptidefragmenten mogelijk de hersenen kunnen bereiken, speculeerden we dat consumptie van NWT-03 ook gunstige effecten zou kunnen hebben op centrale risicofactoren. In Hoofdstuk 3 onderzochten we de effecten van een dagelijkse inname van 5 g NWT-03 gedurende 4 weken op parameters die de cognitieve functie weerspiegelen, evenals op serum BDNF concentraties. In totaal namen 79 mannen en vrouwen met het metaboolsyndroom deel aan deze gerandomiseerde, dubbelblinde, placebogecontroleerde studie. De cognitieve functie werd beoordeeld met de anti-cue reactietijd test die impulscontrole meet, en de psychomotorische vigilantie test die aanhoudende aandacht meet, drie keer per periode met een wash-out van 2-8 weken ertussen. We evalueerden de verandering in cognitieve prestaties tussen het begin en het einde van elke experimentele periode. Consumptie van NWT-03 verminderde de reactietijden van de anticue-reactietijd test aanzienlijk in vergelijking met placebo, maar niet van de psychomotorische vigilantie test. Er werden geen effecten gevonden op serum BDNF concentraties. We concludeerden dat, ongeacht stabiele BDNF concentraties, NWT-03 potentie heeft om de cognitieve functie binnen het executieve domein te verbeteren.

In Hoofdstuk 5 verschoof onze doelgroep van het metabool syndroom naar prediabetes. In totaal werden gegevens van 34 mannen en vrouwen met prediabetes gebruikt in een cross-sectionele correlatieanalyse. We onderzochten of de insulineresistente conditie bij prediabetes al samenhangt met verstoorde cognitieve prestaties zoals die bij diabetes type 2 worden gezien, en of dit samenhangt met verstoringen in de perifere en centrale vaatfunctie. In meer detail evalueerden we de associaties tussen insulinegevoeligheid en perifere vaatfunctie, brein vaatfunctie, en cognitieve prestaties. De insulinegevoeligheid werd gemeten met de hyperinsulinemische euglycemische clamp en uitgedrukt als de M-waarde, of als HOMA-IR. De perifere vaatfunctie werd geëvalueerd met PWA of PWV als maat voor de arteriële stijfheid, terwijl de brein vaatfunctie werd geëvalueerd met grijze stof CBF. Tenslotte werden de cognitieve prestaties geëvalueerd met een reeks neuropsychologische tests van CANTAB. Insulinegevoeligheid vertoonde geen duidelijke correlatie met cognitieve prestaties of brein vaatfunctie, maar correleerde significant met perifere vaatfunctie. Op zijn beurt correleerde de brein vaatfunctie significant met cognitieve prestaties. De correlatie tussen de perifere en de brein vaatfunctie blijft speculatief. We concludeerden dat verstoringen in de perifere vaatfunctie al kunnen worden waargenomen bij prediabetes. Potentiële verstoringen in de periferie en de hersenen zijn echter niet duidelijk met elkaar in verband gebracht. Of dit te wijten is aan het feit dat het te vroeg is in de ontwikkeling van diabetes of dat deze processen epifenomenen zijn die naast elkaar voorkomen, moet verder onderzocht worden.

De cross-sectionele data in Hoofdstuk 5 zijn verkregen in het kader van een voedingsinterventiestudie naar de effecten van amandelen op het glucosemetabolisme, die in Hoofdstuk 6 wordt beschreven. Noten maken deel uit van de voedingsrichtlijnen voor cardiovasculaire preventie vanwege hun lipidenverlagende effecten. Bovendien zou een verbetering van de insulinegevoeligheid en het glucosemetabolisme de ontwikkeling van prediabetes tot diabetes type 2 kunnen voorkomen. De bevindingen over dergelijke gezondheidseffecten van amandelen zijn echter niet eenduidig, wat te maken zou kunnen hebben met schommelingen in het lichaamsgewicht. Daarom onderzochten we de effecten van langdurige amandelconsumptie op het glucosemetabolisme onder vrije leefomstandigheden; zonder gedetailleerde dieetinstructies over hoe de amandelen in het gebruikelijke dieet moesten worden opgenomen. Het glucosemetabolisme werd beoordeeld met een hyperinsulinemische euglycemische clamp om de insulinegevoeligheid te meten, een maaltijdtest om de postprandiale glucoseconcentraties te meten, en een continue glucosemonitor om de glucoseprofielen onder vrije leefomstandigheden te meten. In totaal consumeerden 34 mannen en vrouwen met prediabetes gedurende 5 maanden dagelijks 50 g amandelen in deze gerandomiseerde gecontroleerde studie met een wash-out van

2 maanden. Er werden geen gedetailleerde instructies gegeven met betrekking tot de consumptie van de amandelen, waarmee de omstandigheden in het echte leven werden nagebootst. Aan het einde van elke experimentele periode werden de verschillende methodologieën om de effecten op het glucosemetabolisme te evalueren naast elkaar beoordeeld. Amandelconsumptie verminderde de insulinegevoeligheid aanzienlijk, en verhoogde de postprandiale glucoseconcentraties en de nuchtere insulineconcentraties, vergeleken met de controleperiode. De continue glucoseprofielen bereikten net geen statistische significantie. In de amandelperiode namen ook de BMI en de tailleomtrek significant toe, evenals de energie-inname zoals afgeleid uit de voedingsvragenlijsten. Dit suggereert dat zonder aanvullende voedingsrichtlijnen amandelen werden geconsumeerd bovenop het gebruikelijke dieet, en niet volledig in de plaats kwamen van andere energierijke voedingsmiddelen. We concludeerden dat langdurige amandelconsumptie onder vrije leefomstandigheden nadelige effecten heeft op het glucosemetabolisme.

Op basis van een systematische review, een cross-sectionele studie en drie humane interventiestudies in dit proefschrift, concluderen we dat voedingsinterventies in staat zijn nuchtere BDNF concentraties te beïnvloeden ten gunste van metabole en cognitieve gezondheid, maar niet postprandiale BDNF concentraties. NWT-03 consumptie is een aanvullende manier om de cognitieve gezondheid te verbeteren. Wat prediabetes betreft, wezen correlaties erop dat gelijktijdige verstoringen in de perifere vaatfunctie reeds kunnen worden waargenomen, maar dat de insulinegevoeligheid en het glucosemetabolisme niet kunnen worden verbeterd door amandelen aan het dieet toe te voegen.

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ABOUT THE AUTHOR

Elske Gravesteijn was born on the 13th of August, 1992 in Nuenen, the Netherlands. After completion of secondary school at Pleincollege Eckart in Eindhoven in 2010, she moved to Amsterdam to study Psychobiology at the University of Amsterdam. She obtained her bachelor's degree in 2013 and continued studying Neurobiology as part of the master program in Biomedical Sciences. A master project was performed at Erasmus University Medical Center in Rotterdam at the interface of neuroimaging and childhood obesity. Later she chose a major in Science Communication at the Free University of Amsterdam to learn more about and support the dialogue between science and society. She engaged in a communication project at Hersenstichting in The Hague where she wrote an information brochure about the adolescent brain development. In 2015 she obtained her master's degree.

After working as an assistant research and development engineer at Quantib BV in Rotterdam, Elske started in 2017 as a PhD candidate at Maastricht University. Within the department of Nutrition and Movement Sciences under the supervision of Prof. dr. Jogchum Plat and Prof. dr. Ronald Mensink, she was responsible for a human intervention study with almonds in individuals with prediabetes. Her research focused on dietary effects on metabolic health, vascular function and cognition, of which the results are presented in this thesis.

LIST OF PUBLICATIONS

Gravesteijn, E., Mensink, R.P. & Plat, J. Effects of nutritional interventions on BDNF concentrations in humans: A systematic review. *Nutritional Neuroscience 2021*

Gravesteijn, E., Adam, J.J., Mensink, R.P., Winkens, B. & Plat, J. Effects of the egg protein hydrolysate NWT-03 on cognitive function in men and women with the metabolic syndrome: A randomized, double-blind, placebo-controlled study. *Submitted*

Gravesteijn, E., Mensink, R.P., Smeets, E.T.H.C. & Plat, J. Dietary macronutrients do not differently influence postprandial serum and plasma brain-derived neurotrophic factor concentrations: A randomized, double-blind, controlled cross-over trial. *Frontiers in Neuroscience 2021*

Gravesteijn, E., Mensink, R.P., Joris, P.J. & Plat, J. The associations between whole-body insulin sensitivity, peripheral and brain vascular function, and cognition in prediabetic men and women. *In preparation*

Gravesteijn, E., Mensink, R.P. & Plat, J. The effects of long-term almond consumption on whole-body insulin sensitivity, postprandial glucose responses, and free-living glucose patterns in men and women with prediabetes: A randomized controlled trial. In preparation. *In preparation*