Doctoral Thesis

Influence of exergame training and its combination with omega-3 fatty acids on the elderly brain

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Publication Date:
2017

Permanent Link:
https://doi.org/10.3929/ethz-b-000222607

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INFLUENCE OF EXERGAME TRAINING AND ITS COMBINATION WITH OMEGA-3 FATTY ACIDS ON THE ELDERLY BRAIN

A thesis submitted to attain the degree of

DOCTOR OF SCIENCE of ETH ZURICH
(Dr. sc. ETH Zurich)

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2017
“To get back my youth I would do anything in the world, except take exercise, get up early, or be respectable”

(Oscar Wilde, The Picture of Dorian Gray, 1891)
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Summary

Due to demographic changes, the number of older adults suffering from age-related bodily dysfunctions will increase in the near future. Limitations in motor and cognitive functions have been associated with increased risk of fallings, reduced life quality, and elevated mortality. To improve health and quality of life, especially counteracting the risk of falling, it is important that the elderly have a stable gait pattern when performing daily life activities. Motor and cognitive functions, in particular executive functions (EFs), are important for a stable gait pattern. This motor-cognitive interplay, also active during gait performance, is controlled by the brain. During aging, the brain undergoes age-related neuronal changes at the structural and functional level that affect motor and cognitive processes. The frontal part of the brain ((pre)frontal cortex) in particular starts to degenerate at an early stage. The (pre)frontal cortex controls the EFs that are important factors in gait performance and in many other daily life activities. Therefore, it is essential to counteract and stabilize such brain deterioration using appropriate methods.

Studies have shown that physical exercise (PE) has positive effects on the elderly brain. In particular, video game-based PE, or so-called exergame training, seems to be a promising way to address the neuronal system. Exergame training involves specific whole-body movements triggered by a video game. Furthermore, exergames allow the simultaneous training of motor and cognitive functions. In addition, previous research proposed that the beneficial effects of PE on the brain can be complemented by the concurrent consumption of selected dietary elements. The potential synergy between PE and nutritional supplements could involve common cellular pathways important for energy homeostasis, neurogenesis, synaptic plasticity, and vascular function. In particular, fish oil, which includes omega-3 fatty acids (FA), positively affects brain structure and function in the elderly. Accordingly, this doctoral thesis aims to better understand the effects of exergame training, alone and in combination with nutritional supplementation of omega-3 FA, on the brain structure and function, the cognitive capacities (EFs), and the motor abilities (gait parameters) of the elderly.

As a first step, a systematic review was performed drawing on studies with humans as well as with mammals. The review included studies that examined the effect of PE, nutritional supplementation, and their combination on brain structure, function, and/or metabolism. The review revealed that, at present, no human study with older adults has been able to show enhanced effects when PE was combined with nutritional supplementation. However, rat studies showed enhanced results for brain structure, function, and metabolism when PE was combined with nutritional supplementation, especially with omega-3 FA or selenium. Crucial to this might be the fact that PE and nutritional supplementation have been chosen as they share similar neurobiological cascades, achieving a possible interplay and thus greater beneficial effects.
A first randomized controlled study was performed to investigate the effects of exergame training in comparison to conventional balance training on prefrontal brain activity and its associated cognitive and motor functions in older adults. The results of the electroencephalography (EEG) measurement demonstrated that exergame training had a favorable effect on prefrontal brain activity, while no effects were found to arise from balance training. Furthermore, the results of the Test for Attention Performance (TAP) showed that within the exergame group working memory, divided attention, inhibition, and flexibility improved, whereas within the balance group only flexibility improved. With respect to gait performance, the exergame training enhanced gait parameters primarily under dual-task condition (cognitive task while walking), while the balance training enhanced gait parameters primarily under single-task condition (walking only). These results indicate that, compared to traditional training approaches, exergame training offers additional and highly promising training effects for the promotion of healthy brain aging. The desired effects of exergame training could be evoked by both the integrated motor-cognitive (dual-task) training aspect and the video games, which included training principles and specific cognitive stimuli.

Based on the findings of the systematic review and the first experimental study, a second randomized controlled trial was designed. This study explored whether exergame training in combination with omega-3 FA supplementation (fish oil) would affect brain structure and function as well as the associated cognitive and motor functions differently than exergame training alone in older adults. The results showed that the combination of exergame training and omega-3 FA supplementation did not lead to enhanced effects when compared with exergame training by itself. Nevertheless, further analysis showed positive time main effects (amongst all participants) for prefrontal brain activity using EEG, for divided attention using TAP, and for gait parameters under dual-task condition. These findings indicate that exergame training, which was performed in both groups, was the key factor in evoking the improvements. In addition, the omega-3 FA values, determined by blood samples, might indicate that the body required omega-3 FA during the exergame training period. Because of available omega-3 FA in both groups at the start of training, it cannot be completely ruled out that omega-3 FA acted as a supportive component for the training. However, it can only just be speculated that omega-3 FA were beneficial for the brain, since other bodily processes could also have profited from omega-3 FA. Nonetheless, the results indicate that exergame training functioned as the key factor, while omega-3 FA acted as a supportive component.

The observations of the studies demonstrate that exergame training seems to be a promising motor-cognitive training for older adults. Exergame training improved prefrontal brain activity, EFs, and gait parameters especially under dual-task conditions. For aging humans, an exercise program that effectively addresses these parameters is essential since age-related decline can impact gait, amplify the risk of falling, and reduce life quality. Future studies are needed to explore the effects evoked by prolonged training time and optimized video games. Moreover, further studies are needed that examine
the effects of combined interventions. The second experimental study offered initial insights about a potential interplay of exergame training and omega-3 FA supplementation. Future studies should consider prolonging the combinatory period or including older adults with a lack of omega-3 FA, so that a possible interplay might become more apparent. Finally, neuroimaging methods can provide further information about the effects of exergame training and its combination with omega-3 FA on the elderly brain.
Zusammenfassung


zu sein, dass körperliches Training und Nahrungssupplementierung gleiche neurobiologische Mechanismen teilen, damit ein mögliches Zusammenspiel beider Faktoren und somit ein verstärkter Effekt erzeugt werden kann.


Chapter 1

Prologue
1.1 **Background**

In the near future, the number of elderly people suffering from age-related bodily dysfunction will increase due to demographic changes [1]. The number of people aged over 60 years has been estimated to reach almost two billion by 2050 [2]. The growing proportion of older people and increasing life expectancy result in a larger population affected by age-related bodily dysfunctions. Limitations in motor and cognitive functions have been associated with depression, increased risk of falls and injuries, reduced quality of life, growing health care costs, and elevated mortality [3-6].

1.1.1 **The Aging Body**

Proper bodily functioning is required for the daily activities of independent living. Decreasing bodily capacities, therefore, may affect quality of life by decreasing walking ability and secure stair climbing [7, 8]. Gait instability and falls have been associated with degradation of joints, loss of muscle mass, and the resulting decrease of strength [9, 10]. In addition to reductions in the musculoskeletal system, impairments in vision, reaction time, and balance play an important role [9]. Nevertheless, the weakening of the sensorimotor system is probably not the only cause that explains age-related bodily dysfunction such as impaired gait; a reduction in cognitive functions is believed to play an important role as well [9, 11]. Cognitive functions are “…any mental process that involves symbolic operations–e.g. perception, memory, creation of imagery, and thinking…” [12]. Cognitive aging is characterized by impaired memory, decreased learning ability, slower processing speed, poorer attention, reduced executive skills, and greater anxiety [11, 13-16]. Cognitive functioning can affect motor functioning as both healthy participants and participants with cognitive impairment or subclinical cerebrovascular lesions had reduced gait stability and postural control during dual-task walking [7, 9, 17]. Daily activities frequently involve an interplay of cognitive and motor functions. Therefore, the reliable functioning of both components can be important to protect independence and quality of life in older adults. However, particular cognitive and motor functions are affected by age-related changes of the brain.

1.1.2 **The Aging Brain**

The aging process of the brain is associated with neuroanatomical changes, such as loss of brain tissue (gray and white matter volume) and white matter microstructure disconnections [15, 18, 19]. The brain loses about 15% of the cerebral cortex and about 25% of the cerebral white matter between the ages of 30 and 90 years [20]. Furthermore, the brain also shows modification in neuronal activity patterns [21]. In addition to neuroanatomical and neuronal activity alterations, neurochemical processes also change during aging [22]. The brain exhibits decreased capacity for synthesis and binding of neurotransmitters as dopamine, serotonin, and acetylcholine as well as metabolic changes including mitochondrial dysfunctions [23-30].
Influence on Cognitive and Motor Performance

Studies investigating cognitive aging support the idea that neuroanatomical and -chemical alterations, such as loss of brain tissue, cortical disconnections, and decreased levels of neurotransmitters, might explain poorer cognitive performance among healthy older adults [15, 18-20, 23-27]. In particular, the (pre)frontal lobe is vulnerable to age-related degeneration as shown in cross-sectional and longitudinal magnetic resonance imaging studies [31-35].

Anatomically, cognitive control, or so-called executive functions (EFs), has been linked with the frontal lobe of the brain, particularly with the dorsolateral prefrontal cortex (PFC) and related brain networks [36, 37]. A large PFC volume and greater PFC thickness have been associated with better EFs [38].

During one’s lifetime, the (pre)frontal network undergoes age-related neuronal changes; however, no consensus exists as to the precise pattern of EF alteration [15, 37, 39, 40]. One assumption is that the decline in frontal gray matter might be associated with the deterioration of EFs [41]. Furthermore, it has been proposed that frontal lobe white matter mediates the association of age and performance in tasks involving memory and EFs [39].

EFs are “higher-level” cognitive abilities that control and regulate “lower-level” cognitive processes and goal-directed actions [42, 43], e.g. walking in challenging environments. Different EF components, e.g. “working memory”, “inhibition”, and “divided attention”, partly explain gait performance [44-46]. In particular, divided attention is associated with spatial and temporal dual-task costs of gait (additional cognitive load while walking with a cognitive task, as compared to normal walking) [47]. Furthermore, gait disturbances and falls are believed to be moderated by executive functioning [48, 49]. Therefore, age-related reduction of EFs can impact gait performance and amplify the risk of falling [50].

A greater reliance on cognitive control for motor tasks makes structural differences in the PFC interesting from the perspective of age-related decreases in motor control [51]. Disturbances in cortico-cortical and cortico-subcortical connections, e.g. frontal connections with parietal lobes and basal ganglia, respectively, are classified as higher-level gait disorders [49, 52]. A phenomenon called ‘retrogenesis’ suggests that brain circuits which mature late in ontogeny, e.g. the (pre)frontal cortex, are the most vulnerable to early neurodegeneration [53]. Age-related changes in the (pre)frontal cortex might contribute to the understanding and predictability of disturbances in gait and gait-related motor activity. This suggestion is supported by the recent work of Rosano et al. (2012), which shows that a smaller volume of the prefrontal area is likely to contribute to a slower gait through slower information processing [54].

To sum up, age-related brain deterioration does affect cognition along with motor performance in healthy older adults. Since cognitive and motor decline threatens the independence and quality of life of the elderly, prevention and treatment has become of increasing importance [55].
1.1.3 Interventions Supporting Healthy Brain Aging

Several factors influence age-related brain deterioration, such as biological and behavioral factors (Figure 1.1). Examples of biological risks include genetic, molecular/cellular (e.g. oxidation), and system-level (e.g. homeostasis and stress) factors [56], while lack of physical exercise (PE), dietary habits, and multiple medication belong to behavioral risk factors. Changes in behavioral factors, e.g. PE and nutrition, may help to minimize or even slow down certain biological risk factors that influence brain aging.

**FIGURE 1.1 | Influence of biological and behavioral factors on the aging system.** The brain receives sensory input that is used to generate cognitive and motor outputs. Some processes, e.g. gait performance, required the interaction of cognitive and motor outputs. An age-affected brain can no longer generate proper cognitive and motor outputs. Reduced cognitive and motor outputs lead to weakness, an instable gait pattern, or even to fall events in older adults. Several aspects influence age-related bodily deterioration, e.g. biological and behavioral factors. Changes in behavioral factors, e.g. physical exercise and nutrition, may help to minimize or even slow down biological risk factors that influence brain aging.

**Physical Exercise**

One of the most effective ways to maintain a healthy body and mind is PE [13]. PE lowers blood pressure, increases sensitivity to insulin, contributes to weight loss, improves learning and memory, reduces the risk of neurodegeneration, and delays age-related cognitive decline [57, 58]. Particularly on a brain level, PE affects dendritic spinal properties, enhances long term potentiation, influences brain vasculature through the action of the insulin-like growth factor and vascular endothelial growth factor, and stimulates the brain-derived neurotrophic factor (BDNF), which plays an essential role in synaptic plasticity and cell genesis, growth, and survival [13]. Hence, PE appears to be a powerful trigger for molecular and cellular mechanisms causing brain plasticity [57, 59]. Due to its positive effects on
neurotransmitters, neurotrophins, and vasculature, PE enhances cognitive functioning [14]. For example, in the hippocampus, neurogenesis is associated with improved cognition, and the strongest neurogenic stimulus seems to be PE [13]. To conclude, PE has the ability to strengthen neuronal structures, synaptic plasticity, and therefore cognition [11].

**Video Game-Based Physical Exercise – Exergames**

To modulate neuronal networks and evaluate underlying neuronal mechanisms, video game-based training serves as a powerful tool [60]. Video games have the potential to train cognitive functions, including reaction time, processing speed, attention, and EFs [61-63]. A systematic review concluded that video games are promising for improving cognitive abilities in older adults who have a higher risk of cognitive decline [63].

Video game-based PE, as the name suggests, incorporates video games into a PE program. Video game-based exercise, or a so-called exergame, combines the training of motor with cognitive functions as the participants perform specific whole-body movements in response to video game commands (Figure 1.2). Two recent reviews concluded that combining cognitive and motor exercises in clinical practice enables older adults to move more safely in their physical environment [64, 65]. PE with decision-making opportunities is believed to facilitate the improvement of both cognitive and motor performance [66]. Furthermore, the combined training approach leads to a better general functional status in older adults [67]. A reason for this might be that PE triggers brain plasticity by cell proliferation and synaptic plasticity, while cognitive training supports the survival of newborn neurons and their integration into pre-existing networks in the brain [68-70]. At the very least, computerized interventions seem promising when they include training principles that enhance (motor) learning [7, 71], e.g. direct feedback on performance and rewards for correct responses in the video game-based training scenario.

Exergames that incorporate stepping exercises are effective in reducing to the likelihood of falls as well as improving gait and balance by the concurrent combination of motor and cognitive training [72]. As mentioned before, improved gait performance and reduced fall events can be achieved by the training of EFs [50, 64]. EF includes higher attentional functions (e.g. divided attention), inhibition, interference control, mental flexibility, and working memory [73]. Exergames set up (divided) attention challenges; participants observe cues on a frontal screen and must simultaneously execute well-coordinated movements. Furthermore, the video game application can be individually designed to allow for the implementation of EFs.

In brief, it can be hypothesized that PE which also targets EFs might be an integral training approach for stabilizing or even improving cognitive and motor performance in the elderly [50, 64]. Thus, exergame training could be a promising intervention for the support of healthy brain aging and, therefore, also of cognitive and motor performance.
**FIGURE 1.2 | Composition of an exergame training session.** The picture shows an elderly participant performing an exergame training session similar to that used for this thesis. On a dance plate the participants perform well-coordinated body movements in response to the video games. On the frontal screen, the participants follow the video game stimuli, to which they have to react.

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**Nutritional Supplementation and Physical Exercise**

Nutritional supplements may also exhibit favorable effects on brain health. Studies showed that nutritional supplements such as fish oil, fruits, teas, folate, spices, and vitamins have a positive impact on brain function [74, 75]. Recent studies focusing on underlying mechanisms like neuronal signaling have suggested that certain nutritional supplements seem to share similar cellular and molecular pathways as PE [13, 76].

Previous research proposed that the effects of PE on the brain can be complemented by the concurrent consumption of selected dietary elements [13, 76]. Accordingly, it can be hypothesized that a combination of PE with nutritional supplements has the potential to further enhance the effects of PE on brain structure and function in older adults. The potential synergy between PE and nutritional supplements could involve common cellular pathways that are important for energy homeostasis, neurogenesis, cell survival, synaptic plasticity, and vascular function [13, 74, 76] (Fig. 1.3). In rats, studies have shown that docosahexaenoic acid (DHA, belonging to the omega-3 fatty acids (FA)) as a supplement enhanced the effects of exercise on axonal growth, BDNF-related synaptic plasticity, and cognition [77, 78].
Mitochondrial energy production can be affected by nutrition and exercise. Mitochondrial energy metabolism is important for maintaining neuronal excitability and synaptic function. Additive effects on synaptic plasticity and cognition seem to be caused by the combination of certain diets and exercise. Mitochondria produces ATP, which in turn may activate the brain-derived neurotrophic factor (BDNF) and the insulin-like growth factor 1 (IGF-1). Both factors support synaptic plasticity and cognitive functioning. On the other hand, high caloric intake or strenuous exercise can produce excess energy, resulting in the formation of reactive oxygen species (ROS). When the buffering capacity of the cell is exceeded by overly high ROS levels, synaptic plasticity and cognitive function are compromised. This process might happen because of a reduction in the actions of signal-transduction modulators such as BDNF. An adaptation of nutrition and exercise may reduce the formation of ROS.

Omega-3 FAs are important for the function and integrity of the neuronal plasma membranes (which have DHA, arachidonic acid, and eicosapentaenoic acid as their main components), for energy metabolism, and for perfusion in the brain [74, 79]. In the aging brain, long-chain polyunsaturated FA (LCPUFA) concentration decreases [79]. Therefore, LCPUFA intake could be important as LCPUFA improve cognition, decrease (neuro)inflammation, and reduce vascular risk factors in normal aging adults [79]. Furthermore, LCPUFA may decrease brain deterioration by having positive effects on brain structure, function, and cerebral blood flow [80]. For example, DHA acts as a neurotrophic factor by increasing the level of the BDNF [77]. Previous randomized controlled studies have shown that fish oil, including omega-3 FA, had positive effects on brain structure and function in healthy older adults [81, 82]. The participants showed improved EFs, white matter microstructure integrity, gray matter volume, and vascular parameters.

On the brain level, the interplay components of PE and omega-3 FA seems to include the neuronal plasma membrane as well as the control of energy metabolism and synaptic plasticity by activating similar mechanism [76-78, 83]. For the neuronal plasma membrane, PE might activate mechanism that preserve DHA on the membrane supporting neuronal signaling [76]. Furthermore, the interplay effects of PE and omega-3 FA, especially DHA, involve BDNF-mediated synaptic plasticity [77]. PE can lead to higher BDNF levels through the stimulation of neurotransmitter, including NMDA (N-methyl-D-aspartate receptor) receptors and the noradrenergic system [84-86]. There are several mechanism how
DHA can lead to higher BDNF levels: (1) DHA is converted to Neuroprotectin D1 that can elevate BDNF [77, 87, 88], (2) on plasma membranes, DHA may activate signaling mechanism that can result in more BDNF, (3) antioxidant capacity of DHA reducing oxidative stress that has been shown to decrease BDNF [89], (4) DHA supports the glucose transport across the brain-blood barrier to provide energy source for the neurons [90]. Furthermore, the procedure whereby BDNF affects metabolism and synaptic plasticity seems to involve IGF-1 [91]. IGF-1 can be produced in skeletal muscle, the liver, and the brain, and so it can send peripheral messages to the brain in the context of diet and exercise [74]. BDNF and IGF-1 act at presynaptic and postsynaptic receptors triggering signaling systems, such as the mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase II (CaMKII), and Akt signaling systems, which facilitate synaptic transmission and support long-term potentiation that is associated with learning and memory [74, 92]. However, the focus of this thesis did not include molecular mechanisms.

To conclude, combining PE, in this case exergame training, with omega-3 FA may be a promising intervention design. Exergame training may gain added benefit from the positive effects of omega-3 FA, as omega-3 FA are important for energy metabolism and for the provision of building material for the brain [74, 76].
1.2 Aims and Outline

Due to demographic changes, the number of older adults suffering from age-related bodily dysfunctions will increase in the near future [1]. The brain is one part of the body that is affected by age-related changes [15, 18, 19]. Brain deterioration seems to impair cognitive [15, 18-20, 23-27] and motor performance [49, 52, 54], leading to unstable gait patterns or, even worse, to falls amongst the elderly. Thus, there is widespread agreement about the importance of promoting healthy brain aging in order to facilitate daily life activities and increase quality of life in older adults. To support healthy brain aging, evidence-based interventions are required. However, there is currently incomplete evidence about the effect of exergame training on the elderly brain. Furthermore, no study has so far investigated the effects of exergame training combined with omega-3 FA supplementation on brain structure and function in the elderly. This doctoral thesis, therefore, aims to better understand the influence of exergame training and its combination with omega-3 FA supplementation on healthy brain aging, including brain structure and function as well as corresponding cognitive and motor abilities.

Specifically, the following aims have been defined:

| Aim 1: | Examining whether the combination of physical exercise and nutritional supplementation has greater (additive) effects on brain structure, function, and/or metabolism than their separate administration in mammals and humans. |
| Aim 2: | Comparing exergame training with conventional balance training, focusing on prefrontal brain activity and associated cognitive and motor performance in healthy older adults. |
| Aim 3: | Examining whether the combination of exergame training and omega-3 fatty acids affect brain structure and function differently than exergame training alone in healthy older adults. |

This PhD thesis includes a systematic review, a protocol paper, and two experimental studies. Both experimental studies were conducted in Zurich (Switzerland) with healthy elderly participants (≥ 65 years). The findings of the studies are presented in the study sections of this thesis (Chapters 2, 3, and 5).

Chapter 2 includes a systematic review of the effects of PE combined with nutritional supplementation on brain structure, function, and metabolism in elderly human and mammalian studies, in reference to Aim 1. The systematic review provides an overview of the current state of knowledge about the interplay of PE and nutritional supplementation. The insights from the systematic review were implemented in the first and second experimental studies.
The first experimental study (Chapter 3) investigated the effects of exergame training compared to conventional balance training on prefrontal brain activity and associated cognitive and motor functions in healthy older adults, in reference to Aim 2.

Chapter 4 contains a protocol paper about the second experimental study. The protocol reviews the detailed proposal for the study, including considerations about why exergame training may be combined with omega-3 FA supplementation to support healthy brain aging.

The second experimental study of Chapter 5 built on the knowledge gained from the systematic review and the first experimental study. This study, in reference to Aim 3 of this doctoral thesis, examined the combinatory effect of exergame training and fish oil supplementation on brain structure and function in healthy older adults.

Finally, Chapter 6 provides an overview and a summary of the most important findings, followed by a general discussion. Moreover, limitations are discussed, leading to considerations and proposals for future studies.
Chapter 2

Paper 1

Effects of Physical Exercise Combined with Nutritional Supplements on Aging Brain Related Structures and Functions: A Systematic Review

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Keywords: nutritional supplementation, nutrition, physical exercise, brain function, brain metabolism, aging

Published in¹:

Frontiers in Aging Neuroscience. 2016, 8 (161)

DOI: 10.3389/fnagi.2016.00161

¹Figure, tables, and language errors in the original publication were adapted/corrected for this thesis.
Abstract

Age-related decline in gray and white brain matter goes together with cognitive depletion. To influence cognitive functioning in elderly, several types of physical exercise (PE) and nutritional intervention have been performed. This paper systematically reviews the potential additive and complementary effects of nutrition/nutritional supplements and PE on cognition. The search strategy was developed for EMBASE, Medline, PubMed, Cochrane, CINAHL, and PsycInfo databases and focused on the research question: “Is the combination of PE with nutrition/nutritional supplementation more effective than nutrition/nutritional supplementation or PE alone in effecting on brain structure, metabolism, and/or function?” Both mammalian and human studies were included. In humans, randomized controlled trials that evaluated the effects of nutrition/nutritional supplements and PE on cognitive functioning and associated parameters in healthy elderly (> 65 years) were included. The systematic search included English and German language literature without any limitation of publication date. The search strategy yielded a total of 3129 references of which 67 studies met the inclusion criteria; 43 human and 24 mammalian, mainly rodent, studies. Three out of 43 human studies investigated a nutrition/PE combination and reported no additive effects. In rodent studies, additive effects were found for docosahexaenoic acid supplementation when combined with PE. Although feasible combinations of PE/nutritional supplements are available for influencing the brain, only a few studies evaluated which possible combinations of nutrition/nutritional supplementation and PE might have an effect on brain structure, metabolism and/or function. The reason for no clear effects of combinatory approaches in humans might be explained by the misfit between the combinations of nutritional methods with the physical interventions in the sense that they were not selected on sharing of similar neuronal mechanisms. Based on the results from this systematic review, future human studies should focus on the combined effect of docosahexaenoic acid supplementation and PE that contains elements of (motor) learning.
2.1 Introduction

Thirty percent of people aged 65 and older living in the community experience at least one fall per year, and this proportion increases markedly with age [93]. The elevated incidence of falls in elderly is only one of the many physical dysfunctions that may be encountered with advanced age [9]. Elderly experience a reduction in walking speed, an increased variability in step timing, a decline in gait stability, and are compromised in their learning ability [9, 11]. These reductions in movement functionality often develop already in midlife [3] and have been described as age-related deteriorations in physical functioning [7]. Physical functioning can be defined as the ability to conduct activities that are required for independent living and that may affect quality of life, such as walking and climbing stairs [7, 8]. Limitations in physical functioning have been associated with depression, increased risk for falls and injuries, reduced quality of life, increased health care costs, and mortality [3]. In the near future, the number of elderly people suffering from physical dysfunctions will increase due to demographic changes [1].

Various factors have been proposed causing the decline in physical functioning in the older population. Loss of muscle mass, strength, and degradation of joints have been associated with gait instability and falls [9, 10]. In addition to reductions in the musculoskeletal system, impairments in vision, reaction time, and balance play an important role [9]. However, worsening of the sensorimotor system is probably not the only cause explaining deteriorations in physical functioning: reduction of cognitive functions is believed to play a significant role as well [9, 11]. Cognitive functions are “…any mental process that involves symbolic operations–e.g. perception, memory, creation of imagery, and thinking…” [12].

A decrease in cognitive performance in old age is predominant in most individuals. Ageing associated cognitive decline has a prevalence rate of 28% for people from 65 to 84 years [94]. Another 17% of the population investigated (n = 4785) showed objective evidence of cognitive decline without cognitive complaints, which sums up to a total of 45% of people showing some kind of cognitive impairment without dementia. Cognitive aging is characterized by mental decline [14], memory impairments [11, 13, 15], decreased learning ability [11, 13], greater anxiety and poorer attention [11], slower processing speed [11, 15, 16], and reduction of executive skills [15]. Other studies showed that healthy participants and participants with cognitive impairment or subclinical cerebrovascular lesions had reduced gait stability and postural control during dual-task walking suggesting that cognitive abilities affect gait performance [7, 9, 17].

Studies investigating cognitive aging support the idea that neuroanatomical changes, such as loss of brain tissue and cortical disconnections, might explain the poorer cognitive performance of healthy elderly [15, 18-20]. Age-related structural changes include loss of gray matter volume (frontal and temporal lobes), vulnerability of prefrontal white matter, loss of microstructural white matter integrity, and decrease in hippocampus and cerebellum volumes [15, 18]. For example, frontal lobe white matter
has been proposed to mediate the association of age and performance in tasks assessing executive skills and memory [39]. Structural alterations might partially account for observed age-related declines in cognition [15]. In addition to the neuroanatomical changes, neurochemical processes change during the course of aging [22]. In rodents, for example, an age-related decrease of neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), might contribute to age-related cognitive impairments [95-97].

Since cognitive decline potentially threatens independence and quality of life of older adults, prevention and treatment of cognitive impairment in the elderly has assumed increasing importance [55]. Two factors that may positively effect on cognition are physical activity [98] and nutritional supplementation [74] (Figure 2.1). Physical exercise (PE) has been described to be the most effective way to maintain a healthy body and mind [13]. PE lowers blood pressure, increases sensitivity to insulin, contributes to weight loss, delays age-related cognitive decline, improves learning and memory, and reduces the risk of neurodegeneration [57, 58]. The proposed mechanisms by which PE affects cognition revolve around changes in neurotransmitters, neurotrophins, and vasculature [14]. Neurogenesis in the hippocampus is associated with improved cognition, and the strongest neurogenic stimulus seems to be PE [13]. Moreover, PE appears to affect properties of dendritic spines, to enhance long term potentiation, to influence brain vasculature through the actions of insulin-like growth factor (IGF) and vascular endothelial growth factor, and to affect BDNF which plays an essential role in synaptic plasticity and cell genesis, growth, and survival [13].

**FIGURE 2.1 | Interaction of cognitive and physical functioning.** Physical and cognitive functioning influences each other (yellow arrow). In turn, both can be influenced by physical exercise and nutrition (light green arrows). The motor and sensory system regulates physical functioning (dark green arrows).
Nutrition and nutritional supplements may also exhibit positive effects on brain health [13]. Studies showed that caloric restriction (CR) and nutritional supplements such as fish oil, teas, fruits, folate, spices, and vitamins have the potential to positively affect cognitive functioning [74]. Investigations on the effects of nutrition on brain function have usually focused on neuroprotective properties of nutritional supplements [13]. Recent studies focused on underlying mechanisms like neuronal signaling [13]. In fact, nutritional supplementation and CR seems to affect similar cellular and molecular pathways as PE [13].

From the foregoing, the assumption that PE and nutrition could have additive effects on brain structures and functions that may result in greater benefits on cognition for combinatory interventions seems justified [76]. “Additive” means when two interventions are combined intendedly (PE and nutrition are an integral part of one intervention) to enhance effects and “complementary” in case each intervention stands by itself. Recent studies indicate that exercise is capable of boosting the health effects of certain diets and that selected dietary factors may have the capacity to complement the effects of exercise [76]. However, existing reviews on these effects are either narrative, or, when being performed systematically, are limited in the sense that they focus on the isolated effect of either PE or nutrition [13, 74, 99]. Which combination of selected dietary factors possibly best should be added to PE for additive effects of exercise on cognition in humans remains, therefore, indefinite. To the best of our knowledge, a systematic review focusing on the possible additive effects of PE and nutrition/nutritional supplementation on the elderly brain has not been performed. Therefore, a systematic review was performed on the effect of combined physical and nutritional interventions with the aim of clarifying the relationship between the type of combined intervention and the effects of such an intervention on brain related structure and function in both mammalian and human studies. The following research question guided this systematic review: “Is the combination of PE with nutritional supplementation more effective than nutritional supplementation or PE alone in effecting on brain structure, metabolism, and/or function”?

2.2 Methods

2.2.1 Data Sources and Searches

A search strategy was developed in collaboration with a librarian from the Medical Library of the University of Zurich. The search period covered all years from the inception to October, 2015, and included EMBASE, Medline, PubMed, Cochrane, CINAHL, and PsycInfo. Searches were undertaken using MeSH headings and text words including the following main terms for the population: aged, elder, placental mammals, human, rat, mouse, mice, mammal, mammalia; for nutritional intervention: diet supplementation, diet therapy, protein intake, dietary intake, diet, protein, nutrient, mineral, vitamin, supplementation, supplement, additive, intake, therapy, treatment; for physical exercise: resistance
training, physical, activity, exercise, fitness, strength, training and for the outcome of interest: cognition, executive function, memory, nervous system development, nerve cell plasticity, angiogenesis, neurogenesis, synaptogenesis, neuroplasticity, brain structure, spine density, function, structure, neurotransmitter, vascular endothelial growth factor, insulin-like growth factor, brain, nerve. Furthermore, the bibliographies of all eligible articles and related reviews, as well as recent conference proceedings, were checked through hand searching. To ensure the clarity and transparency of reporting, the PRISMA guidelines [100] were followed.

2.2.2 Selection Criteria

Both studies with mammals and humans were considered for this review. From mammal research knowledge about the effects on molecular, cellular, and neural circuit levels and how these may impact cognitive function can be gained [99, 101]. Higher-level cognition effects and influences on macro- and systems-level change in the central nervous system can be evaluated in human studies [99, 101]. The search strategy included “elderly over the age of 65 years” and “older mammals”. Interventions that focused on PE and nutritional supplementation or the combination of both were considered. Study outcomes were determined on brain structure, function, and metabolism levels. Randomized controlled trials (RCT), the most rigorous way of determining whether a cause-effect relation exists between treatment and outcome [102], were primarily included. Because well-designed observational studies have been shown to provide results similar to RCT [103] case control trials were also included. In addition, reviews on our topic written in English or German with no year restriction were considered for discussion.

2.2.3 Selection Process

The first step was the removal of duplicate citations. Afterwards two reviewers (JS, AS) determined which studies should be included by independently screening of title, abstract, and keywords. A priori set inclusion and exclusion criteria were applied to the articles (Table 2.1). An article was eligible, if the investigator examined complementary or additive effects of PE and nutritional interventions on cognitive functions in humans and mammals and/or associated brain parameters in mammals. Only longitudinal studies were included that carried out an intervention. Interventions that considered aerobe, strength, and/or coordination training were defined as PE. Nutritional interventions were those that considered nutritional supplementation. Studies using pharmacological supplementation or that focused on physical outcomes were excluded. Subsequently, the results from the screening were discussed to exclude any differences in the inclusion decisions. Full text reading of the remaining literature yielded the final list of papers. Studies were included assessing brain structure (e.g. gray and white matter), brain metabolism (e.g. neurotrophic factors), and brain function (e.g. cognitive test batteries) in healthy elderly.
TABLE 2.1 | List of inclusion and exclusion criteria.

<table>
<thead>
<tr>
<th>Area</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Older (&gt;65 years) adults and old mammals</td>
<td>Patients with neurodegenerative diseases</td>
</tr>
<tr>
<td>Intervention</td>
<td>Nutritional supplementation, brain food, physical exercise, exercise trainings, physical activity</td>
<td>Pharmacological interventions</td>
</tr>
<tr>
<td>Outcome</td>
<td>Neurogenesis, synaptic plasticity, brain structure, spine density, angiogenesis, growth factors, neurotransmitter, neurotrophins, cognitive function</td>
<td>Physical benefits</td>
</tr>
<tr>
<td>Study type</td>
<td>Randomized controlled and case control trials</td>
<td>Methodological, theoretical, review, and discussion papers</td>
</tr>
<tr>
<td>Language</td>
<td>English and German</td>
<td>All other languages</td>
</tr>
<tr>
<td>Year</td>
<td>All years</td>
<td></td>
</tr>
</tbody>
</table>

2.2.4 Data Extraction and Data Synthesis

The included studies were sub-divided into human and mammalian studies. Each of these classes was then further subdivided into three groups: PE, nutritional intervention, and studies that investigated a combination of PE and nutritional intervention. In this way, comparisons between different treatments were easier to track. A purpose adjusted individualized data extraction form from Wright et al. [104] was used to collect data from single studies. The extraction of the data included (1) reference information: author and date; (2) characteristics of study population: number of participants, gender, age, genetics (mammals); (3) characteristics of PE intervention: type of exercise, frequency, and duration; (4) characteristics of nutritional intervention: diet or nutritional supplement, amount of intake, and duration; (5) characteristics of outcomes: outcome measures and results. The data is presented in the results section as a descriptive summary of the studies and their results. Furthermore, a qualitative synthesis of the studies was executed. A meta-analysis was not performed due to the high heterogeneity of intervention types and outcome variables among the studies.

2.2.5 Quality Appraisal

Quality evaluation of the studies was done by reporting potential sources of bias [105]. For critical quality appraisal, the purpose-adjusted Downs & Black checklist for randomized and non-randomized studies of health care interventions was used [106]. The quality checklist consisted of 27 items having a theoretical maximum score of 32 points. The checklist scored 5 different domains: the quality of reporting (10 items, maximum 11 points), the external validity (3 items, maximum 3 points), internal validity – bias (7 items, maximum 7 points), internal validity – confounding (selection bias) (6 items, maximum 6 points) and power (1 item with maximum 5 points). A summary of the set criteria (20 for human and 13 for mammals) for quality assessments that were used is displayed in the supplementary material table 1. The quality evaluation procedure was done independently by two reviewers (JS and
AS), as previously advised [105, 107]. The level of agreement was assessed with Cohen’s kappa analysis on all items of the checklist. Landis and Koch’s benchmark for assessing agreement ranges from almost perfect (0.81-1.0), substantial (0.61-0.8), moderate (0.41-0.6), fair (0.21-0.4), slight (0.0-0.2), and poor (<0) [108]. Disagreements were resolved by consensus or by consulting a third reviewer.

2.3 Results

2.3.1 Study Selection

The database search resulted in a total number of 3129 studies. The selection process is illustrated in figure 2.2. After the removal of duplicates (n= 431) and the screening process, 75 studies were left for full text reading. During full text reading, the cited human studies in the reference lists that perhaps would be relevant for the review were kept track leading to an additional 40 papers selected for full text reading. Finally, following full text reading, 67 articles were included in the systematic review. For the mammalian studies, the included studies were performed with rodents only.

FIGURE 2.2 | Search and selection process.
2.3.2 Study Characteristics

Characteristics of Rodent Studies

The total number of mice and rats in the 24 included studies was 1396 (median: 48 rodents per study, range 11-200). The studies examined C57BL/6 mice [109-111], Wistar rats [112-118], Fischer 344 rats [119], Long Evans rats [120], Sprague-Dawley rats [77, 78, 121-125], F344xBN hybrid rats [126-128], and BALB/cJ mice [129]. At the beginning of the interventions, all rodents were disease free. The age of the rodents varied from a few months to a few years. The articles were studies that evaluated the effects of PE [111, 124, 130], of nutritional intervention [109, 110, 114, 119, 120, 123, 126-128], or used a combination of PE and nutritional intervention [77, 78, 112, 113, 115-118, 121, 122, 125, 129]. PE was always an aerobic type of exercise (running or swimming) performed five times per week for 20 to 60 min over a period of two weeks to 13 months, while nutritional interventions consisted of either dietary supplementation (taurin, niacin, amino acid, selenium, fatty acids (FA), and epinephrine) or caloric restriction. Caloric restriction was included as a part of nutritional supplementation in the sense of nutritional depletion. A summary of measured outcomes in rodents is given in table 2.2. The outcome categories that were assessed were behavior, neurogenesis, neurotrophins, synaptic proteins, cell signaling proteins, metabolic homeostasis proteins, and measures of oxidative stress.
### Table 2.2: Study outcomes measured in included rodent studies.

<table>
<thead>
<tr>
<th>Category</th>
<th>Outcomes</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior</td>
<td>Behavioral tests</td>
<td>• Measurement of learning, memory, motor skill, and anxiety like behavior</td>
</tr>
<tr>
<td>Neurogenesis</td>
<td>BrdU labeling</td>
<td>• Detection of proliferating cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Evaluation of neurogenesis</td>
</tr>
<tr>
<td></td>
<td>Ki-67 staining</td>
<td>• Cellular marker for proliferation</td>
</tr>
<tr>
<td></td>
<td>Doublecortin staining</td>
<td>• Marker for neurogenesis</td>
</tr>
<tr>
<td></td>
<td>Immunohistochemistry</td>
<td>• Determination of phenotype of newly generated cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o GFAP: astrocyte protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Neuronal nuclear marker</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o MAP2a: mature neuron-specific protein</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>BDNF-, NT-3-, trkB-, and trkC- mRNAs</td>
<td>• BDNF and NT-3: neurotrophins</td>
</tr>
<tr>
<td>Synaptic proteins</td>
<td>NMDA receptor subunits: NR1, NR2A, and NR2B</td>
<td>• Glutamate receptor</td>
</tr>
<tr>
<td></td>
<td>AMPA receptor subunits: GluR1 and GluR2</td>
<td>• Important for synaptic plasticity and memory</td>
</tr>
<tr>
<td></td>
<td>Synaptophysin</td>
<td>• Non-NMDA glutamate receptor</td>
</tr>
<tr>
<td></td>
<td>STX-1 and STX-3</td>
<td>• Involved in plasticity and synaptic transmission</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Involved in synaptic transmission</td>
</tr>
<tr>
<td></td>
<td>GAP-43</td>
<td>• Plasma membrane syntaxins</td>
</tr>
<tr>
<td></td>
<td>Synapsin</td>
<td>• Present in synaptic membranes and in neuronal growth cones</td>
</tr>
<tr>
<td>Cell signaling</td>
<td>CaMKII</td>
<td>• Growth associated protein</td>
</tr>
<tr>
<td></td>
<td>CREB staining</td>
<td>• Involvement in neurotransmitter release, axonal elongation, and maintenance of synaptic contacts</td>
</tr>
<tr>
<td></td>
<td>Akt protein determination</td>
<td>• Important in learning and memory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Involvement in learning and memory</td>
</tr>
<tr>
<td>Metabolic homeostasis</td>
<td>Glucocorticoids receptor, 11-beta-HSD1, ghrelin</td>
<td>• Molecular systems that play dual roles on metabolism and synaptic plasticity</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Amount of oxidized proteins</td>
<td>• Measurement of oxidative stress</td>
</tr>
</tbody>
</table>

Characteristics of the Human Studies

The total number of participants in the 43 included human subject studies was 19,757. This number includes the participants from large supplementation studies such as the Women’s Health Initiative WHI (n=14,200), the Physicians Health Study II PHSII (n=5,947), the Women’s Health Study WHS (n=6,377), and the Age-Related Eye Disease Study AREDS (n=2,166). The number of participants decreased to 3,847 (median: 58 participants per study, range 12-910) without the aforementioned studies. At the time of recruitment, all participants were healthy elderly living in a nursing home or at home and having a mean age around 65 years or higher. The studies evaluated the effects of PE [59, 131-150], of nutritional intervention [151-169], or a combination of PE and nutritional intervention [170-172]. PE in the human studies consisted of either strength or aerobic training that was usually done three times per week for one hour over a period of four to 12 months. Nutritional intervention included (multi)vitamin supplementation, amino acids, nitrate enriched diets, creatine, FA or protein supplementation. Small nutritional supplementation studies were done over a period of several months, while large nutritional supplementation trials were done for several years. Many different outcomes have been measured in the human trials (Table 2.3). The focus of the majority of the articles was on cognitive tests. Briefly, cognitive tests were administered to the participants to assess general cognitive status, memory, executive function (EF), attention, intelligence, and sensorimotor performance. Measurements concerning the brain included brain volumes, brain activity, and metabolites concentrations in the brain. In addition, blood samples were used to measure serum BDNF, IGF-1, and neurotransmitter/hormone levels.

**TABLE 2.3 | Study outcomes measured in included human studies.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Outcome</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive function</td>
<td>Cognitive tests</td>
<td>Tests for general cognitive functioning, memory, executive functions, intelligence, attention, and sensorimotor performance</td>
</tr>
<tr>
<td>Brain structure</td>
<td>Whole brain volume and regional brain volumes</td>
<td>MRI: voxel-based morphometry</td>
</tr>
<tr>
<td>Brain activity</td>
<td>Electroencephalography: event-related potentials</td>
<td>During a cognitive task or a sensory stimulus (sensory evoked potential)</td>
</tr>
<tr>
<td></td>
<td>Cerebral blood flow</td>
<td>Determined from MRI</td>
</tr>
<tr>
<td></td>
<td>Apparent diffusion coefficients of white and gray matter</td>
<td>Acquired using an eight channel SENSE head coil</td>
</tr>
<tr>
<td></td>
<td>Functional MRI: cortical recruitment</td>
<td>Functional MRI during a cognitive task</td>
</tr>
<tr>
<td>Blood markers</td>
<td>Serum IGF-1, BDNF, dopamine, epinephrine and granulocyte colony-stimulating factor levels, and total antioxidant capacity</td>
<td>Blood samples (cephalic vein)</td>
</tr>
<tr>
<td>Metabolism</td>
<td>N-acetyl aspartate, creatine, choline, and myo-inositol brain concentrations</td>
<td></td>
</tr>
</tbody>
</table>

BDNF: Brain-derived neurotrophic factor, IGF-1: Insulin-like growth factor 1, MRI: Magnetic resonance imaging.
2.3.3 Effects of Physical Exercise and Nutritional Intervention in Rodents

The detailed results of the rodent combinatory studies are listed in table 2.4. The detailed results of the rodent studies examining PE or nutritional intervention are listed in the supplementary material table 4. Ten studies evaluated the effects of PE and nutritional supplementation on behavioral tests [77, 78, 112, 113, 115-118, 122, 129]. Three out of these ten studies found additive effects of PE and nutritional supplementation on Morris Water Maze (MWM) [77] and object recognition task [112, 113]. Cechella et al (2014a, b) found the benefits in 24 months old rats but not in 12 months old rats. However, the other seven studies found no additive beneficial effects on cognitive performance [78, 115-118, 122, 129]. Chytrova et al (2010) and Rachetti et al (2013) found that both PE and nutritional supplementation resulted in improved learning or memory. However, Jacotte-Simancas et al (2013) and Khabour et al (2010, 2013) found that only exercised groups improved spatial learning and memory. On the other hand, Hansalik et al (2006) found no improvements in the MWM for any of the intervention groups.

Four articles assessed BDNF levels, three of them in the hippocampus [77, 116, 117] and one in the cerebral parietotemporal cortex [125]. One study reported an additive effect [77], two studies found increased BDNF levels in the PE group only [116, 117], and one study found no increased levels in any group. Moreover, two studies investigated the combinatory effect on synaptic proteins and both studies reported additive effects [77, 78]. In addition, three studies measured cyclic adenosine monophosphate response element-binding protein (CREB), Akt, or calmodulin-dependent protein kinase II (CaMKII) concentrations in the hippocampus [77, 112, 113]. Wu et al (2008) found that the combinatory intervention had higher cell signaling protein levels. However, Cechella et al (2014a, b) measured increased CREB levels in the PE or in the nutritional intervention group. Akt levels were only increased in the PE group. Gomez-Pinilla and Ying (2010) found an inconsistent pattern of change in leptin and ghrelin receptor protein levels, phosphor-adenosine monophosphate-activated protein kinase, sirtuin 1, glucocorticoid receptor, and 11beta-hydroxysteroid dehydrogenase type 1 levels in the hypothalamus and in the hippocampus in rats [121]. At least, one study showed a positive effect of the combination group related to oxidative stress (decreased carbonyl levels) [77].
### TABLE 2.4 | Included rodent studies combining physical exercise and nutritional intervention.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Groups</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhattacharya et al, 2015</td>
<td>N= 91; male BALB/cJ mice Age: 10 weeks</td>
<td>Running wheel 1.49mg of EGCG per g diet 3.34mg of B-ALA per g diet</td>
<td>Exe, sed, B-ALA: exe or sed, EGCG: exe or sed, B-ALA + EGCG: exe or sed N=11-12 per group</td>
<td>Fear conditioning (contextual and cued) BrdU staining</td>
<td>Exe increased duration of freezing (contextual) Exe approx. 4-fold greater duration of freezing behaviour than sed (cued) No effect of diet or interaction between diet and exercise Exe increased total number of BrdU+ cells in the granule layer of the dentate gyrus approx. 4 fold over sed No effect of diet or interaction between diet and exercise</td>
</tr>
<tr>
<td>Cechella et al, 2014a</td>
<td>Male wistar rats</td>
<td>Swim training: 20min, 5+/ week 1ppm of diphenyl diselenide (selenium) 5 weeks</td>
<td>Exe (I), selenium (II), exe + selenium (III), adult control (IV), aged control (V) N= 4-6 per groups</td>
<td>ORT OLT CREB and Akt in hippocampus</td>
<td>Short term memory: (I)+(II) improved compared to (V), (III) better than all other groups Long term memory: (II)+(III) better than control groups (II) better than aged control, (I) + (III) better than control groups pAkt/ Akt: same for (I)+(III)+(IV)+(V), (I) higher than control groups pCREB/CREB: (I)+(II)+(III) better than aged control</td>
</tr>
<tr>
<td>Cechella et al, 2014b</td>
<td>N= 30; male wistar rats</td>
<td>Swim training: 20min, 5+/ week 1ppm of diphenyl diselenide (selenium) 5 weeks</td>
<td>Exe (I), selenium (II), exe + selenium (III), adult control (IV), aged control (V) N= 4-6 per groups</td>
<td>ORT OLT CREB in hippocampus</td>
<td>Short term memory: (I)+(II)+(III) better than control groups, (III) shows the best results Long term memory: (I)+(II)+(III) better than aged control, (I) better than adult control (I)+(II)+(III) better than aged control pCREB/CREB: (I)+(II) better than control groups</td>
</tr>
<tr>
<td>Chytrova et al, 2010</td>
<td>N= 24; adult male Sprague-Dawley rats</td>
<td>Running wheel DHA enriched diet 12 days</td>
<td>RD + sed (I), DHA + sed (II), RD + exe (III), DHA + exe (IV) N= 6 per group</td>
<td>Synaptic proteins in hippocampus MWM</td>
<td>NR2B: sig. increase for (II)+(III)+(IV), greatest effect for (IV) compared to (III) STX-3 and GAP-43: sig increase for (II)+(III)+(IV), greatest effect for (IV) Latency: (II)+(III)+(IV) decreased compared to (I)</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Sex</td>
<td>Procedure</td>
<td>Treatment Details</td>
<td>Outcome</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gomez-Pinilla and Ying, 2010</td>
<td>24</td>
<td>Male</td>
<td>Running wheel DHA enriched diet 2 weeks</td>
<td>RD + sed(I), DHA + sed(II), RD + exe (III), DHA + exe (IV) N= 6 per group</td>
<td>Leptin: <em>Hyp</em>: increase for (II)+(III)+(IV), greatest for (IV), <em>Hyp</em>: increase for (II)</td>
</tr>
<tr>
<td>Hansalik et al, 2006</td>
<td>200</td>
<td>Male</td>
<td>Running wheel Treadmill: 20min, 5×/week CR 13 months</td>
<td>Baseline (age: 5 months) (I), exe (TM) (II), exe (RW) + CR (III), sed + CR (IV), sed1 (one rat, one cage) (V), sed4 (four rats, one cage) (VI) MWM</td>
<td>Learning and short term memory: no effects comparing various intervention groups at age 10 and 18 months</td>
</tr>
<tr>
<td>Jacotte-Simancas et al, 2013</td>
<td>62</td>
<td>Male</td>
<td>Running wheel Epinephrine: 0.01 or 0.05mg/kg</td>
<td>Sed (I), sed + 0.01 ep (II), sed + 0.05 ep (III), exe (IV), exe + 0.01 ep (V), exe + 0.05 ep (VI) Barnes maze</td>
<td>Distance: (IV)+(V)+(VI) sig. shorter than (I)+(II)+(III) Latency: no sig. results</td>
</tr>
<tr>
<td>Khabour et al, 2010</td>
<td></td>
<td></td>
<td>Voluntary exercise CR 6 weeks</td>
<td>Sed (I), CR (II), exe (III), exe + CR (IV) N= 10-13 per group</td>
<td>Spatial learning and memory formation: (IV)+(III) enhanced compared to (I)+(II), no effect of CR BDNF: (IV)+(III) sig. higher levels compared to (I)+(II) no effect of CR</td>
</tr>
<tr>
<td>Khabour et al, 2013</td>
<td>92</td>
<td>Male</td>
<td>Swimming: 60min, 5×/week CR 6 weeks</td>
<td>Sed (I), CR (II), exe (III), exe + CR (IV) N= 15 per group</td>
<td>Spatial learning and memory formation: (IV)+(III) enhanced learning/memory compared to (I)+(II), no effect of CR (IV)+(III) sig. higher levels compared to (I)+(II) no effect of CR</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Treatment/Intervention</td>
<td>Outcomes/Findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rachetti et al, 2013</td>
<td>N= 45; adult wistar rats</td>
<td>Treadmill: 30 min, 5x/week (until age of 27 days) Fish oil capsules Length: prenatal to 10 months</td>
<td>Exe (I), exe + fish (II), control (III), control + fish (VI) N= 11-12 per group Open field test ORT Plus maze discriminative avoidance task (VI) decrement in location during 2nd exposure compared to the other groups Test session: all groups explored sig. more the novel object compared to familiar object, Re-test session: (II) explored sig. more the novel object compared to familiar object (I)+(II) discriminated the aversive from non-aversive arms and spent sig. less time in aversive arm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strasser et al, 2006</td>
<td>Male Sprague-Dawley rats</td>
<td>Running wheel Treadmill: 20min, 5x/week CR 13 months</td>
<td>Baseline (age: 5 months) (I), exe (TM) (II), exe (RW) + CR (III), sed + CR (IV), sed1 (one rat, one cage) (V), sed4 (four rats, one cage) (VI) BDNF in parietotemporal cortex Decrease for (V), increase for (VI), highest values for (VI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al, 2008</td>
<td>N= 24; Sprague-Dawley rats</td>
<td>Running wheel DHA enriched diet 12 days</td>
<td>RD + sed (I), DHA + sed (II), RD + exe (III), DHA + exe (IV) MWM BDNF, Synapsin I, CREB, Akt, CaMKII Oxidative proteins Latency: (II)+(III)+(IV) shorter than (I), (IV) shorter than (II)+(III), (II)+(III) increased values and even more in (IV) (II)+(III) reduced oxidized protein and even more in (IV)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3.4 Effects of Physical Exercise and Nutritional Intervention in Humans

The detailed results of the human studies that combined PE with a nutritional intervention are listed in table 2.5. The detailed results of the human studies examining PE or nutritional intervention in isolation are listed in the supplementary material table 5. Three studies evaluated the combined effects of PE and nutritional supplements on cognitive functions [170-172]. None of these studies found additive effects of PE and nutritional supplementation. Alves et al (2013) investigated the effects of resistance training with creatine supplementation and found no change for any of the cognitive tests [170]. Van de Rest et al (2014) executed a similar protocol but with a protein drink [172]. The results indicated that PE in combination with protein and the group performing PE only showed improvements in different cognitive domains. No interaction effect was found between the treatments. Cetin et al (2010) looked at the effects of endurance exercise with vitamin E supplementation [171]. They found positive effects for the exercise group and no effect for the vitamin E supplementation for electroencephalography recordings. In addition, they found no changes in the antioxidant capacity for any intervention group [171].
Table 2.5 | Included human studies combining physical exercise and nutritional intervention.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Groups</th>
<th>Outcomes</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alves et al, 2013</td>
<td>N= 56; women</td>
<td>Strength exercise 3 sets for 7 exercise, 2×week</td>
<td>Creatine (I), exe (II), creatine + exe (III), non creatine + non exercise (IV)</td>
<td>MMSE, Stroop test, TMT, Digit span test, delay recall test</td>
<td>No sig. diff. for any of the variables</td>
</tr>
<tr>
<td></td>
<td>Age range: 60-80 years</td>
<td>2×weeks</td>
<td>N= 14 per group</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Creatine: 5g/day 2×weeks</td>
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<tr>
<td>Cetin et al, 2010</td>
<td>N= 57; sedentary</td>
<td>Aerobic exercise 90min, 3×week</td>
<td>Exe (I), vitamin (II), exe + vitamin (III), non exe + non vitamin (IV)</td>
<td>EEG (auditory oddball paradigm)</td>
<td>P3 amplitude: no diff.</td>
</tr>
<tr>
<td></td>
<td>Age range: 69.6-73.1</td>
<td>Vitamin E 6 months</td>
<td>N= 14-15 per group</td>
<td></td>
<td>P3 latency: (I), (II), (III) shorter latency compared to pre-treatment and (I)+(II) shorter latency compared to control</td>
</tr>
<tr>
<td></td>
<td>years</td>
<td></td>
<td></td>
<td></td>
<td>No diff. to control group or within a group after 6 months</td>
</tr>
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<tr>
<td>Van de Rest et al, 2014</td>
<td>N= 127; frail and pre-</td>
<td>Strength exercise 2×week Protein shake: twice daily</td>
<td>Exe + protein (I), exe + placebo (II), non exe+ protein (III), non exe + placebo (IV)</td>
<td>Word learning test, Digit Span Task, TMT A&amp;B, Stroop Colour-Word Test, Verbal Fluency Test Finger pre-cuing task</td>
<td>- (I) vs (III): improvement: information processing speed</td>
</tr>
<tr>
<td></td>
<td>frail</td>
<td>2×weeks</td>
<td>N= 62 (exe), 65 (non-exe)</td>
<td></td>
<td>- (II) vs (IV): improvement: attention, working memory</td>
</tr>
<tr>
<td></td>
<td>Mean age: 79±8 years</td>
<td></td>
<td></td>
<td></td>
<td>Reaction time: improved over time in all groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No sig. interaction on any of the cognitive domains</td>
</tr>
</tbody>
</table>

EEG: Electroencephalography, Exe: Exercise, MMSE: Mini Mental Status Examination, TMT A/B: Trail Making Test A/B.
2.3.5 Quality Evaluation

The agreement on study quality criteria between the two reviewers was substantial with an estimated kappa value of 0.65 (95% confidence interval between 0.63 and 0.67). The percentage of agreement between the two raters was 85.15% for the human and rodent studies. The results of the PE, nutrition, and combination intervention of human and rodent studies are summarized in the supplementary material table 2 and 3.

Rodent Studies

None of the 24 studies reached the maximum possible score of 13 points. The quality scores ranged from a minimum of 9 points to a maximum of 12. The mean quality score was 10.29 points (range: 9-12 points), the median value was 10.5 points and the mode was 11 points. The mean score for reporting was 5.04 points (maximum: 6 points; range: 4-6 points), for internal validity (bias) 4.08 points (maximum: 5 points; range: 4-5 points), for internal validity (bias) 0.58 (maximum: 1 points; range: 0-1 points), and for power 0.58 (maximum: 1 points; range: 0-1 points). The mean score for PE studies was 11 points (maximum: 12 points; range: 10-12 points), for nutritional supplementation studies was 9.77 points (maximum: 11 points; range: 9-11 points), and for combination studies was 10.5 points (maximum: 12; range: 9-12 points).

Human Studies

One study from the total 43 studies reached the maximum possible score of 21 points [162]. The average score was 14.12 points ranging from a minimum of 6 to a maximum of 21 points. The median value was 14 points and the mode was 15 points. The mean score for reporting was 7.21 points (maximum: 9 points; range: 3-9 points), for external validity 0.65 points (maximum: 2; range: 0-2 points), for internal validity (bias) 4.02 (maximum: 5 points; range: 2-5 points), for internal validity (confounding) 1.88 (maximum: 4 points; range: 0-4 points), and for power 0.35 (maximum: 1 points; range: 0-1 points). The mean score for PE studies was 13 points (maximum: 20 points; range: 6-20 points), for nutritional supplementation studies was 14.68 points (maximum: 21 points; range: 10-21 points), and for combination studies was 16.33 points (maximum: 19; range: 15-19 points).
2.4 Discussion

2.4.1 Summary

The aim of this systematic review was to evaluate whether the combination of PE and nutritional supplementation has greater benefits (additive effects) on brain structure and function than their separate administrations. Studies measured cognitive functioning with the help of behavioral tests and associated parameters including metabolic and structural neuronal changes on brain level. In human trials, the combination of PE and nutritional supplementation did not lead to any additive effects. In rodents, four studies showed additive effects on different outcomes [77, 78, 112, 113]. Wu et al (2008) showed additive effects on behavioral level, on BDNF level, on synaptic protein levels, and on oxidative stress using a combination of running exercise and docosahexaenoic acid (DHA) supplementation [77]. Moreover, Chytrova et al (2010) found additive effects on synaptic protein levels using a combination of running and DHA supplementation. Cechella et al (2014a, b) found additive effects on behavioral level using a combination of swim training and selenium [112, 113].

The search strategy led to the identification of many articles with different brain function related outcomes. However, the studies that used a combined approach had a poor to moderate quality and were rather heterogenic which, in turn, led to difficulties comparing the results and performing a meta-analysis. The limited availability of high-quality prospective studies that used a combined approach warrants further targeted future research investigating the effects of combined approaches on the brain. Based on our findings, we will discuss and suggest directions for future research related to combined interventions with PE and nutritional supplements. Through this review, it became apparent that isolated interventions of either PE or nutrition were able to effect on brain in both mammals and humans. However, combinations of these components were not having an effect. It appears that many of the included studies have been using a “complementary” approach where the administration of PE and nutritional components were not combined with the intention to cause an effect on similar mechanisms. No studies explicitly used an “additive” approach with the aim to enhance effects of the combined physical-exercise-&-nutrition-approach because both components shared similar mechanisms. For elucidating this seemingly contradiction, we continue this discussion by considering the effects of studies applying either PE or nutritional supplementations with the aim to effect on brain. Practices of various interventions are discussed together with the underlying mechanisms that theoretically would explain the effects of the used intervention components.

2.4.2 Physical Exercise Interventions

Results from the three studies that looked at the effects of PE on cognition in rodents showed better performances in learning and memory abilities. This finding is in agreement with existing literature reviews suggesting that PE in rodents benefits cognitive functioning [13, 98]. Interestingly, other studies
showed that the PE component, especially forced PE, is able to activate neuronal brain metabolism [173, 174]. Furthermore, providing opportunities for PE is the critical element of environmental enrichment explaining the influence on neurogenesis [175]. The mechanisms by which PE improves cognition are, however, not yet fully understood. In the last couple of years, research seems to support the idea that PE affects cellular and molecular systems associated with synaptic plasticity and energy metabolism [98]. BDNF plays an essential role through the interaction with energy metabolism and growth factors (IGF-1) to influence downstream effectors mediating synaptic plasticity and neurogenesis, especially in the hippocampus [98]. The hippocampus is an important area for learning and memory [98]. In this systematic review, two studies found increased neurogenesis in the dentate gyrus of running mice [111, 130]. In the hippocampus of running mice, van der Borght et al (2007) found increased phosphorylated CREB levels, a downstream effector of BDNF [76].

In humans, aerobic exercise improved general cognitive functioning, EF, perceptual speed, and to some extent also memory. A meta-analytic review by Smith et al (2010) found that aerobic exercise led to modest improvements in attention and processing speed, EF, and memory but did not affect working memory [176]. In contrast, a systematic review by Van Uffelen et al (2008) found only weak evidence for better cognition after PE; only five out of fifteen studies showed significant improvements on some measures of cognition [177]. In addition, a meta-analysis by Colcombe et al (2003) investigated the effects of fitness interventions on cognitive functions [178]. They showed that the largest benefits of PE appear to be on EF. Hence, results from this systematic review and other reviews seem to support the idea that PE has the most reliable effects on EF. Moreover, one might speculate that improvements in tests measuring general cognitive functioning might have been originated from improvements in subtests assessing EF. Although, we do not know the sub-scores of the Mini Mental State Examination (MMSE), the study by Moul et al (1995) using the Ross Information Processing Assessment to assess general cognitive functioning supports this hypothesis [150]. The improved total score resulted from improvements in two attentional demanding tasks (organization and auditory processing). Differentiating the types of PE, three studies found positive effects on memory examined following strength training. Two studies used strength training [135, 146] and one study combined strength training with endurance exercise [149]. Moreover, resistance training improved general cognitive functioning [132] and EF [135, 142]. A review by Chang et al (2012) on resistance exercise and cognition suggests that resistance exercise improves cognitive functions including information processing speed, attention, memory formation, and EF [179]. Interestingly, participants who underwent coordination training improved in perceptual speed tasks [144, 148].

The evaluation of neurogenesis and brain growth factors concentration is limited in human studies. In the studies included in our systematic review, the authors used magnetic resonance imaging techniques to assess brain volumes. Erickson et al (2011) found that aerobic type of exercise was able to increase the hippocampus volume [139]. The results support the idea that PE induces neurogenesis in the
hippocampus. Furthermore, positive effects of aerobic type of exercise on brain volumes were found in the anterior cingulate cortex, the supplementary motor area, the right inferior frontal gyrus, the left superior temporal gyrus, and the anterior white matter [136, 147]. The brain regions have been associated with critical cognitive processes (prefrontal cortex) and memory (temporal lobes) [136]. Moreover, the prefrontal cortex is considered to play an important role in EF [180] and has been shown to be susceptible to aging [141]. In fact, Raz et al (2005) argue that the brain regions that are late to mature (i.e. frontal regions) are also the most vulnerable to cognitive decline [18]. In addition, cognitive aging seems to affect mainly tasks that require substantial mental effort and novel stimuli, such as EF, whereas semantic knowledge appears to be well preserved [15]. On the other hand, strength training decreased whole brain volume [142]. This finding seems paradoxical, since decreased brain volume is usually associated with impaired function [181]. However, Liu-Ambrose and colleagues concluded that this phenomenon needs to be further investigated [142]. Interestingly, participants who underwent coordination training increased globus pallidus and caudate volumes [144, 148]. The two sub-regions of the basal ganglia are involved in prefrontal cognitive processes such as planning and working memory [182]. Since the dorsal part of basal ganglia is involved in motor learning [144], it is not surprising that coordination training affected this area of the brain. This fact would also support the improvement in the perceptual speed task.

In an event-related potential study, latency of the P3 component has been attributed to information processing speed, attention, and working memory [183]. Ozkaya et al (2005) found better early sensory processing for the strength but not for their endurance training group [145]. Kamijo et al (2009) investigated the effects of one bout of aerobic exercise and found improved P3 latencies [141]. Two studies used functional magnetic resonance imaging to evaluate brain activation during a flanker task [137, 148], and they showed greater activity in attentional control areas and reduced activity in the anterior cingulate cortex (ACC) that could be attributed to more efficient information processing [148]. Colcombe et al (2004) argued that the successful completion of the incongruent flanker task requires activation of the frontal and parietal circuitry involved in spatial attention and a decreased activation of the ACC involved in response conflict [137].

With respect to blood markers, no increased serum BDNF or catecholamine levels were found after aerobic exercise. Erickson et al (2011) found no significant changes in BDNF levels, but they found that changes in BDNF levels correlate with changes in hippocampal volume [139]. However, Ruscheweyh et al (2011) also found no significant increase in BDNF levels after PE [147]. The authors argued that the absence of increased BDNF levels in the blood could be due to two reasons: (1) levels could tail off after approximately one month of training, (2) or cerebral BDNF levels might have been a better measure than blood levels to measure the impact of PE on this parameter, although BDNF has been shown to pass the blood brain barrier. However, one study showed that resistance training for six months increased serum IGF-1 levels [135], and this increase correlated with cognitive performance. The results
support the hypothesis that IGF-1 plays an important role in cognition. Thus, more studies are needed to clarify the effect of aerobic exercise and resistance exercise on serum IGF-1 levels and on BDNF levels, respectively.

Overall, the results suggest that different types of PE affect different cognitive domains through different mechanism. This is in line with previous research showing differing effects in the brain based on different exercise approaches. Where aerobic training increases activation in the sensorimotor network, coordination training leads to a higher activation of the visuospatial network [148], and strength training has the potential to change the hemodynamic activity of brain regions associated with response inhibition processes [184]. This is an indication that the types of physical training are likely to have task specific effects on the brain. Hence, combining aerobic exercise, strength training, and coordination training might be more beneficial for cognitive functioning than performing just one type of PE. This kind of combined intervention was also suggested by Kramer and colleagues [59]. A recent study indeed demonstrated that a multicomponent simultaneous cognitive-physical training program was able to boost particularly EF (including shifting attention and working memory) in healthy older adults compared to an exclusively physical multicomponent program [185], and that depending on the type of cognitive-physical training program applied differential training specific adaptations in brain function related walking parameters may be observed [186]. However, it seems important that the principles of exercise training are consistently followed and accurately reported for PE interventions [187]. Application of PE principles (specificity, overload, progression, initial values, reversibility, and diminishing returns) ensures that the dose and type of PE is planned to maximize the benefits for the recipients [187]. Such information was difficult to derive from the majority of studies included in this systematic review. It can be hypothesized that the lack of effect of PE on the brain in some of the reports is partly due to not considering the quantity and quality of the exercise needed to trigger responses in the brain.

2.4.3 Nutritional Supplementation

In rodent studies, four out of five studies showed positive effects on learning, and only one study showed positive effects on memory using CR. However, these results have to be interpreted with caution. First of all, CR has also been shown to have negative effects on cognition. CR impairs memory assessed with object recognition [127] and increases anxiety like behavior, probably due to increased cortisol levels [109]. Moreover, the study of Carter et al (2009) evaluated whether the beneficial effects of CR are attributed to increased physical activity [127]. They showed that CR rats had significantly higher activity levels than ad libitum (AL) fed rats. Moreover, the distance to reach the platform in the MWM task, which is not confounded by fitness, was the same in AL and CR rats. However, Kuhl et al (2013) found improvements in spatial learning and working memory in CR rats that moved less than AL rats [109]. Hence, the question whether CR has beneficial effects on cognition remains controversial. CR has often been used as an intervention because excess calorie intake might reduce synaptic plasticity through
increased oxidative stress and subsequent cell damage [74]. In mice and rats, Lee et al (2000, 2002) found that CR enhances neurogenesis by increasing survival of newly generated cells but not proliferation [110, 123]. This finding is interesting because PE and CR appear to control different mechanisms; PE increased newly generated cells in the hippocampus whereas CR promoted survival of cells in the hippocampus. The hypothesis that PE is the strongest neurogenic stimulus is also supported by a review by van Praag (2009) on exercise and the brain [13]. If PE and nutritional supplementation act differently on neurogenesis, their combined effects on neurogenesis could provoke additive results. However, a study by van Praag et al (1999) showed that running increased both cell proliferation and survival in the hippocampus of mice [188]. Furthermore, the confounding effect of physical activity in CR mice or rats cannot be fully excluded [110]. In addition to neurogenesis, Lee et al (2000, 2002) showed increased BDNF [110, 123] and NT-3 [110] levels in the hippocampus after CR [110]. They argued that this might mediate the positive effects of CR on neurogenesis [110]. These findings are in line with a review by Gomez-Pinilla (2008) on the effects of nutrients on brain function and a study by Duan et al (2001) on CR [74, 189]. Both studies suggested that CR increases BDNF levels and that this might mediate the effects on synaptic plasticity. Moreover, the authors illustrated that CR in rats was able to stabilize the decrease in key synaptic protein levels occurring with age. Again, these synaptic proteins are thought to be associated with synaptic plasticity in the hippocampus [126].

Studies that evaluated nutritional supplements showed no benefits for learning or memory using taurine or niacin [114, 120]. For example, Young et al (2007) showed that niacin supplementation worsened spatial learning ability, probably due to increased brain nicotinamide adenine dinucleotide and cyclic adenosine diphosphate ribose levels that facilitate long term depression and impair long term potentiation [120]. In addition, epinephrine supplementation did not lead to significant improvements in learning and memory [115]. On the other hand, DHA and diphenyl diselenide supplementation resulted in improved learning and memory [77, 78, 112, 113, 118, 121]. The finding is in agreement with a review by Su (2010) that illustrated the positive effects of DHA on learning and memory performance in rodents [190]. Results from this systematic review suggest that the effects of the diphenyl diselenide supplementation on learning and memory involve CREB phosphorylation without altering the levels of Akt [113].

In humans, vitamin and multivitamin supplementation did not seem to positively affect scores of cognitive tests. No evidence was found for EF, processing speed, attention, or intelligence. A systematic review and meta-analysis by Grima et al (2012) on the effects of multivitamin supplementation on cognitive performance revealed minimal benefits after vitamin supplementation [191]. They showed that only immediate free recall memory seemed to profit from vitamin supplementation but not the other cognitive domains. On the other hand, cross sectional studies show associations between vitamin status and cognitive functioning. For example, a recent systematic review showed that low vitamin D status is associated with lower outcomes in cognitive tests [192]. In addition, Cockle et al (2000) list many other
studies that showed associations between vitamin status and cognitive functioning, for example vitamin B12 and memory or folate and spatial copying ability [151]. Smith et al (1999) found no improvements in cognitive functions after multivitamin supplementation. However, a subgroup analysis revealed that individuals with low baseline levels of vitamin C improved in cognition after the supplementation [164]. We think that the discrepancy between cross sectional and interventional studies arises because studies in our systematic review investigated the possible causal effects of supplementation in healthy individuals without known vitamin deficiencies.

The other supplementation studies showed different effects on cognitive functioning in humans. L-carnitine or anserine plus carnosine improved MMSE scores. It is noteworthy that improvements were only seen in very old people. For example, Malaguarnera et al (2007) investigated the effects of L-carnitine on centenarians and Szczesniak et al (2014) found improved MMSE scores after anserine and carnosine supplementation only in people aged 81-94 but not in those aged 65-80 [78, 158]. In addition, participants from the study by Malaguarnera et al (2007) had very low baseline scores of the MMSE, averaging 16.5 points. Furthermore, fish oil together with lycopene and gingko biloba improved general cognitive functioning, memory, and processing speed and attention [169]. Other studies that investigated the effects of n-3 polyunsaturated FA on cognitive functions found no positive effects on cognition [193, 194]. McMorris et al (2007) found that creatine supplementation improved memory scores, but the study quality was rather low [159]. A higher ranked study (15 points) combined creatine with resistance exercise and reported no improvement in memory, EF, or MMSE in the creatine group [170]. In addition, nitrate supplementation showed no beneficial effects on cognition [156]. The inconsistent results of this systematic review limit the strength of the evidence that supports the intake of supplements on cognition.

Our search strategy detected no studies that evaluated the effects of nutritional supplementation on brain volumes or neurotrophin blood levels in humans due to the fact that the proposed mechanisms of supplements, especially of vitamin supplementation, usually involve antioxidant properties. Antioxidant foods have been claimed to favor cognition because of the susceptibility of the brain to oxidative damage [74]. However, in our systematic review few antioxidant supplements had positive effects on cognition. Reasons could be that participants were too healthy [151, 156]. In addition, antioxidant supplementation might protect against the deleterious effects of diets rich in saturated fats and sugars which have been shown to increase oxidative stress and decrease hippocampal BDNF levels [76]. Overall, results from vitamin studies seem to support the idea that vitamin supplementation is beneficial for cognition only in participants with low baseline vitamins status.

Two studies hypothesized that nitrate would be converted to nitric oxide that results in a vasodilation and consequently increases blood flow to the brain [156, 161]. However, Kelly et al (2013) found no positive effect on any of the measured outcomes (cognitive tests, apparent diffusion coefficients, and brain metabolite concentrations) [156]. Furthermore, Presley et al (2011) found no differences in global perfusion [161]. Both studies used very short intervention periods of two to two-and-a-half days. Hence,
studies with longer nitrate supplementation periods seem necessary to evaluate long term effects on brain perfusion and cognition [156].

The search strategy did not yield studies that investigated the effects of CR in elderly humans. However, hand searching yielded a study that showed beneficial effects of CR (30% reduction) on memory performance in healthy elderly [195]. Higher synaptic plasticity and stimulation of neurofacilitatory pathways might be due to improved insulin sensitivity and reduced inflammatory activity.

2.4.4 Combination of Physical Exercise and Nutritional Supplementation

In rodent studies, DHA or diphenyl diselenide in combination with PE evoked additive effects [77, 78, 112, 113]. DHA is a dietary omega-3 fatty acid and has the potential to affect synaptic plasticity and cognition [74, 76]. A review on brain foods described why DHA is important for cognition and brain health: DHA constitutes more than 30% of phospholipids of plasma membranes of neurons, and thus plays a crucial role for synaptic function [74]. More importantly, DHA can affect molecules such as BDNF and IGF-1 which in turn can activate signaling systems such as mitogen-activated protein kinase, CaMKII, and phosphoinositide 3-kinase/Akt/mammalian target of rapamycin [74]. Therefore, DHA seems to have the potential to facilitate synaptic transmission, to modulate synaptic plasticity and cognitive function, and to support long term potentiation which is associated with learning and memory [74]. Interestingly, the effects of PE seem to depend on similar mechanisms involving BDNF mediated synaptic plasticity and energy homeostasis [76]. Thus, DHA supplementation could complement the actions of PE resulting in an effective strategy to counteract cognitive decline [76]. In both studies, the combination had greater effects on hippocampal BDNF levels, synaptic protein and signaling molecules levels, on proteins involved in metabolic homeostasis, and on oxidative stress [77, 78]. However, the positive effects were only seen in two studies [77, 78] which used an identical design: 12 days of 1.25% DHA supplementation with or without free access to a running wheel on 24 Sprague-Dawley rats. Both studies using diphenyl diselenide and swimming expected increased CREB level as mediator for improved memory [112, 113]. As the CREB levels did not change, the question about the underlying neurobiological mechanism remains to be elucidated.

In humans, none of the three studies that combined PE and nutritional supplementation showed additive effects [170-172]. The reason for no additive effects might be that the combination of intervention components was not explicitly selected based on a shared mechanism and, therefore, evoked complementary effects at best. This means that the chosen single components (PE or nutritional intervention) of the combined administration act not on the same neurobiological cascade to produce additive effects.

To evoke possible additive effects, three items seemingly should be taken into account: (1) training principles to ensure quality and quantity of the exercise component, (2) dose and duration of diet or nutritional supplementation, and (3) the selected nutritional component(s) and PE should act on the same
neurobiological cascade. The results can be interpreted in the sense that so far there seems to be a mismatch in many studies between the exercise program offered and the nutritional supplements given. It seems reasonable to assume that the nutritional supplements should be selected based on the theoretical effect they have on the brain; e.g. they preferably should share similar mechanisms with exercise [76] and, thus, theoretically have the potential to complement the action of exercise. Possible additive effects might be achieved, if both components (PE and nutrition) act complementary on the same molecular mechanisms. Other influencing factors are the genetic component and the living environment that are very individual in humans, but more or less identical in rodents. In humans, the individual genetic variability influences individual response to nutritional intervention [196].

2.4.5 Strengths and Limitations

Review

The standards of reporting animal experiments lag behind those of human RCTs [197], which is a potential concern for bias. Furthermore, publication bias related to overstatement of efficacy may negatively affect the interpretation of animal studies [198]. A further limitation relates to the focus on older animals and humans. The precise correlation between the age of rodents and humans is subject of debate implying, when age is an important factor, differences between animals and humans should be taken into consideration [199].

For the human trials, the majority of included studies resulted from the reference list search. Hence, we cannot guarantee that all studies examining nutritional supplementation and PE on cognition in healthy elderly are included in this systematic review. Moreover, we performed no gray literature search, and thus cannot guarantee that there was no publication bias. Another limitation is that the included studies are very heterogeneous regarding included participants, interventional design, and outcomes. This heterogeneity hindered a meta-analytical approach which would have been a more objective way to quantify the results.

Individual Studies

Generally, the included studies in this systematic review were of good quality. In the quality evaluation of rodent studies, the question assessing power had a low average score because it was often not possible to be determined (the results were displayed in graphs and not tabulated). Question 25 addressing confounding averaged only 0.58 points due to insufficient information regarding the number of rodents that were used for the analysis. The small differences in scores between studies might be explained through the very similar study designs. However, in human studies quality scores varied much more, probably due to heterogeneous study designs. Moreover, it is important to keep in mind that quality scores rely upon the quality of reporting rather than the quality of the actual study conduct [105].
Furthermore, the number of cognitive tests used in human studies is huge, and almost every study used different tests. Moreover, it was common that some authors used the same specific test but evaluated different cognitive domains. In other words, no standardized way is present to evaluate cognitive functioning. In addition, it can be argued that the applied tests were not always appropriate causing a suspected misfit between the targets of the intervention and the used (un)specific outcome tests. For example, many authors used the MMSE to evaluate general cognitive functioning. However, the MMSE is a diagnostic tool not designed to measure change or improvement and might, therefore, suffer from ceiling effects [165]. In animals, the MWM was often used to evaluate learning and memory. However, Fitting et al (2008) suggested that the improvements in latency after CR were due to preservation of motor function and not due to cognition [128]. In addition, Jacotte-Simancas et al (2013) argued that exercised animals might perform better than sedentary animals in the MWM because they cope better with the physical effort and stress generated by the task [115]. Hence, motor fitness could be a confounding factor for cognitive performance evaluation in the MWM. An additional possible limitation of the studies with rodents relates to the gender distribution of the investigated animals. The fast majority of the animal studies identified through the systematic literature search were carried out on male animals only. From human studies we know that some brain related impairments affect women more than men; e.g. sex disparity in stroke prevalence persists with women being more affected then men [200]. It seems important that future studies test interventions in both sexes.

### 2.4.6 Conclusion

In healthy elderly humans, no additive effects were identified for nutritional supplementation and PE. In rodents, DHA and PE or selenium and PE resulted in additive effects on learning and on neurobiological measures. The main interventions that resulted in improved cognition or associated parameters were aerobic type of exercise, strength training, coordination training, CR, and DHA supplementation. It can, thus, be speculated that a combination of these interventions might provide better cognitive outcomes than just their sole-administration. More research is needed examining the possible additive effects of PE and nutritional intervention in humans. This systematic review reveals that applications of targeted exercise in combination with nutritional supplements with the aim to effect on the brain are still at a fledgling stage. There are, however, interesting first results in rodent studies that encourage further work in this field and which hold promise for utilizing the combined exercise-nutrition approach as a therapeutic tool.

### 2.4.7 Future Direction

A central element of successful cognitive rehabilitation for older adults should be the design of interventions that either re-activate disused or damaged brain regions, or that compensates for decline in parts of the brain through the activation of compensatory neural reserves [201]. Based on the results of the systematic review, we would design further combinatorial studies as follows: Based on the findings
of this review the nutritional supplements should be selected such that they share similar mechanisms with exercise and, thus, theoretically have the potential to support the action of exercise. PE would base on a combination of aerobic and strength exercise that also includes a cognitive component. Considering the cognitive part, previous research suggests a focus on executive functioning processes including enriched environments that provide physical activities with decision-making opportunities because these are believed to be able to facilitate the development of both motor performance and brain functions [66]. The use of virtual reality environments for virtual augmented exercise has recently been proposed as having the potential to increase exercise behavior in older adults in combination with the potential to influence cognitive abilities [202]. At present there is evidence that specific types of video games are able altering brain structure [203] and function [204] and, when added to a multicomponent exercise program, improve certain aspects of cognitive functioning [185, 186]. Future research should develop, implement and evaluate for example virtual reality based training scenarios that allows the combination of aerobic and strength exercises together with cognition. Moreover, video games allow the implementation of FITT (Frequency, Intensity, Type, and Time) training principles to ensure that the dose and type of PE is planned to maximize the benefits for the recipients. For nutrition, a diet including omega-3 FA, is assumed to have the potential to affect synaptic plasticity and cognition. A previous study performed in humans showed beneficial effects on cognitive functioning and memory [81]. Furthermore, one study investigated the effects of CR in healthy elderly humans [195]. In rodents, CR showed positive effects on brain function, but CR studies should be controlled for the confounding factor of increased physical activity. In future studies, authors should agree upon a standardized set of tests in order to compare the results between studies, since there is a myriad of tasks that have been proposed to evaluate cognitive functioning. However, physical activity and nutrition are closely linked together, and positive effects of PE might be confounded by better nutrition. Controlling for this factor appears necessary, if one wants to evaluate the additive effects of PE and nutrition on cognition.

**Author contribution:** AS, KB, and JS developed the research question under the lead of PW and ED. The concept and design part was established by AS, KB, and JS while PW and ED acted as methodological councils. AS, KB, and JS did articles acquisition as well as analysis and interpretation of the articles which was edited and improved by PW and ED. AS and JS produced an early version of the manuscript. KB, PW, and ED substantially revised the manuscript to bring it to its current version. All authors have read and approved the final manuscript.

**Acknowledgements:** The authors would like to thank Dr. Martina Gosteli of the Q8 Medicinal Library of the University of Zurich for her help in elaborating the search strategy. This article was supported by the ETH Foundation through ETH Research Grant ETH-17 13-2.

**Conflict of interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

**Supplementary material:** The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2016.00161.
Adaptations of Prefrontal Brain Activity, Executive Functions, and Gait in Healthy Elderly Following Exergame and Balance Training: A Randomized-Controlled Study

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Keywords: elderly, exergame, balance, executive function, gait performance, prefrontal brain activity

Published in: Frontiers in Aging Neuroscience. 2016, 8 (278)

DOI: 10.3389/fnagi.2016.00278

1Figure, tables, and language errors in the original publication were adapted/corrected for this thesis
Abstract

During aging, the prefrontal cortex (PFC) undergoes age-dependent neuronal changes influencing cognitive and motor functions. Motor learning interventions are hypothesized to ameliorate motor and cognitive deficits in older adults. Especially, video game-based physical exercise might have the potential to train motor in combination with cognitive abilities in older adults. The aim of this study was to compare conventional balance training with video game-based physical exercise, a so-called exergame, on the relative power (RP) of electroencephalographic (EEG) frequencies over the PFC, executive function (EF), and gait performance. Twenty-seven participants (mean age 79.2 ± 7.3 years) were randomly assigned to one of two groups. All participants completed 24 trainings including three times a 30min session/week. The EEG measurements showed that theta RP significantly decreased in favor of the exergame group [L_(14) = 6.23, p = 0.007]. Comparing pre- vs. post-test, EFs improved both within the exergame (working memory: \( z = -2.28, p = 0.021 \); divided attention auditory: \( z = -2.51, p = 0.009 \); divided attention visual: \( z = -2.06, p = 0.040 \); Go/No-go: \( z = -2.55, p = 0.008 \); set-shifting: \( z = -2.90, p = 0.002 \)) and within the balance group (set-shifting: \( z = -2.04, p = 0.042 \)). Moreover, spatio-temporal gait parameters primarily improved within the exergame group under dual-task conditions (speed normal walking: \( z = -2.90, p = 0.002 \); speed fast walking: \( z = -2.97, p = 0.001 \); cadence normal walking: \( z = -2.97, p = 0.001 \); stride length fast walking: \( z = -2.69, p = 0.005 \)) and within the balance group under single-task conditions (speed normal walking: \( z = -2.54, p = 0.009 \); speed fast walking: \( z = -1.98, p = 0.049 \); cadence normal walking: \( z = -2.79, p = 0.003 \)). These results indicate that exergame training as well as balance training positively influence PFC activity and/or function in varying proportion.
3.1 Introduction

The human brain undergoes age-dependent changes that affect motor and cognitive performances of daily living of older adults. The brain aging process is associated with neuroanatomical changes, such as loss of brain tissue and white matter disconnections [15, 18, 19], decrease in synthesis and binding of dopamine, serotonin, and acetylcholine [23-27], metabolic changes including mitochondrial dysfunction [28-30], and changes in neuronal activity pattern [21]. Especially the (pre)frontal lobe is vulnerable to age-related degeneration as shown in cross-sectional [31-33] and longitudinal magnetic resonance imaging studies [34, 35]. Prefrontal volume and thickness are associated with executive function (EF) [38]. EFs are “higher-level” cognitive abilities that control and regulate “lower-level” cognitive processes and goal-directed actions [42, 43]. An interaction of different EF components, e.g. “working memory” [44], “divided attention” [45], and “inhibition” [46], is needed for gait performance. Reduced gait stability and postural control during dual-task walking suggest that cognitive abilities may affect gait performance [7, 9]. Therefore, age-associated reduction of EFs can impact gait and amplify the risk of falling [50]. The assumption is supported by recent work showing that a smaller volume of the prefrontal area is likely to contribute to slower gait speed through slower information processing [54]. A greater reliance on cognitive control for motor tasks makes structural differences in the prefrontal cortex interesting from the perspective of age-related decrements in motor control [51]. Moreover, disturbances in cortico-cortical and cortico-subcortical connections, e.g. frontal connections with parietal lobe or with basal ganglia, respectively, are classified as higher-level gait disorders [49, 52].

To improve motor and cognitive performance in elderly, it can be hypothesized that a physical training that also targets EFs might be an important component [50, 64]. In aging humans, physical exercise (PE) can strengthen neuronal structures, synaptic plasticity, and cognitive functions [11]. Therefore, PE might be a trigger to support molecular and cellular mechanisms for brain plasticity [57, 59]. Traditional PE programs of elderly are usually associated with conventional balance exercises. Balance training has been shown to be beneficial for maintenance and improvement of motor performance through changes on subcortical structures [205]. Balance training performed with the help of video games that incorporate stepping exercises are effective in reducing falls next to improving gait and balance [72]. Furthermore, compared to conventional balance training this type of training differently modulates prefrontal brain activity and EFs [204]. It seems important, in this context, that interventions provide physical activity with decision-making opportunities because these are believed to facilitate the improvement of both motor performance and cognitive function [66].

Video game-based training serves as a powerful tool to modulate neural networks and to evaluate underlying neuronal mechanisms [60]. Moreover, video games seem to have the potential to train cognitive functions [61] including reaction time (RT), processing speed, attention, and EFs [62, 63]. A systematic review concluded that video games are promising for improving cognitive abilities in older adults who have a higher risk of cognitive decline [63]. An interactive exergame challenges (divided)
attention; participants observe cues on a frontal screen and concurrently execute well-coordinated movements. Furthermore, the combined training of physical and cognitive functions leads to better cognitive function and general functional status in older adults [67]. Two recent reviews, focusing on the interplay between physical function and cognition, concluded that it seems important to combine motor and cognitive exercises into clinical practice to enable older adults to move safer in their physical environment [64, 65]. Especially, computerized interventions seem promising [7, 71], in particular, when they consider training principles that enhance (motor) learning [71]; e.g. direct feedback on performance and rewards for correct responses in the video game-based training scenario.

Despite existing evidence that the older brain shows adaptations associated with motor-cognitive training, the type of training that best promotes these adaptations remains to be elucidated. The aim of this study was, therefore, to compare exergame training with conventional balance training focusing on prefrontal brain activity, EFs, and gait performance. We hypothesized that a combined motor and cognitive training would differently affect prefrontal brain activity, EFs, and spatio-temporal gait parameters when compared to more conventional motor training.

### 3.2 Materials and Methods

#### 3.2.1 Study Design and Participants

The study was a randomized controlled trial (RCT) including two parallel running intervention groups. From May through June 2015, potential participants were recruited through public advertisements in local newspapers, on the homepage of the Senior University Zurich (Switzerland), pensioner community ETH Zurich (Switzerland), and in local senior residency dwellings (Zurich, Switzerland). Measurements and interventions were performed in a senior residence dwelling in Zurich (Möhrlistrasse, 8006 Zurich). The intervention started in two blocks, one block started in the middle of June 2015 and the second at the beginning of July 2015. Study measurements were performed before and after the intervention. The ethics committee of the ETH Zurich, Switzerland (EK 2015-N-10) approved the study protocol. The study is registered at Current Controlled Trials under ISRCTN73384012 (http://www.isrctn.com). Before any measurements were performed, all eligible participants had to sign written informed consent according to the Declaration of Helsinki. CONSORT 2010 guidelines were used for the reporting of this parallel-group RCT [206].

The potential participants were screened using the Mini Mental State Examination (MMSE) to assess cognitive status and the Geriatric Depression Scale (GDS) to screen for depression. Furthermore, the participants completed a health questionnaire including questions about physical impairments, medical history, anthropometric data, and physical activity level. Participants fulfilling all of the following inclusion criteria were eligible for the study: (1) age ≥ 65 years, (2) live independently or in a senior residency dwelling, (3) non-smoker, (4) healthy (self-reported), (5) able to walk at least 20 meters with
or without walking aids. Participants were excluded from the study in case they exhibit one of the following exclusion criteria: (1) mobility impairments, (2) severe health problems (e.g. recent cardiac infarction, uncontrolled diabetes, or uncontrolled hypertension), (3) orthopedic or neurological diseases that prevent training participation, (4) Alzheimer disease or dementia, (5) rapidly progressive or terminal illness, (6) acute or chronic illness, (7) history of stroke, (8) history of dizziness or individuals with a recent head injury, (9) medications that act on neuronal level (e.g. Psychotropic medications), (10) cognitive impairments (MMSE < 22 points), (11) signs of an upcoming depression.

The intended study size of 30 to 40 participants was based on similar training studies [64, 207]. For study group randomization, a randomization program was used (www.randomization.com). With the specification of two intervention groups and the intended number of participants, the program automatically generated a list including exergame and balance training in a randomized and balanced fashion and a block size of two. The participant assignment started at one and was hence continuously filled-up according to the chronological measurement protocol. The random allocation sequence was performed by a study investigator while the participant enrollment and assignment was done by another study investigator. Blinding of the supervisors was not possible, because they performed the measurements and the intervention. Participants were blinded to the expected study outcome.

### 3.2.1 Training Interventions

In the period from June 2015 to September 2015, the participants performed 30min sessions three times per week on separate days. The trainings were scheduled individually from Monday to Friday with certain regularity for each week and a guideline of no more than one training per day. The 24 training sessions were distributed within a period of eight to ten weeks, while a maximum of two weeks holiday interruption was allowed. Each training session included a warm-up (5min), a training (20min), and a cool-down phase (5min). Three postgraduate students instructed the participants and ensured that training principles of progression and overload were present in both groups [187]. Training intensity was individually adapted to achieve a moderate to vigorous training level [208]. The time frame and training intensity was based on studies illustrating positive training effects in older adults performing a video game on a dance plate [64, 67, 204] and on current recommendations for achieving physical fitness and fall prevention in elderly [208]. The participants trained in groups to enhance motivation and motor learning [67].
Exergame Training

The exergame group performed a motor-cognitive training including an interactive video game-based PE. On a pressure sensitive plate (Impact Dance Platform, 87.5cm×87.5cm×2.5cm, Positive Gaming BV, BZ Haarlem, Nederland) the participants performed specific whole-body movements driven by a video game presented on a frontal screen. Electronic sensors, on the platform, detected position and timing information that were then used to provide participants with real-time visual feedback. Through foot pushes on the plate arrows (right, left, top, and bottom), the participants interacted in the gaming interface. The dance platform was connected by USB to a desktop computer and to a beamer projecting the video game on a wall.

The exergame allowed the implementation of training principles as previously described [209]; a feedback system to facilitate training, offering individual difficulty zones to catch each participant’s training level, individually adjusted task difficulty to facilitate retention and offering variability of training to enhance task transfer. The participants played four different games developed and provided by dividat (Schindellegi, Switzerland) (Table 3.1). The games specifically train EFs that are (1) associated with the prefrontal brain area [38], (2) are important for the control and regulation of “lower-level” cognitive processes and goal-directed actions [42, 43], and (3) can impact on gait and amplify the risk of falling [50]. Each game included different difficulty levels to ensure adaption and progression to the abilities of each individual. Furthermore, each game was accentuated with music. One session included six to seven rounds while one round of a game lasted about three minutes. The intervention started with “Balloon” and “Step” and was extended with “Space” and “Season” in case “Balloon” and “Step” were mastered by an individual during the intervention period. The participants trained 18 to 21 minutes including individual break sessions.

**TABLE 3.1 | Video game description.**

<table>
<thead>
<tr>
<th>Video game</th>
<th>Task</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balloon</td>
<td>If a balloon meets one of the four circles, the participant has to push the corresponding arrow on the plate. The balloons come from random sides of the game play screen and move towards one of the four circles. The balloons may exhibit different colors and the participant has to inhibit moving, in case the balloon exhibits the “wrong” color.</td>
</tr>
<tr>
<td>Step</td>
<td>If an object meets one of four target object points (corresponding to front, back, left, and right arrows), the participant has to push the relevant arrow direction on the plate. The objects appear in the middle of the play screen and run to one of the four target objects. The participant has to inhibit moving when the shape of the appearing objects (e.g. triangles) is incompatible with the four target objects (e.g. squares).</td>
</tr>
<tr>
<td>Season</td>
<td>Participants view four different scenes (four seasons). Each of the four scenes corresponds to one arrow direction of the plate. If within a scene a moving animation (e.g. bird) appears, the participant has to push the corresponding arrow on the plate as quickly as possible.</td>
</tr>
<tr>
<td>Space</td>
<td>The game is based on the rules of the tile-matching video game Tetris. The participant arranges the tile objects using the relevant arrow directions on the plate.</td>
</tr>
</tbody>
</table>
Balance Training

The balance group trained conventional balance training. Participants performed repetitive static and dynamic exercises on stable and unstable surfaces to challenge their balance. To ensure a progression in difficulty levels, the exercises were increased in difficulty by requiring exercise performance in either bipedal or monopedal stance positions, by opening or closing the eyes, and through causing external perturbations or using a moving base of support.

3.2.2 Measurement of Spatio-Temporal Gait Parameters

Temporal and spatial gait parameters were measured with the Physilog (Gait up Sàrl, Lausanne, Switzerland) via wearable standalone movement sensors (50×37×9.2mm, 19grams, anatomical curved shape) containing inertial sensors. The focus of this study was on gait speed, cadence, and stride length. The validity of the Physilog has been previously established [210-212]. At the right and left forefoot, the sensors were fixed with elastic straps for flat over ground gait analysis. A button on the sensors was used for the start and stop of measurements. For further analysis, a micro-USB port allowed data transfer to the computer.

Over a 10m walkway, participants performed a single-task walking condition (both preferred and fast gait speed) and a dual-task walking condition, i.e. preferred and fast gait speed whilst counting backwards in steps of seven. Application of the dual-task paradigm aims to quantify the automaticity of movement [213-215]. For the single-task, participants were instructed to position themselves at the beginning of the walkway and were asked to walk with their comfortable self-paced (or fast) speed. For the dual-task, the participants got a random starting number from which they had to count backwards in steps of seven while walking with their comfortable self-paced (or fast) speed. The instructions were as follows: (1) “Walk with your comfortable preferred/fast speed right to the end of the walkway.” (2) “Walk with your comfortable preferred/fast speed right to the end of the walkway counting backwards from [223, 238, and 245].” The participants had to count loud and regularly; otherwise, the trial was recorded as failure. The participants performed the following procedure: (1) self-paced single-task walking, (2) self-paced dual-task walking, (3) fast single-task walking, and (4) fast dual-task walking. Each tested condition was repeated three successful times to obtain representative samples. The means out of the three successful runs and from the left and right foot were used for further data analysis. To analyze steady state walking, acceleration and deceleration steps were removed from the data and not counted.
3.2.3 Measurement of Executive Functions

Participants performed four tests from the test battery Test for Attentional Performance (TAP) (PSYTEST, Psychologische Testsysteme, Herzogenrath) testing different forms of EFs. The selected tests are geared to the component factors underlying EFs: inhibition and switching, working memory, and (selective) attention [42, 216]. The TAP is valid as the subtests measure different and statistically independent attentional and executive aspects [217]. On a personal computer, the participants performed the following test procedure: (1) working memory (5min): The software presented two-digit numbers. The participant compared presented numbers with previously exposed numbers and had to push the button when the presented number is the same as the penultimate number; (2) set-shifting/ flexibility task (minimum 1min and 45sec): The software presented a letter and a number – one on the left and one on the right side of the computer screen in a randomized order. The participant had to react on the target stimulus (e.g. “letter” – “number” – “letter”...) pushing the right (appearance on the right side) or left (appearance on the left side) button. The button press triggered the presentation of the next stimulus; (3) divided attention (3min 25s): The software concurrently presented visual and acoustic signals. In a 4x4 matrix, the visual task consisted of crosses appearing in a random configuration. The participant had to push the button when the crosses formed the corners of a square. The acoustic part consisted of low and high beeps playing in a randomized sequence. The participant had to push the button when a sequence of two similar tones appeared, e.g. low-low or high-high, respectively; (4) Go/No-go/ inhibition task (2min): The software randomly presented stimulus (x) or (+). The participant had to push the button in the presence of the key stimulus (x) and had to inhibit the push in the presence of the non-key stimulus (+). For each test, the program stored the RT. To avoid learning effects each test was preceded by a short pre-test version to familiarize with the test.

3.2.4 Measurement of Prefrontal Brain Activity

Electroencephalography Procedure

A portable electroencephalography (EEG) device (HeadCoach™, Alpha-Active Ltd, Devon, UK) was used in order to record scalp electro-voltage activity (sampling frequency: 128 Hz, band-pass hardware filter: 1Hz to 32Hz) from two active (positive inputs) electrodes (Ambu® White Sensor, Ambu A/S, Denmark), placed on Fp1 and Fp2 according to the 10-20 EEG system [218]. Three additional electrodes were used: a passive Driven-Right-Leg (DRL) reference electrode [218] and two active (negative inputs) Common Mode Sense (CMS) electrodes, placed at Fpz and M1/M2 (mastoid process), respectively. DRL and CMS electrodes were used in order to reduce participant’s electromagnetic interference and to improve the common-mode rejection ratio of the two recording positive inputs channels. Before electrode placement, the skin was prepared with an abrasive skin preparation gel (Nuprep™, Weaver and Company, Aurora, USA) and then cleaned with an alcohol-free wipe.
The brain signals acquisition was carried out during the divided attention task of the TAP that included the simultaneous presentation of visual and auditory stimuli (see description in paragraph measurement of EFs). EEG measurements were time-locked to both auditory and visual stimuli of the correct responses (visual: 17 correct responses out of 100 total stimuli; auditory: 16 correct responses out of 200 total stimuli). EEG analysis was triggered with the stimulus onset from the cognitive test, in both auditory and visual stimuli, using the following naïve approach: 1) the onset of the first auditory stimulus was determined and recorded using the .wav file (produced by the EEG software and chronological to the EEG data file), 2) the onset of the first visual stimulus, as well as all the subsequent auditory and visual stimuli were determined calculating the time lasting from the first auditory stimuli and the defined time intervals between the onset of either auditory and visual stimuli (as defined by the TAP software).

Moreover, the protocol included the measurement of brain signals during gait performance. The measurements were performed concurrent with spatio-temporal gait measurements (see description in paragraph measurement of spatio-temporal gait parameters). The EEG measurement was controlled by pushing the start button when participants started to walk and by pushing the stop button when participants stopped to walk. For each run and condition, the same EEG procedure was performed. To enable the measurement during walking, an extension cable was used to connect the EEG system with the personal computer.

**Data Processing**

Off-line signal processing was performed using a custom written script and both EEGLAB [219] and ERPLAB [220] toolboxes for MATLAB (Mathworks, Natick, MA). To facilitate further EEG analysis, raw signals were first resampled to 100 Hz, and subsequently time-locked to either auditory or visual stimuli in 4s epochs (-1500 ms pre- and 2500 ms post-stimulus onset), which were analyzed separately. Then, EEG epochs containing artifacts (e.g. ocular movements and eye blinks) were automatically rejected using an absolute voltage threshold criterion (epochs rejected when peaks amplitude ≥ ± 200 µV).

EEG spectral power was calculated through Power Spectrum Density (PSD) extraction, using the pwelch method [221] implemented in the DK_PSD function [222]. The following frequency bands were determined a priori and then extracted: total bandwidth (1-30 Hz), delta (1 - 3.5 Hz), lower theta (3.5 – 5.5 Hz), upper theta (5.5 – 7.5 Hz), lower alpha (7.5 – 10 Hz), upper alpha (10 – 12.5 Hz), and beta (12.5 – 30 Hz). All the further EEG analysis was performed clustering Fp1 and Fp2 channels. RP density was computed for each frequency band in each participant as previously described [223, 224], using the following equation: RP(λ) = \( \frac{AP(\lambda)}{MP(TB)} \) where RP = relative power, λ = frequency band, AP = absolute power peak, MP = mean power spectra, TB = total bandwidth. RP values were then natural-log normalized. The analysis of event-related potentials was not performed because of the naïve triggering approach and the use of a low-density EEG device.
3.2.5 Fear of Falling, Cognitive Status, and Depression

The short falls efficacy scale international (FES-I) was used to measure concern about falling through the combination of phrases and matching pictures. The scale assesses both easy and difficult physical and social activities and contains seven items with a 4-point scale (1 = not at all concerned, 2 = somewhat concerned, 3 = fairly concerned, 4 = very concerned). The short FES-I is a feasible scale to assess fear of falling in elderly [225].

The cognitive status was determined using the MMSE, a reliable and valid test to quantitatively estimate severity of cognitive impairments [226, 227]. The test has a maximal score of 30 points and is categorized into seven categories: 1. orientation to time, 2. orientation to place, 3. registration of three words, 4. attention and calculation, 5. recall of three words, 6. language, and 7. visual construction.

The GDS, for valid and reliable depression screening [228], was used to identify depression status in older adults. The short form has 15 questions focusing on worries of the individual, and the way they conceive and interpret their quality of life [229]. The self-report questionnaire can be answered with yes/no responses.

3.2.6 Statistical Analysis

Statistical Package for Social Sciences 22.0 for Windows (SPSS, Inc; Chicago, Illinois) was used for all statistical analyses. A per protocol analysis was performed. Normality of the sample was tested by Shapiro-Wilk. Given that the assumption of normality was not met, the data were rank-ordered in order to perform, for each variable, a two-way repeated measures analysis of variance (ANOVA) with one within-subjects factor (time: pre-test/post-test) and one between-subjects factor (intervention group: balance/exergame). This allows to compare main effects of time and time × group interaction effects, using the Puri and Sen L Statistics for ranked data [230]. L value was calculated using Pillai’s Trace. Post Hoc analyses to determine differences in time (within-group) were performed using the Wilcoxon signed-rank test. Baseline (pretest) comparisons were undertaken using Mann-Whitney U test. A p-value ≤ 0.05 was considered significant. Effect sizes assessing meaningfulness of differences within-group design were calculated and expressed using the following equation: \( r = \frac{Z}{\sqrt{N}} \) where \( Z \) = Z-score and \( N \) = amount of participants. An effect size of \( r = 0.1 \) is considered a “small” effect, around \( 0.3 \) a “medium” effect, and \( 0.5 \) and above a “large” effect. Effects sizes assessing differences between × within group design were calculated and expressed as \( \eta^2 \) where percentage of the total variance can be accounted for by group membership [231], using the following equation: \( \eta^2 = \frac{SS_{effect}}{SS_{effect} + SS_{error} + SS_{error within}} \) where SS = sum of squares.
3.3 Results

A total of 29 older adults were randomized into the two groups: exergame and balance training. 27 participants completed the whole training procedure with each participant completing all 24 scheduled training sessions. The study flow chart is presented in figure 3.1. The analysis does not consider intention-to-treat analysis because of a clear description of the reason(s) for drop-out (CONSORT 2010 guidelines [206]). Two participants were excluded from analysis for not completing the study. Drop-out reasons (one personal and one health issue) were not associated with the intervention. Table 3.2 summarizes demographic data of the participants.

FIGURE 3.1 | Study flow chart. Participants were randomly assigned to either EXERGAME or BALANCE group. Both groups trained 24 times within a period of 8 to 10 weeks. The screening included a health questionnaire, geriatric depression scale, mini mental status examination, and short falls efficacy scale international. Prefrontal brain activity, executive functions, and spatio-temporal gait parameters were assessed before and after the intervention.
TABLE 3.2 | Demographic characteristics and screening values.

<table>
<thead>
<tr>
<th></th>
<th>Exergame group</th>
<th>Balance group</th>
<th>p (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female/male)</td>
<td>5/8</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>80 (73; 83)</td>
<td>80 (72.25; 81.75)</td>
<td>.685</td>
</tr>
<tr>
<td>Mini mental status examination</td>
<td>29 (29; 30)</td>
<td>28.5 (27; 29)</td>
<td>.259</td>
</tr>
<tr>
<td>Geriatric depression scale</td>
<td>1 (0; 2)</td>
<td>2.5 (1; 4.75)</td>
<td>.085</td>
</tr>
<tr>
<td>Short falls efficacy scale international</td>
<td>7 (7; 8)</td>
<td>8.5 (7; 10)</td>
<td>.076</td>
</tr>
</tbody>
</table>

Data are number of participants or median (interquartile range) values as indicated. p ≤ 0.05.

3.3.1 Prefrontal Brain Activity

For the analysis, some participants had to be excluded due to technical problems (the included number of participants is illustrated in tables 3.3 and 3.4). The total amount of epochs, used for analysis, were for the exergame group pre (N = 147), post (N = 161) and for the balance training group pre (N = 107), post (N = 122). In every participant, eight epochs had to be excluded because epoch time windows overlapped. Table 3.3 summarizes the interaction effects for RP values separately for both auditory and visual stimuli. Significant time × group interaction effect was present for theta RP (auditory stimuli) (L(14) = 6.230, p = 0.007). The results of the pre- vs. post-tests comparisons are presented in table 3.4. The exergame group significantly decreased theta RP (auditory stimuli) (z = -2.37, p = 0.016). The naïve triggering modalities and the low-density EEG device allowed no analysis of the data during gait performance.
TABLE 3.3 | Interaction effects (time × intervention) of repeated measures analyses of ranked data for relative power values.

<table>
<thead>
<tr>
<th>Auditory stimuli</th>
<th>Pillai’s trace $r^2$</th>
<th>$L = (N-1)r^2$</th>
<th>$p$</th>
<th>$\eta_G^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>0.218</td>
<td>3.052</td>
<td>.079</td>
<td>0.01</td>
</tr>
<tr>
<td>Theta</td>
<td>0.445</td>
<td>6.230</td>
<td>.007**</td>
<td>0.02</td>
</tr>
<tr>
<td>Alpha low</td>
<td>0.216</td>
<td>3.024</td>
<td>.081</td>
<td>0.08</td>
</tr>
<tr>
<td>Alpha high</td>
<td>0.223</td>
<td>3.122</td>
<td>.076</td>
<td>0.00</td>
</tr>
<tr>
<td>Beta</td>
<td>0.125</td>
<td>1.750</td>
<td>.195</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual stimuli</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>0.020</td>
<td>0.280</td>
<td>.618</td>
<td>0.00</td>
</tr>
<tr>
<td>Theta</td>
<td>0.215</td>
<td>3.010</td>
<td>.082</td>
<td>0.00</td>
</tr>
<tr>
<td>Alpha low</td>
<td>0.260</td>
<td>3.640</td>
<td>.052</td>
<td>0.01</td>
</tr>
<tr>
<td>Alpha high</td>
<td>0.174</td>
<td>2.436</td>
<td>.122</td>
<td>0.00</td>
</tr>
<tr>
<td>Beta</td>
<td>0.240</td>
<td>3.360</td>
<td>.064</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$N = 15$; exergame group $N = 7$ and balance group $N = 8$. *$p \leq 0.05$, **$p \leq 0.01$, p-values are one-tailed and based on normalized data. $\eta_G^2$: effect size.

TABLE 3.4 | Pre- vs. post-test of relative power values for exergame and balance group.

<table>
<thead>
<tr>
<th>Exergame group</th>
<th>Pre (N=8)</th>
<th>Post (N=9)</th>
<th>$z$</th>
<th>$p$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory stimuli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>1.79 (1.61; 2.07)</td>
<td>1.59 (1.33; 1.95)</td>
<td>-1.52</td>
<td>.156</td>
<td>-0.37*</td>
</tr>
<tr>
<td>Theta</td>
<td>1.86 (1.55; 2.42)</td>
<td>1.70 (1.24; 1.86)</td>
<td>-2.37</td>
<td>.016*</td>
<td>-0.57b</td>
</tr>
<tr>
<td>Alpha low</td>
<td>1.24 (1.16; 1.38)</td>
<td>1.00 (0.80; 1.35)</td>
<td>-1.18</td>
<td>.297</td>
<td>-0.29</td>
</tr>
<tr>
<td>Alpha high</td>
<td>1.14 (0.67; 1.38)</td>
<td>0.83 (0.55; 1.16)</td>
<td>-1.18</td>
<td>.297</td>
<td>-0.29</td>
</tr>
<tr>
<td>Beta</td>
<td>-0.21 (-0.6; 10.06)</td>
<td>-0.19 (-0.44; 0.14)</td>
<td>-1.18</td>
<td>.297</td>
<td>-0.29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual stimuli</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>-0.08 (-0.63; 0.07)</td>
<td>-0.22 (-0.46; 0.14)</td>
<td>-1.01</td>
<td>.375</td>
<td>-0.25</td>
</tr>
<tr>
<td>Theta</td>
<td>-0.82 (-1.29; -0.38)</td>
<td>-0.60 (-0.96; -0.28)</td>
<td>-1.18</td>
<td>.297</td>
<td>-0.29</td>
</tr>
<tr>
<td>Alpha low</td>
<td>-0.57 (-1.23; -0.21)</td>
<td>-0.32 (-0.78; 0.02)</td>
<td>-1.70</td>
<td>.109</td>
<td>-0.41a</td>
</tr>
<tr>
<td>Alpha high</td>
<td>-0.77 (-1.14; -0.09)</td>
<td>-0.44 (-1.14; -0.09)</td>
<td>-0.85</td>
<td>.469</td>
<td>-0.21</td>
</tr>
<tr>
<td>Beta</td>
<td>-0.70 (-0.90; -0.74)</td>
<td>-0.31 (-0.74; -0.04)</td>
<td>-1.52</td>
<td>.078</td>
<td>-0.37a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Balance group</th>
<th>Pre (N=10)</th>
<th>Post (N=9)</th>
<th>$z$</th>
<th>$p$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory stimuli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>1.70 (1.28; 2.10)</td>
<td>1.99 (1.55; 2.29)</td>
<td>-0.56</td>
<td>.641</td>
<td>-0.13</td>
</tr>
<tr>
<td>Theta</td>
<td>1.81 (1.63; 2.02)</td>
<td>1.92 (1.65; 2.10)</td>
<td>-0.70</td>
<td>.547</td>
<td>-0.16</td>
</tr>
<tr>
<td>Alpha low</td>
<td>0.79 (0.44; 1.17)</td>
<td>1.30 (0.69; 1.47)</td>
<td>-1.54</td>
<td>.148</td>
<td>-0.35a</td>
</tr>
<tr>
<td>Alpha high</td>
<td>0.79 (0.29; 1.28)</td>
<td>1.26 (0.77; 1.46)</td>
<td>-1.82</td>
<td>.078</td>
<td>-0.42a</td>
</tr>
<tr>
<td>Beta</td>
<td>-0.16 (-0.33; 0.20)</td>
<td>-0.26 (-0.90; 0.21)</td>
<td>-1.54</td>
<td>.148</td>
<td>-0.35a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual stimuli</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>-0.12 (-0.48; 0.18)</td>
<td>-0.14 (-0.68; 0.15)</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
</tr>
<tr>
<td>Theta</td>
<td>-0.24 (-1.49; 0.03)</td>
<td>-0.77 (-1.55; -0.43)</td>
<td>-1.40</td>
<td>.195</td>
<td>-0.32a</td>
</tr>
<tr>
<td>Alpha low</td>
<td>-0.27 (-1.12; -0.13)</td>
<td>-0.60 (-0.90; -0.37)</td>
<td>-0.70</td>
<td>.547</td>
<td>-0.16</td>
</tr>
<tr>
<td>Alpha high</td>
<td>-0.21 (-1.81; -0.07)</td>
<td>-0.97 (-1.74; -0.32)</td>
<td>-1.12</td>
<td>.313</td>
<td>-0.26</td>
</tr>
<tr>
<td>Beta</td>
<td>-0.35 (-1.38; 0.07)</td>
<td>-0.84 (-1.16; -0.28)</td>
<td>-0.70</td>
<td>.547</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

Normalized data from pre-post are median values (interquartile range) as indicated. *Significant within-group differences pre-post ($p \leq 0.05$) calculated with Wilcoxon signed-rank test (p-values are two-tailed). For effect size $r$, $r = 0.1$-0.29 indicates a small effect, $r = 0.3$-0.49 indicates a medium effect, and $r \geq 0.5$ indicates a large effect.
3.3.2 Executive Functions

For the analysis, some participants had to be excluded because of technical problems (the included number of participants is illustrated in table 3.5 and 3.6). No significant interactions were present for any of the TAP tests (Table 3.5). The results of the pre- vs. post-tests comparisons are presented in table 3.6. Comparing pre- vs. post-test for the exergame group, all TAP tests showed a significant decrease of the RT (working memory: \( z = -2.28, p = 0.021 \); divided attention auditory: \( z = -2.51, p = 0.009 \); divided attention visual: \( z = -2.06, p = 0.040 \); Go/No-go: \( z = -2.55, p = 0.008 \); set-shifting: \( z = -2.90, p = 0.002 \)). The balance group showed a significant decrease of the RT for the set-shifting test (\( z = -2.04, p = 0.042 \)).

**TABLE 3.5 | Interaction effects (time \( \times \) intervention) of repeated measures Puri & Sen-analyses of ranked data for Test for Attention Performance.**

<table>
<thead>
<tr>
<th></th>
<th>Pillai’s trace ( r^2 )</th>
<th>( L = (N-1)r^2 )</th>
<th>( p )</th>
<th>( \eta^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working memory†</td>
<td>0.144</td>
<td>3.456</td>
<td>.061</td>
<td>0.00</td>
</tr>
<tr>
<td>Divided attention auditory++</td>
<td>0.040</td>
<td>1.000</td>
<td>.326</td>
<td>0.09</td>
</tr>
<tr>
<td>Divided attention visual</td>
<td>0.043</td>
<td>1.118</td>
<td>.297</td>
<td>0.02</td>
</tr>
<tr>
<td>Go/No-go</td>
<td>0.063</td>
<td>1.701</td>
<td>.206</td>
<td>0.11</td>
</tr>
<tr>
<td>Set-shifting</td>
<td>0.023</td>
<td>0.598</td>
<td>.446</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Test for Attentional Performance measured reaction time [ms]. \( N = 27; \) exergame group \( N = 13 \) and balance group \( N = 14 \). *\( N = 25; \) exergame group \( N = 12 \) and balance group \( N = 13 \); **\( N = 26; \) exergame group \( N = 12 \) and balance group \( N = 14 \). \( p \leq 0.05, p \)-values are one-tailed. \( \eta^2 \): effect size.

**TABLE 3.6 | Pre- vs. post-test of Test for Attention Performance for exergame and balance group.**

<table>
<thead>
<tr>
<th>Exergame group</th>
<th>Pre (N=13)</th>
<th>Post (N=13)</th>
<th>( z )</th>
<th>( p )</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working memory</td>
<td>876.5 (701.8; 1104.5)†</td>
<td>750 (649.5; 907.5)</td>
<td>-2.28</td>
<td>.021*</td>
<td>-0.46a</td>
</tr>
<tr>
<td>DA auditory</td>
<td>631.5 (580; 756.25)++</td>
<td>598 (533.5; 629)</td>
<td>-2.51</td>
<td>.009**</td>
<td>-0.50b</td>
</tr>
<tr>
<td>DA visual</td>
<td>1109 (835; 1291)</td>
<td>945 (879.5; 1082)</td>
<td>-2.06</td>
<td>.040*</td>
<td>-0.40a</td>
</tr>
<tr>
<td>Go/No-go</td>
<td>450 (426; 496)</td>
<td>421 (410.5; 451.5)</td>
<td>-2.55</td>
<td>.008**</td>
<td>-0.50b</td>
</tr>
<tr>
<td>Set-shifting</td>
<td>1723 (1143.5; 2273.5)</td>
<td>1405 (855; 1727.5)</td>
<td>-2.90</td>
<td>.002**</td>
<td>-0.57b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Balance group</th>
<th>Pre (N=14)</th>
<th>Post (N=14)</th>
<th>( z )</th>
<th>( p )</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working memory</td>
<td>802 (682.3; 871.5)</td>
<td>767 (726; 926.5)+++</td>
<td>-0.31</td>
<td>.787</td>
<td>-0.06</td>
</tr>
<tr>
<td>DA auditory</td>
<td>720 (606.25; 787.75)</td>
<td>665.5 (604.25; 733.5)</td>
<td>-1.10</td>
<td>.296</td>
<td>-0.21</td>
</tr>
<tr>
<td>DA visual</td>
<td>961 (841.75; 1082.25)</td>
<td>907.5 (855.5; 1054.25)</td>
<td>-0.60</td>
<td>.583</td>
<td>-0.11</td>
</tr>
<tr>
<td>Go/No-go</td>
<td>472 (448.5; 503.75)</td>
<td>466 (417.3; 509)</td>
<td>-0.25</td>
<td>.802</td>
<td>-0.05</td>
</tr>
<tr>
<td>Set-shifting</td>
<td>1196 (1045.5; 1752.25)</td>
<td>1110 (984; 1409.5)</td>
<td>-2.04</td>
<td>.042*</td>
<td>-0.39a</td>
</tr>
</tbody>
</table>

The table presents the reaction time [ms] for each test. Data from pre-post are median values (interquartile range) as indicated. *Significant within-group differences pre-post (*\( p \leq 0.05 \) and ** \( p \leq 0.01 \)) calculated with Wilcoxon signed-rank test (\( p \)-values are two-tailed). For effect size, \( r = 0.1\)-0.29 indicates a small effect, \( r = 0.3\)-0.49 indicates a medium effect, and \( r \geq 0.5 \) indicates a large effect. DA = divided attention, *\( N = 12 \); ++ \( N = 12 \); +++ \( N = 13 \).
3.3.3 Spatio-Temporal Gait Parameters

No significant interactions were present for any of the gait parameters (Table 3.7). The results of the pre- vs. post-tests comparisons are presented in table 3.8. Comparing pre- vs. post-test, the exergame group significantly improved gait speed during dual-task walking at preferred \((z = -2.90, p = 0.002)\) and at fast \((z = -2.97, p = 0.001)\) speed. Furthermore, cadence was significantly enhanced for dual-task walking at preferred speed \((z = -2.97, p = 0.001)\) and stride length was significantly improved for dual-task walking at fast speed \((z = -2.69, p = 0.005)\). Comparing pre- vs. post-test, the balance group significantly improved gait speed for single-task walking at preferred \((z = -2.54, p = 0.009)\) and at fast \((z = -1.98, p = 0.049)\) speed and for dual-task walking at fast speed \((z = -1.98, p = 0.049)\). Moreover, gait cadence was significantly improved for single-task walking at preferred speed \((z = -2.79, p = 0.003)\).

TABLE 3.7 | Interaction effects (time × intervention) of repeated measures Puri & Sen-analyses of ranked data for spatio-temporal gait parameters.

<table>
<thead>
<tr>
<th></th>
<th>Pillai’s trace (r^2)</th>
<th>(L = (N-1)r^2)</th>
<th>(p)</th>
<th>(\eta_G^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speed [m/s]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>0.001</td>
<td>0.026</td>
<td>.880</td>
<td>0.01</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>0.068</td>
<td>1.768</td>
<td>.189</td>
<td>0.00</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>0.001</td>
<td>0.026</td>
<td>.882</td>
<td>0.01</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>0.004</td>
<td>0.104</td>
<td>.753</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Cadence [steps/min]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>0.003</td>
<td>0.078</td>
<td>.774</td>
<td>0.05</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>0.108</td>
<td>2.808</td>
<td>.094</td>
<td>0.08</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>0.032</td>
<td>0.832</td>
<td>.371</td>
<td>0.00</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>0.031</td>
<td>0.806</td>
<td>.376</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Stride length [m]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>0.000</td>
<td>0.000</td>
<td>.915</td>
<td>0.00</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>0.007</td>
<td>0.182</td>
<td>.682</td>
<td>0.01</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>0.014</td>
<td>0.364</td>
<td>.554</td>
<td>0.01</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>0.070</td>
<td>1.820</td>
<td>.183</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(N = 27;\) exergame group \(N = 13\) and balance group \(N = 14\). \(p \leq 0.05\), \(p\)-values are one-tailed. \(\eta_G^2\): effect size, normal: preferred speed, fast: fast speed.
TABLE 3.8 | Pre- vs. post-test of spatio-temporal gait parameters for exergame and balance group.

<table>
<thead>
<tr>
<th>Exergame group</th>
<th>Pre (N=13)</th>
<th>Post (N=13)</th>
<th>z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed [m/s]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>1.13 (0.92; 1.22)</td>
<td>1.19 (1.06; 1.29)</td>
<td>-1.64</td>
<td>.110</td>
<td>-0.32*</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>1.54 (1.31; 1.63)</td>
<td>1.49 (1.38; 1.64)</td>
<td>-1.29</td>
<td>.216</td>
<td>-0.25</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>1.02 (0.88; 1.18)</td>
<td>1.08 (0.95; 1.29)</td>
<td>-2.90</td>
<td>.002**</td>
<td>-0.57b</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>1.40 (1.13; 1.47)</td>
<td>1.45 (1.10; 1.62)</td>
<td>-2.97</td>
<td>.001**</td>
<td>-0.58b</td>
</tr>
<tr>
<td>Cadence [steps/min]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>103.7 (95.9; 109.3)</td>
<td>106.3 (100.9; 113.3)</td>
<td>-1.78</td>
<td>.080</td>
<td>-0.35*</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>129.7 (114.9; 142.6)</td>
<td>125.6 (118.48; 134.0)</td>
<td>-0.45</td>
<td>.685</td>
<td>-0.09</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>104.4 (94.37; 107.3)</td>
<td>104.1 (96.8; 116.7)</td>
<td>-2.97</td>
<td>.001**</td>
<td>-0.58b</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>116.7 (106.6; 129.8)</td>
<td>124.2 (110.3; 131.1)</td>
<td>-1.43</td>
<td>.168</td>
<td>-0.28</td>
</tr>
<tr>
<td>Stride length [m]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>1.26 (1.14; 1.34)</td>
<td>1.30 (1.26; 1.37)</td>
<td>-1.92</td>
<td>.057</td>
<td>-0.38a</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>1.39 (1.35; 1.48)</td>
<td>1.43 (1.37; 1.48)</td>
<td>-1.15</td>
<td>.273</td>
<td>-0.23</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>1.18 (1.10; 1.30)</td>
<td>1.21 (1.15; 1.32)</td>
<td>-1.57</td>
<td>.127</td>
<td>-0.31a</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>1.33 (1.21; 1.39)</td>
<td>1.36 (1.29; 1.53)</td>
<td>-2.69</td>
<td>.005**</td>
<td>-0.53b</td>
</tr>
<tr>
<td>Balance group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed [m/s]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>1.06 (0.92; 1.25)</td>
<td>1.12 (1.04; 1.41)</td>
<td>-2.54</td>
<td>.009**</td>
<td>-0.48b</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>1.44 (1.29; 1.62)</td>
<td>1.56 (1.40; 1.78)</td>
<td>-1.98</td>
<td>.049*</td>
<td>-0.37a</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>1.00 (0.87; 1.05)</td>
<td>1.04 (0.90; 1.24)</td>
<td>-1.54</td>
<td>.135</td>
<td>-0.29</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>1.22 (1.16; 1.47)</td>
<td>1.40 (1.17; 1.54)</td>
<td>-1.98</td>
<td>.049*</td>
<td>-0.37a</td>
</tr>
<tr>
<td>Cadence [steps/min]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>108.2 (100.1; 112.8)</td>
<td>115.1 (101.1; 116.9)</td>
<td>-2.79</td>
<td>.003**</td>
<td>-0.53b</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>133.1 (117.78; 147.4)</td>
<td>137.4 (129.6; 150.2)</td>
<td>-1.92</td>
<td>.058</td>
<td>-0.36a</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>101.9 (92.0; 111.8)</td>
<td>111.1 (99.8; 115.4)</td>
<td>-1.85</td>
<td>.068</td>
<td>-0.35a</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>115.9 (110.4; 131.7)</td>
<td>127.0 (117.5; 135.0)</td>
<td>-1.35</td>
<td>.194</td>
<td>-0.26</td>
</tr>
<tr>
<td>Stride length [m]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task normal</td>
<td>1.18 (1.06; 1.34)</td>
<td>1.29 (1.04; 1.39)</td>
<td>-1.92</td>
<td>.058</td>
<td>-0.36a</td>
</tr>
<tr>
<td>Single-task fast</td>
<td>1.32 (1.11; 1.53)</td>
<td>1.40 (1.15; 1.56)</td>
<td>-0.91</td>
<td>.391</td>
<td>-0.17</td>
</tr>
<tr>
<td>Dual-task normal</td>
<td>1.17 (1.02; 1.28)</td>
<td>1.20 (0.97; 1.34)</td>
<td>-0.79</td>
<td>.463</td>
<td>-0.15</td>
</tr>
<tr>
<td>Dual-task fast</td>
<td>1.30 (0.93; 1.53)</td>
<td>1.37 (1.07; 1.42)</td>
<td>-1.10</td>
<td>.296</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

Data from pre-post are median values (interquartile range) as indicated. *Significant within-group differences pre-post (*p ≤ 0.05 and ** p ≤ 0.01) calculated with Wilcoxon signed-rank test (p-values are two-tailed). For effect size r, r = 0.1-0.29 indicates a small effect, * r = 0.3-0.49 indicates a medium effect, and b r ≥ 0.5 indicates a large effect. Normal: preferred speed, fast: fast speed.
3.4 Discussion

This study compared exergame training with conventional balance training focusing on prefrontal brain activity (RP), EFs, and gait performance. We hypothesized that a combined motor and cognitive training would differently affect prefrontal brain activity, EFs, and spatio-temporal gait parameters when compared to more conventional motor training. The results of the brain activity measurement showed that theta RP (auditory stimuli) significantly decreased post-intervention in favor of the exergame group. Comparing pre- vs. post-test, four EFs improved within the exergame group (working memory, divided attention, go-/no-go, and set-shifting) and one within the balance group (set-shifting). In addition, spatio-temporal gait parameters primarily improved within the exergame group under dual-task conditions and within the balance group under single-task conditions, respectively.

3.4.1 Prefrontal Brain Activity

The results showed that theta RP (auditory stimuli) significantly decreased in the exergame group. This decrease in theta power band frequency contrasts the behavior we might expect due to the aging process; this parameter is rather expected to increase with age [21]. Furthermore, previous research findings suggest that an increased frontal theta power reflects an aging mechanism in which slow cortical waves (e.g. theta oscillations) move from a stable state (lower EEG power) to a relatively unstable state (higher EEG power) [232], a mechanism called age-related “slowing” [21]. The results of our study, therefore, might mean that exergame training is able to ameliorate age-related “slowing” of EEG that is, in turn, linked to superior cognitive functioning, improved motor performance, and enhanced sensory processing [21]. However, we should bear in mind that the findings on the slowing effect are not consistently reported [21], since there are also studies reporting that no increase in theta power can be observed in older adults [233], or spontaneous delta power is higher in young compared to healthy older adults [234].

According to the de-differentiation view, an increase of theta RP frequency reflects a difficulty in recruiting specialized neural mechanisms [235]. One might speculate that the exergame training helped to increase connections between different brain areas, because theta RP decreased, thus enabling better control and eased recruitment of specialized neural mechanisms [232, 236]. However, these relations can be only hypothesized so far, because it still remains unclear how age-related changes affect EEG (slow) oscillations and whether they seem to mediate control processes. It might well be that reduced frontal theta RP reflects an executive impediment in top-down control due to increasing task demands [237], instead of being due to an increase in brain connectivity. Furthermore, theta RP linked to the auditory stimuli might also be enhanced because of working memory improvements by exergame training. Perceptual operations of acoustic inputs are encoded by working memory and these processes are affected by age-limited processing resources [238]. Considering the exergame group, it might be that working memory improved to such an extent that an elevation of the processing resources for
acoustic inputs was achieved. Our results warrant further research in the functional and structural changes of the brain caused by exergaming.

### 3.4.2 Executive Functions

No time × group interaction effects were present for any of the measured EFs. The exergame group, however, significantly improved in four measures, while for the balance group only set-shifting improved. A previous study investigating exergame and balance training in elderly and measuring EFs, especially shifting, working memory, and inhibition, came to a similar result [204].

Video games serve as a powerful tool to enhance cognitive abilities in elderly [60]. On the other hand, a review concluded that computerized cognitive training leads to small to moderate improvements in certain cognitive domains with no effects on EFs [239]. We assume that the video game-based training efficacy might be ensured by video game characteristics that are close to the cognitive outcome of interest [240]. A study concluded that different genres of video games may not have equal positive effects on the same cognitive aspect [241]. The current video games included cues that specifically trained divided attention, inhibition (Go/No-go), set-shifting, and working memory. For example, cognitive flexibility might be trained by video games that train manipulation of multiple information sources [242]. Furthermore, the outcomes should be considered with caution since different cognitive abilities were defined as EF components measured with different kinds of EF assessments. Finally, the combination of PE and cognitive training leads to improvement of general cognitive functions and memory in older adults [67]. Thus, PE might be executed in a cognitively challenging environment to effectively induce cognitive benefits [243, 244]. Exergames are a specific concurrent combination of cognitive and motor training including a video game-based PE. Our interactive exergame challenges different EFs; participants observe specific cues on a frontal screen and concurrently are expected to execute well-coordinated movements. The video games included progressive levels of difficulty. Interestingly, cognitive benefits from exergaming increase with the dose of interactive mental challenge [245]. To summarize, our exergame included a specific EF training under user-matched training levels that might trigger the beneficial effects on the measured EFs. On the other hand, balance exercise consisted of highly variable exercises, which in fact is an important and effective method to train motor learning [209], and might have, therefore, helped participants learn to adapt to new and changing situations, an important aspect for flexibility [246]. Finally, both interventions elicit beneficial effects on EFs while the exergame seems to be a more specific and efficient training method to improve EFs.

### 3.4.3 Spatio-Temporal Gait Parameters

No time × group interaction effects were present for any of the measured gait parameters. The balance group demonstrated significant within-group improvements of several gait parameters, however, merely in the single-task conditions. Balance training improves motor performance by changes on subcortical
structures [205]. Furthermore, the significant improvement for the walking speed parameter during fast and fast cognitive (dual-task) walking might indicate that improved balance abilities impact gait performance by changes of gait speed, especially during fast walking. Similarly, one recent study showed improved gait speed after balance training [247]. Furthermore, PE positively influences dual-task walking by increasing the walking speed [248]. On the other hand, the exergame group showed significant within-group improvements for gait parameters under dual-task conditions. These significant positive within-group differences for gait parameters in the dual-task conditions confirm findings from previous pilot studies with similar results for dual-task related costs [207, 249]. Exergame training combines enhancements of motor and cognitive functions that, therefore, positively influence gait performance under dual-task conditions [7, 64, 65]. Moreover, divided attention is trained by concurrent observation of cognitive stimuli and performance of well-coordinated movements. Even more, participants were expected to observe the virtual environment for drifting symbols or figures and, at the same time, initiate steps on a pressure sensitive area. When using an outward step, participants needed to rapidly unload the leg they were falling towards to allow to take a step. This may be challenging from a cognitive, RT, and/or muscle power generation perspective [250]. The crucial point is that the exergame does not only require well-coordinated leg movements, but also requires cognitive work, e.g. sensing of stimuli, paying attention, and making quick decisions [202]. Thus, a repeated practice of exergaming (dual-tasking), using task-specific training, might improve dual-task interference [248]. A transfer of the trained cognitive abilities on the concurrent performance of multiple tasks might happen [251].

### 3.4.4 Limitations

Some limitations of the study were the quite fit elderly participants, small sample size, and probably the short training intervention time. A modification of these factors might lead to interaction effects between the groups. Furthermore, the training time and intensity of the experimental and control group were not compatible because matching of the training content was not possible. Moreover, for the exergame training, each participant played the same amount of games, but no strict order of the game existed. For the EEG, two circumstances might limit the measurement: the naïve triggering approach and the use of a low-density EEG device.

### 3.4.5 Conclusion

For aging humans, an exercise program that effectively addresses prefrontal brain activity and function, especially EFs and gait performance, might be important since age-associated reduction of EFs can impact gait and amplify the risk of falling [50]. Further, two recent reviews, focusing on the interplay between physical functions and cognition, concluded that it seems important to combine motor and cognitive therapy into clinical practice to enable older adults to move safer in their physical environment [64, 65]. The present study illustrated that especially exergame training affects prefrontal theta RP and
that exergame training and balance training positively influence EFs and gait performance to different extents. Thus, exergame training might be a promising future training strategy targeting prefrontal brain activity, EFs, and gait in elderly. Especially promising seems the effect that exergame is able to influence dual-task walking. Future studies should investigate cortical activity in additional brain areas during EF testing, i.e. using a high-density EEG device to substantiate the presented results. In addition, studies should focus on larger study sample sizes, longer intervention duration, and a strict training content for all the participants. Furthermore, the exergame (visual and acoustic) stimuli should be targeted on the specific EFs that have to be improved in elderly.

Author contribution: AS and RA developed the research question under the lead of EDB. The concept and design part was established by AS and RA while EDB acted as methodological council. AS and RA did data acquisition as well as, together with FG, analysis and interpretation of the results which was edited and improved by EDB. AS and RA produced an early version of the manuscript. FG and EDB substantially revised the manuscript to bring it to its current version. All authors have read and approved the final manuscript.

Acknowledgements: The authors would like to thank Christina Pizio and Christina Chirila for instructing trainings and helping with data acquisition. Furthermore, we would like to thank all participants for their kindness and enthusiasm during the training sessions and measurement period. The trial was supported by the ETH Foundation through ETH Research Grant ETH-17 13-2.

Conflict of interest: The authors AS, FG, and RA 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. EDB was a co-founder of dividat, the spin-off company that developed the video games used in this study, and is associated to the company as an external advisor. No revenue was paid (or promised to be paid) directly to EDB or his institution over the 36 months prior to submission of the work.
Combining Exergame Training with Omega-3 Fatty Acid Supplementation: Protocol for a Randomized Controlled Study Assessing the Effect on Neuronal Structure/Function in the Elderly Brain

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Keywords: older adults, exergame training, video game, nutritional supplementation, omega-3 fatty acid, brain function, brain structure

Published in¹:

Frontiers in Aging Neuroscience. 2016, 8 (283)

DOI: 10.3389/fnagi.2016.00283

¹Figure, tables, and language errors in the original publication were adapted/corrected for this thesis
Abstract

A common problem in the older population is the risk of falling and related injury, immobility, and reduced survival. Age-related neuronal changes, e.g. decline in gray and white matter, affect neuronal, cognitive, and motor functioning. The improvement of these factors might decrease fall events in elderly. Studies showed that administration of video game-based physical exercise, a so-called exergame, or omega-3 fatty acids (FA) may improve motor and/or cognitive functioning through neuronal changes in the brain of older adults. The aim of this study is to assess the effects of a combination of exergame training with omega-3 FA supplementation on the elderly brain. We hypothesize that an intervention using a combination approach differently affects the neuronal structure and function of the elderly's brain as compared to the sole-administration of exergame training. The study is a parallel, double-blinded, randomized controlled trial lasting 26 weeks. Sixty autonomous living, non-smoking, and right-handed healthy older (>65 years) adults who live independently or in a senior residency are included, randomized, and allocated to one of two study groups. The experimental group receives a daily amount of 13.5 ml fish oil (including 2.9 g of omega-3 FA), whereas the control group receives a daily amount of 13.5 ml olive oil for 26 weeks. After 16 weeks, both groups start with an exergame training program three times per week. Measurements are performed on three time-points by treatment blinded investigators: pre-intervention measurements, blood sample after 16 week, and post-intervention measurements. The main outcomes are motor evoked potentials of the right M. tibialis anterior (transcranial magnetic stimulation) and response-related potentials (electroencephalography) during a cognitive test. For secondary outcomes, reaction time during cognitive tests and spatio-temporal parameters during gait performance are measured. Statistics will include effect sizes and a 2 × 2-ANOVA with normally distributed data or the non-parametric equivalent for data not fulfilling normal distribution. The randomized controlled study is the first to investigate the effectiveness of exergame training combined with omega-3 FA in counteracting age- and behavioral-dependent neuronal changes in the brain. This study has been registered in the Swiss National Clinical Trials (SNCTP34995201623) and the ISRCTN (ISRCTN12084831) Portals.
4.1 Introduction

In general, the human brain undergoes age-dependent changes by losing about 15% of the cerebral cortex and about 25% of the cerebral white matter between the ages of 30 and 90 years [20]. Age-associated alterations in gray matter and white matter integrity [15, 252, 253] and a decrease in synthesis and binding of dopamine (produced in substantia nigra and ventral tegmental area), serotonin (produced in raphe nuclei), and acetylcholine (produced in pedunculopithine nucleus and laterodorsal tegmental nucleus, medial septal and diagonal band nuclei, and nucleus basalis) [23-27] are connected to deteriorations of cognitive functioning, e.g. working memory and executive function (EF). EFs are interrelated cognitive abilities that control and guide goal-directed actions [43]; e.g. walking in challenging environments. Different EF components, e.g. “working memory” [44], “divided attention” [45], and “inhibition” [46], partly explain gait performance. Especially spatial and temporal dual-task cost characteristics of gait are associated with divided attention [47]. Gait disturbances and fall events, caused by sensory and motor impairments, are believed to be moderated by executive functioning [48, 49]. Training of EFs in older adults might contribute to improved gait performance [64] and might reduce fall events as EF performance predicted the risk for future falls [50]. However, so far no direct cause and effect relationship was demonstrated between EF and gait [37].

Anatomically, EFs have been linked with the frontal lobe of the brain, in particular the dorsolateral prefrontal cortex (PFC) and related brain networks [36, 37]. A large PFC volume and a greater PFC thickness were associated with better EFs [38]. During lifetime, the (pre)frontal network undergoes age-dependent neuronal changes; however, no consensus exists to the precise pattern of EF altering [15, 37, 39, 40]. One assumption is that the decline in frontal gray matter might be associated with the deterioration of EFs [41]. Moreover, disturbances in cortico-cortical and cortico-subcortical connections, e.g. frontal connections with parietal lobes and basal ganglia, respectively, are classified as higher-level gait disorders [49, 52]. A phenomenon coined ‘retrogenesis’ implies that brain circuits that mature late in ontogeny are most vulnerable to early neurodegeneration [53] and might contribute to the understanding and prediction of disturbances in higher-level gait and gait-related motor activity. This suggestion is supported by recent work of Rosano et al. (2012) showing that a smaller volume of the prefrontal area is likely to contribute to slower gait through slower information processing [54].

So far, training of cognitive abilities (e.g. EFs) may represent an important strategy to preserve brain function and also prevent mobility disability [54, 207, 249, 254]. Furthermore, recent reviews focusing on the interplay between physical functions and cognition concluded that it seems important to combine motor and cognitive training into clinical practice to enable older adults to move safer in their physical environment [65, 68, 207]. Especially, computerized interventions seem promising [68, 71, 207] when considering training principles that enhance (motor) learning [71]. Video games might have the potential to train cognitive functions [61]. A video game-based physical exercise (PE), or a so-called exergame, allows the recommended combinatory training of motor and cognitive abilities. It is believed that PE
interventions with decision-making opportunities might facilitate the development of both motor performance and cognitive function [66].

Recent research indicates that the effects of PE on the brain can be enhanced by concurrent consumption of natural products [13]. This means it can be hypothesized that a combination of physical training with a nutritional supplement has the potential to further enhance the effects of physical training on the level of brain structure and function in older persons. The potential synergy between nutrition and PE could involve common cellular pathways important for neurogenesis, cell survival, synaptic plasticity, and vascular function [13].

A systematic review revealed that previous interventions using a combined approach of PE and NS to effect on the brain were not particularly successful because of the misfit between the combinations; the intervention components were not selected based on sharing of similar neuronal mechanisms [255]. The review indicates, however, that especially fish oil, containing omega-3 fatty acids (FA), might be an effective nutritional supplementation supporting the positive effects of PE. Omega-3 FA are important for energy metabolism and for the composition of the plasma membranes in the brain [74]. Another review showed that long-chain polyunsaturated FA (LCPUFA) might improve cognition, decrease (neuro)inflammation, and reduce vascular risk factors in normal aging adults [79]. Omega-3 LCPUFA may provide decreased brain deterioration through the positive effects on brain structure, function, and cerebral blood flow [80]. A recent randomized-controlled study showed that fish oil had positive effects on brain structure and function in healthy older adults [81]. The participants showed improved EFs, white matter microstructure integrity, gray matter volume, and vascular parameters.

So far, no study investigated the combined effect of exergame training and omega-3 FA on the elderly brain’s structure and function. This study, therefore, aims to investigate the effects of a combination of exergame training and omega-3 FA. The following research question will guide through the research process: “Does the combination of exergame training and fish oil differently affect neuronal system levels in the elderly brain compared to exergame training alone?” The main objectives of the trial are (1) to determine the effects of the intervention on the neuronal structural level of the brain (neuronal excitability) and (2) to assess the effects on functional level in the brain (neuronal activity). We hypothesize that the combination will differently affect these parameters.
4.2 Materials and Methods

4.2.1 Ethics and Reporting

The study procedure has been approved by the local ethics committee (EC Zurich Switzerland, EC number: 2015-0190) and conforms to the Declaration of Helsinki and the guidelines of Good Clinical Practice E6 (R1). No data was recorded before written informed consent was given by the participants. The trial protocol follows the Consolidated Standards of Reporting Trials (CONSORT) statement on randomized trials of non-pharmacological treatment [256] and Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidance for protocol reporting [257].

4.2.2 Design and Setting

The study is a randomized double-blinded, placebo-controlled study involving elderly adults above 65 years. The study is designed to examine the effect of omega-3 FA supplementation and exergame training on the endpoints of neuronal structure and function before and after a 26-weeks intervention period. The measurements and data collection, the exergame training, and data analysis are conducted at the same study site (ETH Höggerberg, Zurich, Switzerland). At home, the participants are expected to take the nutritional supplementation regularly.

4.2.3 Blinding, Randomization, and Allocation

The nutritional supplementation, packed in bottles equal in outer appearance, were blinded by an external center (Kantonsapotheke Zurich, Switzerland) to achieve double blinding. The external center created a computer-generated list including numbers from 001 to 060 that correspond to either fish oil or olive oil, respectively. The list number does not correspond to the participants’ identification (ID) number. The list consists of six blocks of ten whereas fish oil and olive oil are randomly and equally distributed in all blocks. The investigators continuously assigned the volunteering women to the numbers starting with 001 and ending with 030 and the men starting with 031 and ending with 060. The randomization list is stored by a non-involved investigator and out of reach and sight of the involved investigators. For statistics, the groups will be referred to without specification of nutritional supplementation (e.g. group A and B).

4.2.4 Participants

Participants were recruited from the Senior’s University Zurich (Switzerland), senior residency dwellings in Zurich (Switzerland), and through public advertisement. The public advertisement included a brief study description and study site contact information. All those who were interested received a study information sheet including the design, procedure, benefits, and risks of the study. Before the study procedure started, the participants had to provide signed written informed consent forms.
Participants fulfilling all of the following inclusion criteria were eligible for the study: (1) age above 65 years, (2) live independently or in a residency dwelling, (3) non-smoker, and (4) healthy (self-reported). Participants were excluded if they exhibited one of the following exclusion criteria: (1) mobility impairments, (2) orthopedic or neurological diseases that prevent training participation, (3) rapidly progressive or terminal illness as well as acute or chronic illness, (4) history of heart attack, stroke, or epilepsy, (5) medication that interacts with nutritional supplementation (e.g. hypoglycemic medication and anticoagulants), (6) medication that acts on neuronal level (e.g. psychotropic medications), (7) cognitive impairment (Mini Mental Status Examination < 22 points), (8) signs of an upcoming depression (Geriatric Depression Scale), (9) electronic or metallic head implants, and (10) personal history of dizziness.

4.2.5 Interventions

The study interventions are described in detail according to the Template for Intervention Description and Replication (TIDieR) guidelines [258] in table 4.1 to allow readers and other researchers to use or replicate the intervention.
TABLE 4.1 | Description of study intervention based on the Template for Intervention Description and Replication (TIDieR) checklist [258].

<table>
<thead>
<tr>
<th>Item</th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brief name</td>
<td>Fish oil + exergame training</td>
<td>Olive oil + exergame training</td>
</tr>
<tr>
<td>2. Why?</td>
<td>Exergame training [68] as well as omega-3 FA [81] have positive effects on the elderly brain. The combination of exergame training and omega-3 FA might improve brain structure and function more effectively than their sole-administration.</td>
<td>Olive oil is not expected to induce better effects as omega-3 FA. Olive oil acts as a good comparator because of similarity in taste, composition, consistency, and color. Exergame training can improve brain structure and function, but on a lower level as compared to the experimental group.</td>
</tr>
<tr>
<td>3. What materials?</td>
<td>Participants receive bottles including the fish oil, measuring cups, and a NS diary to record adherence. On pressure sensitive dance plates, participants perform whole-body movements driven by VGs presented on a frontal screen.</td>
<td>Participants receive bottles including the olive oil, measuring cups, and a NS diary to record adherence.</td>
</tr>
<tr>
<td>4. What procedure?</td>
<td>The participants take the NS daily. The PE includes six different VGs whereas each VG adapts the difficulty level to the participant’s abilities. Each exergame is designed to train different executive and physical functions. One 30min-training includes one session of each VG (4min) with short breaks (~1min) for game change.</td>
<td></td>
</tr>
<tr>
<td>5. Who provides?</td>
<td>Investigators instructed to NS and exergame training</td>
<td></td>
</tr>
<tr>
<td>6. How?</td>
<td>For the NS, both intervention groups receive initial instruction about intake, duration, and dosage by an exercised investigator. The PE is performed in small groups supervised by experienced investigators (master students in human movement sciences at ETH Zurich).</td>
<td></td>
</tr>
<tr>
<td>7. Where?</td>
<td>The participants take the NS at home. The PE is performed in training rooms at ETH Hönggerberg (Switzerland).</td>
<td></td>
</tr>
<tr>
<td>8. When and how much?</td>
<td>For 26 weeks, the participant takes 13.5ml of the NS daily. After 16 weeks, the participants continue with the NS and start with the PE. The PE takes place 3 times per week (30min) for 10 weeks.</td>
<td></td>
</tr>
<tr>
<td>9. Tailoring</td>
<td>The PE is tailored to the abilities of each individual participant by the integrated progression algorithm. If a participant gets better/worse in performance, the VG automatically adapts and becomes more difficult/easier.</td>
<td></td>
</tr>
</tbody>
</table>

FA: fatty acids, NS: nutritional supplementation, PE: physical exercise, VG: video game.
**Nutritional Supplementation**

Participants randomized to the experimental group take a liquid (oily consistency) fish oil (San Omega AS, Akersbakken 35B, NO-0172 Oslo). Participants randomized to the control group take olive oil as placebo (Oro del Desierto, Ctra. Nacional 340, 04200 Tabernas, Almeria, Spain). The reasons for choosing olive oil as comparator are the similarity of taste, composition, consistency, and color. Thus, olive oil is the most commonly used placebo for omega-3 FA studies [259].

Over 26 weeks, the participants take a daily amount of 13.5ml of fish oil, including 2.9g of omega-3 FA, or 13.5ml of olive oil. The first 16 weeks, the participants take the nutritional supplementation with the aim of reaching a steady state [260-262]. A review on omega-3 FA suggests that a duration of 16 weeks is needed to account for potential interaction effects of gender and age [262]. Moreover, the time frame of 16 weeks is the minimum time needed for red blood cells to reach a steady state [260, 261]. The duration and dosage of the omega-3 FA was based on findings of previous studies. Two studies showed no detectable cognitive benefits when considering an intake of 0.7g for 24 months [263] or 1.8g for 26 weeks [264], respectively. A possible explanation might be that the dosage level is more important than the time frame. Witte et al. (2014) utilized 2.2g for 26 weeks and achieved a significant increase of EF and beneficial effects in white matter microstructure integrity and on gray matter volume [81]. A review identified low to moderate side effects in form of gastrointestinal upset, fishy aftertaste, worsening glycemia, and rise in low density lipoprotein cholesterol for 1 to 3g/d of omega-3 FA [265].

At home, the nutritional supplementation can be taken undiluted or can be added to food (e.g. salads) or drinks. At intervention start, the participants receive bottles including the nutritional supplementation, measuring cups (13.5ml), and oral as well as written information about duration, dosage, and intake. To check for intake adherence, the participants are supplied with a nutritional supplementation diary including week days and daytime.

**Exergame Training**

On a pressure-sensitive dance plate (Impact Dance Platform, 87.5cm×87.5cm×2.5cm, Positive Gaming BV, BZ Haarlem, Nederland), the participants perform specific whole-body movements triggered by a video game presented on a frontal screen. The dance pad is connected by USB to a desktop computer and with symbols projected on to a wall using a beamer. Electronic sensors in the dance pad detect position and timing information that are used to provide participants with real-time visual and auditory feedback. Through foot pushes on the plate arrows (right, left, top, and bottom), the participants interact with the game. The video games (dividat, Schindellegi, Switzerland) are designed to train different aspects of EFs (divided attention, working memory, inhibition, and shifting) and physical functions. The exergame training allows the implementation of training principles as described in the paper of Healy et al. (2014) [209]: a feedback system to facilitate training, individual levels of difficulty according to individual skills and abilities, adjustable task difficulty to facilitate retention, and variability of training.
to enhance task transfer. Additionally, the FITT training principles are implemented; Frequency: three times per week, Intensity: individually adapted video game (allowing training progression), Type: combination of cognitive and motor training, and Time: 30min training sessions.

After 16 weeks of nutritional supplementation intake, all participants start to perform the exergame training that lasts 10 weeks. The participants train for 30min., three times per week. Based on the results of a meta-analysis, the time frame of 10 weeks was chosen in terms of an expected effect size of 0.478 [178]. The time frame and training intensity were, furthermore, based upon studies illustrating positive training effects in older adults performing a video game on a dance plate [64, 207]. Training includes one session of each video game (4min) in a pre-defined order and short breaks (~1min) for game change. In training rooms (ETH Hönggerberg, Switzerland), the participants perform their exercises in small groups supervised by experienced investigators. To control for adherence, the participants receive a training plan including dates and time schedule. Furthermore, the investigators control adherence by a training adherence checklist. A previous trial testing the effects of similar games in older adults showed that this program will effect on EFs [266].

4.2.6 Staff Eligibility

All involved investigators received training for data collection and handling in accordance with the study measurement protocols. Additionally, the investigators were instructed on how to prepare the participants for correct maintenance of the diary and nutritional supplementation intake. Furthermore, the investigators guiding the PE got instructions about the handling of the game console, game software, and the training procedure. The trained investigators supervise PE to explain the video game (if needed) and to minimize the risk of falls.

4.2.7 Outcomes

All measurements are performed at pre- and post-intervention. The primary and secondary outcomes are listed in table 4.2.
TABLE 4.2 | Trial outcomes.

<table>
<thead>
<tr>
<th>Assessment methodology</th>
<th>Outcomes</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>Motor evoked potential (right M. tibialis anterior)</td>
<td>Excitability of neuronal system, indirect measure of synaptic plasticity [99]</td>
</tr>
<tr>
<td>EEG</td>
<td>Response-related potential</td>
<td>Neuronal activity</td>
</tr>
<tr>
<td><strong>Secondary outcome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAP</td>
<td>Reaction time</td>
<td>Cognitive functioning</td>
</tr>
<tr>
<td>Gait</td>
<td>Temporal and spatial parameters</td>
<td>Motor functioning</td>
</tr>
<tr>
<td>DTC</td>
<td>FA levels</td>
<td>Cognitive cost</td>
</tr>
<tr>
<td>Blood sample</td>
<td></td>
<td>Indicator for NS</td>
</tr>
<tr>
<td><strong>Other outcomes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short FES-I</td>
<td>Points (7-28)</td>
<td>“Concern” about falling</td>
</tr>
<tr>
<td>MMSE</td>
<td>Points (0-30)</td>
<td>Mental status</td>
</tr>
<tr>
<td>GDS</td>
<td>Points (0-15)</td>
<td>Depression status</td>
</tr>
</tbody>
</table>


Transcranial Magnetic Stimulation

Participants sit comfortably on an adjustable chair with hip, knee, and ankle joint angles of 100°, 120°, and 90°, respectively. Given the symmetrical nature of transcranial magnetic stimulation (TMS)-related measurements of the lower limb, only the dominant side is assessed [267]. Cortical stimulation is applied by means of a TMS stimulator MAGSTIM 200 (Magstim Company Ltd., Whitland, Dyfed, UK) with a “figure of eight” coil placed over the cortical motor area to stimulate the right M. tibialis anterior (TA). In healthy participants, TMS-related measurements of the TA are reliable [267].

Muscle activity is recorded by Telemyo DTS (Noraxon, Cologne, Germany). Before the measurement, the skin of the shank is shaved (if needed) and prepared with an abrasive gel (OneStep AbrasivPlus, H+H Medizinprodukte, Münster, Germany). The electrodes (Ambu® Blue Sensor N, Cambridgeshire, UK) are placed with an inter-distance of two centimeters on the muscle belly of the right TA. The muscle belly is defined through contraction of the TA.

In the first step, the participants are handed a bathing cap that fits tightly on the head. On the top of the cap, a grid is drawn using the vertex as initial position. The vertex is determined as half distance from nasion to inion and half distance from right to left pre-tragus. As previously suggested, the optimal activation of the TA is obtained if the coil is placed parallel to and approximately 0.5–1.0 cm lateral to the midline and its mid-point is aligned anterior-posteriorly against the vertex (Cz) [268]. To maintain consistent coil positioning across sessions, detailed distance recordings are made from the nasion, inion, and bilateral pre-tragus to the vertex. In the second step, the optimal stimulation point is assessed (hotspot). The hotspot corresponds, on the grid, to the lowest motor threshold that evokes a motor evoked potential (MEP) response [269]. The third step involves determination of the resting motor...
threshold (RMT) defined as the lowest intensity of magnetic stimulation required to evoke MEPs of 50μV in peak-to-peak amplitude in at least six of ten consecutive trials [269]. In the fourth step, a recruitment curve (RC) of increasing intensities of 10% steps is obtained in ten trials per step. The stimuli intensities from 90% RMT to 140% RMT are applied in a random order. The interval between the stimuli is seven seconds with a 20% variance to avoid familiarization. The analysis of peak-to-peak amplitude (MEP) and RC will be performed in Matlab™ for Windows (Mathworks Inc., Natick, MA, USA).

Electroencephalography

For electroencephalography (EEG) measurement, the participants wear a 20-channel dry-electrodes Enobio device (Neuroelectrics, Barcelona, Spain) [270, 271]. The EEG system records and visualizes 24 bit EEG data at 500Hz. The device sends the data via wireless connections to a personal computer where data can be monitored in real-time. During the EEG recording, the participants perform a Go/No-go task including the suppression of a response in the presence of irrelevant stimuli. The stimuli presentation of the Go/No-go task stems from the Test for Attentional Performance (TAP). The task is presented on a personal computer screen in front of the participants for about 10 minutes (five times two minutes). On a keyboard, the participants have to push a predefined button when the relevant stimuli appear. One investigator records the right and wrong event-related responses of the participants comparing the stimuli of the Go/No-go task and the trigger appearing on the EEG screen. At the time point of clicking, a trigger is recorded and integrated into the EEG data recording. The trigger time points will be used for further analysis of the EEG data including response-related potentials (RRP). The analysis will be performed in Matlab™ for Windows (Mathworks Inc., Natick, MA, USA).

Test for Attentional Performance

The TAP (D-TAP 2.3 VL, PSYTEST, Psychologische Testsysteme, Herzogenrath, Germany) was initially developed to assess deficits in attention. The TAP is a valid test with the subtests measuring different and statistically independent attentional aspects [217]. On a frontal screen, the participants see each test running on a personal computer. A button, placed in front of the participants, is used to record the reaction time and failure rate of the participants. Before the main test starts, the participants perform a pre-test to clarify the procedure and to minimize possible learning effects. The participants execute two tests: (1) Working memory (5 minutes): The participant has to compare presented double-digit numbers on the screen with previously exposed double-digit numbers. By pressing the button, the participants indicate the repetition of a number within a short interval; and (2) Divided attention (3.25 minutes): This subtest consists of visual and acoustic signals presented in an asynchronous way. In a 4x4 matrix, the visual task consisting of crosses appearing in a random configuration. The acoustic part consists of low and high beeps playing in a regular sequence. The participant has to detect whether the cross forms the corners of a square or whether the beeps have an irregularity in their sequence.
Gait Analysis

Temporal (time) and spatial (distance) gait parameters are measured with the Physilog (Gait up Sàrl, Lausanne, Switzerland) via wearable standalone movement sensors (50×37×9.2mm, 19 grams, anatomical curved shape) containing inertial sensors. A button on the sensors allows the start and stop of measurement. A micro-USB port allows data transfer to the personal computer for further analysis of gait performance data. Physilog provides objective, quantitative, and valid assessment of gait movement [210-212, 272]. The sensors are fixed with elastic straps at the right and left forefoot of the participants for flat over ground gait analysis over a distance of 10m. Participants perform a single-task condition (preferred walking) and a dual-task condition, i.e. preferred walking whilst counting backwards in sevens from a random given number. The participants are instructed to position themselves at the beginning of the walkway and are asked to walk with their comfortable speed to the end of the walkway. Thereafter, the participants are asked to perform the same walking task while counting. For counting, the participants get a random number between 200 and 250 at the start. The instructions are standardized as follows: (1) “Walk with your comfortable speed right to the end of the walkway.” (2) “Walk with your comfortable speed right to the end of the walkway counting backwards from [random number between 200-250].” The participants have to count loud and don’t stop walking; otherwise, the trial is recorded as failure. Instructions are given that no one task should be prioritized over the other. Assistive devices like canes, crutches or walking frames can be used if necessary. Each tested condition is repeated three successful times to obtain representative samples and the means of the three successful trials will be used for further data analysis. For each participant the relative dual-task costs (DTC) of walking, as percentage of loss relative to the single-task walking performance, according to the formula $\text{DTC} \,[\%] = 100 \times \frac{\text{single-task score} - \text{dual-task score}}{\text{single-task score}}$ will be calculated [273].

Blood Sample

Venous blood samples are collected by a qualified investigator and stored in 2.7ml EDTA tubes (S-Monovette, K3 EDTA, 75×13mm, Sarstedt, Germany). Blood samples are taken to analyze FA values in erythrocytes. This direct method for FA analysis is reliable and accurate with Limits of Detection of the FAs profiles ranging between 0.23 - 3.19 μg [274]. Pre- and post-dosage FA values will be assessed and compared with reference values previously reported [275]. The FA parameters will be analyzed by Omegametrix GmbH (Martinsried, Germany). The laboratory meets the strict criteria of the quality standard DIN ISO 15189.

Mini Mental State Examination

The Mini Mental State Examination (MMSE) is a reliable and valid test to quantitatively estimate the severity of cognitive impairment [226, 227]. The 30 questions of the MMSE are categorized into seven categories: 1. orientation to time, 2. orientation to place, 3. registration of three words, 4. attention and
calculation, 5. recall of three words, 6. language, and 7. visual construction. An investigator performs the test with the participants by giving zero points or one point for incorrect or correct answer, respectively.

**Geriatric Depression Scale**

The Geriatric Depression Scale (GDS) is a self-report questionnaire to identify depression in older adults [228]. The GDS is a valid and reliable depression screening [228]. The short form has 15 questions focusing on worries of the participants, and the way they conceive and interpret their quality of life [229]. The questionnaire can be answered in a yes/no response.

### 4.2.8 Data Collection

All consenting participants received a case report form (CRF) to ensure that eligibility criteria are met and to ensure all measurements are performed. The CRF includes a confirmation that participants read the information sheet and signed the informed consent. Furthermore, the list of inclusion and exclusion criteria is included to confirm the eligibility of the participants. For the measurements, the steps are cross-checked for digital recording data (TMS, EEG, TAP, and gait), results are noted (short FES-I, GDS, MMSE) and blood taking is confirmed. The data from TMS, EEG, TAP, and gait are stored on a personal computer for further analysis. Any digital data, blood samples, questionnaires, and CRFs are coded with the individuals’ ID.

### 4.2.9 Sample Size

To avoid a type I or II error, the power calculation was based on a study examining the effect of omega-3 FA on EEG frequency band distribution during a sustained attention test [276]. The aforementioned study was used for sample size calculation because so far there exists no study that examined the influence of exergame training in combination with omega-3 FA on neuronal systems using TMS or EEG methodology. Due to the values of EEG frequency band distribution during sustained attention tests (with values K+K = 27.70 ± 5.2; K+K = 31.49 ± 8.6), an estimated sample size of 24 participants would result in 80% power at an alpha-level of 0.05 for this parameter. To account and compensate for expected drop-outs, the study includes 30 participants in each group. Drop-outs are expected because of the long study duration of 26 weeks, the age of the participants, and numbers are based on previous reports on adherence of non-institutionalized older adults to exercise programs [277].

### 4.2.10 Statistics

The data analysis will be performed at the end of intervention including the measurement values from pre- and post-intervention measurements. Data will be tested for normal distribution using Shapiro-Wilk test and Q-Q-plots. A 2x2-ANOVA will be used with normally distributed data, the non-parametric
equivalent for data not fulfilling assumptions of normal distribution. The test will be used to compare
the two interventions over time (from pre- to post-measurement) on changes on the main dependent
variables MEP, RRP, RT of cognitive tasks, spatio-temporal gait parameters, DTC, and FA levels. In
addition to statistical significance testing effect size calculation ($r=Z/\sqrt{N}$) will be performed. The
participants’ FA blood levels, demographic, and health information will be examined in relation to the
outcome measures in order to interpret the results in context. All statistical procedures will be conducted
with the IBM Statistical Package for the Social Science software package. A probability level of $p < 0.05$
will be considered to be statistically significant.

4.3 Stepwise Procedure

The stages of the study procedure are illustrated in figure 4.1. The study includes two measurement time
points (pre- and post-intervention) which are performed in a laboratory at the ETH Zurich (Hönggerberg,
Switzerland). The two data collection sessions are performed by treatment-blinded investigators
including the following assessments: TMS measurement, EEG measurement, TAP performance, gait
performance, and questionnaires (short FES-I, MMSE, and GDS). The pre-measurement consists of a
screening and measurement part. The screening part contains data of the MMSE, GDS, and health
questionnaire (including questions about physical impairments, medical history, anthropometric data,
and physical activity level) and is used to determine eligibility for the measurement part and study
participation, respectively. The measurements for each session take about two hours and are conducted
to determine the effects of the interventions on brain structure and function. The pre-intervention
measurement is planned to be performed in the week before the intervention starts. The post-intervention
measurement is planned for the first week after the intervention, while the possibility consists to postpone the measurement by one week. In addition, blood samples are taken at pre-intervention
measurement, after 16 weeks of nutritional supplementation intervention, and at post-intervention
measurement.

The intervention starts on Monday of the week following the pre-intervention measurement and lasts 26
weeks. Each day for 26 weeks, the participants have to take the same amount of nutritional
supplementation regularly at home, while the intake time point should be as consistent as possible. The
investigators can reproduce the intake using the individual intake protocol of each participant. After 16
weeks, the participants start with the exergame training lasting for 10 weeks. In small groups, each
participant trains three times per week for 30min. The training time points are individualized for each
participant and may also be variable from week to week for individual participants. Each participant has
to attend at least 70% of training to be considered for (per protocol) analysis.
4.4 Anticipated Results

The results of this study can be influenced by several factors. One might be the adherence to regular intake of the nutritional supplementation. As countermeasures an intake protocol supports the participant and helps the investigator to control the intake. Blood sample analysis is an additional control measure. Moreover, the training attendance is checked by the investigators, so that each participant reaches at least 70% attendance. However, a higher drop-out rate may be expected because of the long trial duration. The aforementioned sample size calculation included the calculated 25 participants and five additional participants for each intervention group because of the expected drop-outs. Moreover, TMS is used in this study as an indirect proxy measure of synaptic plasticity. Based on the results of this study future studies should focus on more direct measures of synaptic plasticity by using either magnetic resonance tomography [278] or positron emissions tomography scans [279] for comparing the brain scans before and after the intervention.
The aim of this study is to investigate the neuronal effects of an intervention that combines exergame training with omega-3 FA supplementation. Several animal studies combined omega-3 FA and PE focusing on the neuronal effects and the possible underlying mechanism. One study concluded that omega-3 FA interacts with PE in improving the axonal growth, synaptic plasticity, and cognitive function of the adult rat brain [78]. Omega-3 FA and PE might act both on the energy metabolism of hypothalamus and hippocampus thereby influencing brain plasticity and cognitive function [121]. Moreover, the combination may increase the level of brain-derived neurotrophic factor resulting in the activation of CREB and synapsin I [77]. The activated metabolic pathway supports neuroplasticity and cognition. So far, no comparable human study exists that combines PE, especially exergame training, and omega-3 FA. The trial design is based on the results of the aforementioned animal studies and of the sole-administration studies of either exergame training or omega-3 FA for older participants focusing on the brain. An exergame intervention was chosen because a combination of cognitive and motor training is expected to have positive effects on the elderly brain [67, 68, 255] and is able to ameliorate EFs in older adults [204, 266]. Motor training builds new synaptic connections while the cognitive part supports the preservation of the new build structure. In aging humans, PE can strengthen neuronal structure, synaptic plasticity, and transmission as well as cognitive function [11]. PE might trigger molecular and cellular mechanisms supporting brain plasticity [14]. Furthermore, video game-based training serves as a powerful tool to train cognitive abilities [61, 63], including attention and EFs [62] as well as to evaluate functioning of underlying neuronal mechanisms explaining cognitive control [60]. In normal aging humans, omega-3 FA might improve cognition [79] and ameliorate brain deterioration through the positive effects on brain structure, function, and cerebral blood flow [80]. A recent randomized-controlled trial showed that omega-3 FA had positive effects on EF, white matter microstructure integrity, gray matter volume, and vascular parameters [81]. Both interventions, exergame training and omega-3 FA, are believed to act on the same (metabolic) brain pathways and, therefore, complement each other [74]. The brain gets trained during the exergame training and the omega-3 FA might, in this case, provide the needed substance and energy to build up new structures and to support metabolic pathways. An interaction is created that might be more effective for brain structure and function compared to the sole-administration of an individual intervention component.

**Author contribution:** AS and EDB developed the research question. AS developed the study design and measurements protocol while EDB acted as methodological council. EDB edited and revised the study protocol from AS. Both authors have read and approved the final manuscript.

**Acknowledgements:** The authors wish to thank San Omega AS for supplying the fish and olive oil and Swiss Medical Plus for the helpful support. The trial is supported by the ETH Foundation through ETH Research Grant ETH-17 13-2.

**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.
Interplay of Exergame Training and Omega-3 Fatty Acids on the Elderly Brain: A Randomized Double-Blind Placebo-Controlled Trial

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Keywords: nutritional supplementation, nutrition, physical exercise, brain function, brain metabolism, aging

Submitted for publication¹

¹Figure, tables, and language errors in the original publication were adapted/corrected for this thesis
Abstract

Elderly people often suffer from age-related neuronal changes affecting proper neuronal functioning and, therefore, cognitive and motor functions. The improvement of these factors might decrease fall events and related injuries and immobility. Previous studies showed that video game-based physical exercise, so-called exergames, or omega-3 fatty acids (FA) may improve motor and cognitive functioning through neuronal changes in the brain of older adults. The aim was to assess the effects of exergame training when combined with omega-3 FA supplementation on motor evoked potentials (transcranial magnetic stimulation), response-locked potentials (electroencephalography) during a cognitive test, executive functions, and gait parameters. We hypothesized that the combined intervention would differently affect these factors compared to the sole administration of exergame training. Fifty-nine participants were randomly assigned to one of two groups and forty-three (mean age 69.4 ± 4.6 years) completed the entire study. The study was a parallel, double-blind, randomized controlled trial lasting 26 weeks. The experimental group received daily fish oil, whereas the control group received daily olive oil for 26 weeks. After 16 weeks, both groups started with an exergame training program three times per week. Measurements were performed pre, during, and post intervention. The results showed no significant time × group interaction effects. For time main effects, response-locked peak amplitudes before and after response-related onset showed a significant increase in prefrontal-located channels. Furthermore, the number of errors for the divided attention test significantly decreased. Moreover, gait parameters significantly improved predominantly under dual-task walking and, therefore, also dual-task cost of walking. The main component, evoking time main effects, seems to be exergame training, while omega-3 FA may have acted as a supportive component. Future studies should consider neuroimaging methods to define neuroplastic brain changes. Furthermore, a longer exergame training period might be needed for the interplay between exergame and omega-3 FA to become apparent.
5.1 Introduction

Age-dependent changes in the human brain involve structural, functional, and metabolic levels. Age-associated alterations in white matter integrity and grey matter [1-3] and a decrease in synthesis and binding of dopamine, serotonin, and acetylcholine [4-8] go along with deteriorations of cognitive functioning, e.g. executive functions (EFs). EFs are higher-level cognitive functions that control and guide lower-level cognitive functions and goal-directed actions [9], such as walking in challenging environments. Gait performance is partially explained by different EF components, e.g. “working memory” [10], “inhibition” [12], and “divided attention” [11]. Especially divided attention is associated with temporal and spatial dual-task cost characteristics of gait [13]. Gait disturbances and connected falls are believed to be moderated by EFs [14, 15].

The prefrontal cortex (PFC), in particular the dorsolateral prefrontal cortex and related brain networks, has been linked with EFs, [16, 17]. Better EFs are associated with a greater PFC thickness and a larger PFC volume [18]. During life, the (pre)frontal network undergoes age-related transformations; however, no consensus exists to the precise pattern of EFs adjustment [1, 17, 19, 20]. One assumption is that the decline in frontal grey matter and white matter integrity might be associated with the deterioration of EFs. Rosano et al. (2012) illustrated that a smaller volume of the PFC is likely to contribute to slower gait through slower information processing [21]. Furthermore, disruption of cortico-cortical and cortico-subcortical networks, e.g. frontal connections with parietal lobes and basal ganglia, respectively, are classified as higher level gait disorders [15, 22]. Therefore, training of EFs might contribute to improved gait performance [23] and might reduce fall events as EFs performance predicted the risk for future falls [24] in older adults.

Until now, training of cognitive abilities (e.g. EFs) may represent an important approach to preserve brain functioning and also prevent mobility disability [21, 25-27]. Nonetheless, recent reviews focusing on the interplay between cognitive and physical functions concluded that a combined motor-cognitive training seems to be important for clinical practice to enable older adults to move safer in their physical environment [27-30]. In the brain, physical training triggers brain plasticity by cell proliferation and synaptic plasticity, while cognitive training seems to support the surviving of newborn neurons and their integration in pre-exiting networks [28, 31, 32]. Especially, computerized interventions seem promising [27, 28, 33] when providing training principles that enhance (motor) learning [33]. A video game-based physical exercise, or a so-called exergame, allows concurrent training of motor and cognitive abilities. Incorporated video games might have the potential to train cognitive functions [34, 35]. Physical exercise (PE) interventions with decision-making opportunities are potentially able to improve both motor performance and cognition [36]. Recent studies showed positive effects of exergame training on EFs and gait performance under dual-task condition in elderly [37, 38] and a meta-analysis revealed that physical-active video games benefit both healthy older adults and clinical populations with conditions associated with neurocognitive impairments [39].
In various review articles, it is hypothesised that the effects of PE on the brain can be enhanced by concurrent consumption of natural products [40-44]. This would mean, as a way of example, that a combination of physical training with a nutritional supplement (NS) has the potential to further enhance the effects of physical training on the level of brain structure and function in older adults. The potential interplay between PE and nutrition is derived from the involvement of common cellular pathways important for neurogenesis, cell survival, synaptic plasticity, and vascular function [40-44].

Nonetheless, a recent systematic review concluded that previous interventions using a combined approach of PE and NS to effect on the human brain were not particularly successful because of the misfit between the combinations; the intervention components were not selected based on sharing of similar neuronal mechanisms [45]. The review argues, however, that especially omega-3 fatty acids (FA), present in fish oil, might be an effective NS supporting the positive effects of PE. Omega-3 FA are important for energy metabolism, for the function and integrity of the neuronal plasma membranes (with docosahexaenoic acid (DHA), arachidonic acid, and eicosapentaenoic acid (EPA) as their main components), and for blood perfusion in the brain [46, 47]. Particularly, older adults may profit from FA supplementation, as in the aging brain the concentration of long chain polyunsaturated FA (LCPUFA) concentration decreases [46]. LCPUFA intake improves cognition, decreases (neuro)inflammation, and reduces vascular risk factors in normal aging adults [46]. Moreover, LCPUFA may affect the brain through positive effects on neuronal structure, function, and cerebral blood flow [48]. For example, DHA acts as a neurotrophic factor by increasing the level of the brain-derived neurotrophic factor [49]. Previous randomized-controlled studies showed that fish oil had positive effects on brain structure and function in healthy older adults, and participants reportedly improved working memory, EFs, white matter microstructure integrity, grey matter volume, and vascular parameters [50, 51].

So far, studies could show that DHA supplementation enhanced the effects of exercise on axonal growth, brain derived neurotrophic factor-related synaptic plasticity, and cognition in rats [49, 52]. However, no study exists that investigated the combined effect of exergame training and omega-3 FA on the human elderly brain’s structure and function. This study, therefore, aims to investigate the effects of a combination of exergame training and omega-3 FA. The following research question guided through the research process: “Does the combination of exergame training and fish oil differently affect neuronal system levels in the elderly brain compared to exergame training alone?” The main objectives of the study were (1) to determine the effects of the intervention on the neuronal structural level of the brain (neuronal excitability) and (2) to assess the effects on functional level in the brain (neuronal activity). We hypothesized that the combination would differently affect these parameters.
5.2 Methods

5.2.1 Study Design

The study was a randomized double-blinded, placebo-controlled study involving elderly adults above 65 years. Detailed information about the study procedure and protocol has been previously described in detail [53]. Between December 2015 and June 2016, potential participants were recruited from the Senior’s University Zurich (Switzerland), senior residency dwellings in Zurich (Switzerland), and through public advertisement and flyers. The study was divided in three blocks, the first block started at the beginning of March 2016, the second block at the beginning of Mai 2016, and the third at the beginning of June 2016. Measurements and data collection, exergame training, and data analysis were conducted at the same study site (ETH Hönggerberg, Zurich, Switzerland). The participants were provided with NS for home supplementation and expected to take the NS regularly. Measurements were performed before and after the intervention period of 26 weeks. For blood samples, an additional measurement was performed before the training started (after 16 weeks). The study procedure has been approved by the local ethics committee (EC Zurich Switzerland, EC number: 2015-0190) and conforms to the Declaration of Helsinki and the guidelines of Good Clinical Practice E6 (R1). No data was recorded before written informed consent was given by the participants. This study has been registered in the Swiss National Clinical Trials (SNCTP000001623) and the ISRCTN (ISRCTN12084831) portals. The study followed the Consolidated Standards of Reporting Trials (CONSORT) statement on randomized trials of non-pharmacological treatment [54] (Table S1).

5.2.2 Participants

Participants fulfilling all of the following inclusion criteria were eligible to partake: (1) age at or above 65 years, (2) live independently or in a senior residency dwelling, (3) healthy (self-reported), and (4) non-smoker. Participants were excluded, if they showed one of the following exclusion criteria: (1) mobility impairments, (2) rapidly progressive or terminal illness as well as acute or chronic illness, (3) orthopedic or neurological diseases (e.g. stroke or epilepsy) that prevent training participation, (4) history of heart attack, (5) medication that interacts with NS (i.e., oral hypoglycemic drugs or anticoagulants), (6) medication that acts on neuronal level (i.e., psychotropic medications), (7) cognitive impairment (Mini Mental Status Examination < 22 points), (8) signs of an upcoming depression (Geriatric Depression Scale), (9) electronic or metallic head implants (self-reported), and (10) personal history of dizziness (self-reported).

The intended sample size of 60 participants was calculated by a power calculation and based on a study examining the effect of omega-3 FA on electroencephalography (EEG) frequency band distribution during a sustained attention test [55]. So far no study directly investigated the combined approach, therefore, this study was used for sample size calculation. Based on the values of EEG frequency band
distribution during sustained attention tests (with values $K+K=27.70\pm5.2$; $K+K=31.49\pm8.6$), an estimated sample size of 24 participants per group would result in 80% power at an alpha-level of 0.05 for this parameter. The study included 30 participants in each group, to account and compensate for expected drop-outs. Due to the long study duration of 26 weeks and the age of the participants, drop-outs were expected. Moreover, the numbers were based on previous reports on adherence of non-institutionalized older adults to exercise programs [56].

### 5.2.3 Intervention

The study interventions have been previously published in detail in line with the Template for Intervention Description and Replication (TIDieR) guidelines [57]. Table S2 summarizes the TIDieR guidelines with the help of a checklist to facilitate readers and other researchers using or replicating the intervention.

**Nutritional Supplementation**

The NS were packed in bottles identical in outer appearance. An external center (Kantonsapotheke Zurich, Switzerland) was responsible for blinding to achieve double blinding. The external center created a computer-generated list including numbers from 001 to 060 that corresponded to either fish oil or olive oil, respectively. The list number was different to the participants’ identification (ID) number. The list consisted of six blocks of ten whereas fish oil and olive oil were randomly and equally distributed in all blocks. The volunteering women were continuously assigned to the numbers starting with 001 and ending with 030 and the men starting with 031 and ending with 060. The individual who maintained the master randomization list (EDB) was responsible for assigning randomization codes, securely storing all randomization files out of reach and sight of the other investigators, and notifying appropriate study staff that the participant had been randomized. The groups were referred to without specification of NS (e.g., group A and B) for statistical analysis.

**Exergame Training**

The participants performed specific whole body movements on a pressure-sensitive dance plate (Impact Dance Platform, 87.5cm×87.5cm×2.5cm, Positive Gaming BV, BZ Haarlem, Nederland) triggered by a video game (VG) presented on a frontal screen. A desktop computer, connected to the dance pad via USB, presented the game interface. Participants were provided with real-time visual and auditory feedback using electronic sensors in the dance pad that detected position and timing information. The participants interacted with the VG through foot pushes on the plate arrows (right, left, top, and bottom). The VGs (dividat, Schindellegi, Switzerland) were designed to train different aspects of EFs. Training principles similar as described by Healy et al. (2014) [64] were implemented; a feedback system to facilitate training, individual levels of difficulty according to individual skills and abilities, variability
of training to enhance task transfer, and adjustable task difficulty to facilitate retention. In addition, the FITT training principles were applied; Frequency: three times per week, Intensity: individually adapted VG, Type: motor-cognitive training, and Time: 30min training per session.

After 16 weeks, all participants started to perform the exergame training lasting 10 weeks. The participants trained three times per week, where one session took 30min. Exergame training contained one session of each VG (4min) in a predefined order and with short breaks (~1min) for game change. In training rooms, the participants performed their exercises alone or in small groups of two to four supervised by experienced investigators. The participants received a training plan including dates and time schedule to enhance and control adherence. Furthermore, a training adherence checklist was used by the investigators.

### 5.2.4 Outcomes

**Transcranial Magnetic Stimulation**

On an adjustable chair, participants were comfortably seated with hip, knee, and ankle joint angles of 100°, 120°, and 90°, respectively. Single pulse cortical stimulation was applied by means of a transcranial magnetic stimulation (TMS) stimulator MAGSTIM 200 (Magstim Company Ltd., Whitland, Dyfed, UK) with a “figure of eight” coil placed over the cortical motor area to stimulate the right M. tibialis anterior (TA). Only the dominant side was assessed because of the symmetrical nature of TMS-related measurements of the lower limb [66]. TMS-related measurements of the TA are reliable in healthy participants [66].

A recruitment curve (RC) of increasing intensities of 10% steps was obtained in ten trials per step. The stimuli intensities from 90% to 140% resting motor threshold (RMT) were applied in a random order [69]. Muscle activity was recorded by Telemyo DTS (Noraxon, ColognFe, Germany). The electrodes (Ambu® Blue Sensor N, Cambridgeshire, UK) were placed with an inter-distance of two centimeters on the muscle belly of the right TA. The analysis of peak-to-peak amplitude of motor evoked potential (MEP) and RC was performed in Matlab™ for Windows (Mathworks Inc., Natick, MA, USA). The peak-to-peak amplitudes were normalized to the individual maximal pre-measurement RMT (140%).

**Electroencephalography**

The participants wore the 20-channel dry-electrodes Enobio device (Neuroelectrics, Barcelona, Spain) [70, 71]. The device sent the data via Bluetooth connection to a personal computer where data were monitored in real-time using Neuroelectrics Instrument Controller 1.4.8 software. During data recording, the participants performed a Go/No-go task (Test for Attentional Performance (TAP; see following paragraph for company details)). On a personal computer screen, the task was presented in front of the participants for about 10 minutes (five times two minutes). On a keyboard, the participants
had to push a predefined button when the relevant stimuli (Go stimuli) appeared \( (N_{\text{Total}} = 100) \). One investigator recorded the right (Go stimuli) and wrong (No-go stimuli) responses of the participants comparing the stimuli of the Go/No-go task and the trigger appearing on the EEG screen. A trigger was recorded at the time point of clicking and integrated into the EEG data. The trigger signals were used for further analysis of the EEG data including response-locked potentials (RLP). For time reasons and due to technical difficulties, EEG analysis was focused on RLP and no EEG acquisition was performed during gait performance.

Off-line signal processing was performed using EEGLAB [72] and ERPLAB [73] toolboxes for Matlab™ for Windows (Mathworks Inc., Natick, MA, USA). Based on previous studies, the focus of analysis was set to prefrontal-located channels Fp1 and Fp2 as well as midline-located channels from frontal to parietal (Fz, Cz, and Pz) [38, 74, 75]. First, the five runs of the Go/No-go task were merged and divided by 1000 to build one dataset in \( \mu \)V for each participant. Then, dataset was filtered (zero phase FIR filters) using high-pass filter at 0.1 Hz, notch filter \( (48 \text{ Hz} - 52 \text{ Hz}) \), and low-pass filter at 30 Hz. Afterwards, bad channels were rejected using joint probability of the recorded electrodes (kurtosis above 5 standard deviations from the mean of all channels). The next step was re-referencing of the data to the average followed by deletion of events (clicks) appearing at “irrelevant” stimuli (No-go signals). Then, RLP were averaged for 1000 ms preceding the response onset and 1400 ms following it including baseline correction \([-1000 \text{ms} \text{ to } -600 \text{ms}]\) according to Luck (2014) [76]. In addition, the epochs containing values exceeding \( \pm 50 \mu \text{V} \) were rejected. The analysis resulted in repeatable RLP for the prefrontal located channels Fp1 and Fp2. Therefore, further analysis was performed focusing on channel Fp1 and Fp2. For Fp1 and Fp2, peak amplitude was analysed for positive peak before and for negative peak after response onset.

**Test for Attentional Performance**

The TAP (D-TAP 2.3 VL, PSYTEST, Psychologische Testsysteme, Herzogenrath, Germany) was initially established to measure deficits in attention. The TAP is a valid computer-based assessment including subtests that measure different and statistically independent attentional aspects [77]. Each test was played on a screen of a personal computer in front of the participants. To clarify the procedure and to minimize possible learning effects, the participants first performed a pre-test. The participants executed two tests: (1) Working memory test (5 minutes) and (2) Divided attention test (3.25 minutes). Cognitive testing focused on the TAP test battery, while predefined Attention Network Test was discarded to prevent too high participant burden of testing.

**Gait analysis**

Spatial (distance) and temporal (time) gait parameters were measured with the Physilog (Gait up Sàrl, Lausanne, Switzerland) via wearable standalone movement inertial sensors \( (50\times37\times9.2\text{mm}, 19 \text{ grams,} \)
anatomical curved shape). This device provides objective, quantitative, and valid assessment of gait movement [78-81]. Elastic straps were used to fix the sensors at the right and left forefoot of the participants for flat over ground gait analysis over a distance of 10m. Participants executed a single-task condition at preferred walking and a dual-task condition, i.e. preferred walking whilst counting backwards in sevens from a random given number between 200 and 250. First, participants were instructed to position themselves at the beginning of the walkway and were asked to walk with their comfortable speed to the end of the walkway. Second, participants were asked to perform the same walking task while counting. The participants had to count loud and not stop walking; otherwise, the trial was recorded as failure. The participants, if necessary, could have used assistive devices like canes, crutches. Each tested condition was repeated three successful times to obtain representative samples. The means of the three successful trials were used for further analysis. For further analysis, a micro-USB port allowed data transfer to the personal computer. In addition to spatio-temporal gait parameters, the relative dual-task cost (DTC) of walking, as percentage of loss relative to the single-task walking performance was calculated, according to the formula DTC [%] = 100 * (single-task score – dual-task score)/ single-task score [82]. Acceleration and deceleration steps were removed from the data and not counted to analyze steady state walking.

Blood sample

A qualified investigator collected venous blood samples and stored the samples in 2.7ml EDTA tubes (S-Monovette, K3 EDTA, 75×13mm, Sarstedt, Germany). To analyze FA concentrations in erythrocytes, blood samples were taken at pre-, middle- (after 16 weeks), and post-measurement. In this study, the focus was on the omega-3 index that includes the content of LCPUFA EPA and DHA within red blood cells. Omega-3 index is defined as the percentage of EPA and DHA in the red cell membrane, with the remaining FA building up to 100% [83]. The FA parameters were analyzed using a standardized method (gas chromatograph) under rigorous quality control by Omegametrix GmbH (Martinsried, Germany). The laboratory meets the strict criteria of the quality standard DIN ISO 15189.

Cognitive Status, Depression, and Fall Efficacy Scale

The Mini Mental State Examination (MMSE) is a valid and reliable test to quantitatively estimate the cognitive status [84, 85]. The 30 questions are grouped into seven categories: 1. orientation to time, 2. orientation to place, 3. registration of three words, 4. attention and calculation, 5. recall of three words, 6. language, and 7. visual construction. The participants performed the test with an investigator who gave zero points or one point for incorrect or correct answers, respectively. The Geriatric Depression Scale (GDS) is a reliable and valid self-report depression screening questionnaire to identify depression in older adults [86]. The short form has 15 questions including worries of the participants, and the way they conceive and interpret their quality of life [87]. The questionnaire can be answered in a yes/no response. The short form falls efficacy scale international (FES-I) was used to assess concern about
failing. The scale measures both easy and difficult physical and social activities and contains seven items with a 4-point scale (1 = not at all concerned, 2 = somewhat concerned, 3 = fairly concerned, 4 = very concerned). The short FES-I is a feasible scale to determine fear of falling in elderly [88].

5.2.5 Statistics

All statistical procedures were conducted with the IBM Statistical Package for the Social Science software package, version 22. A per protocol analysis was performed. Data were tested for normal distribution using Shapiro-Wilk test and Q-Q-plots. In case of non-normal distribution, the data were rank-ordered in order to perform, for each variable, an analysis of variance (ANOVA) with one within-subjects factor (time: pre-, (middle-), and post-measurement) and one between-subjects factor (intervention group: fish oil and exergame/ olive oil and exergame). Bonferroni correction for multiple comparisons was used. The analysis allowed to compare time main effects and time × group interaction effects, using the Puri and Sen L Statistics for ranked data [89]. L value was calculated using Pillai’s Trace: \( L = \frac{(N - 1)r^2}{1} \) where \( N \) = amount of participants and \( r^2 = \) Pillai’s Trace. Moreover, baseline (pretest) comparisons were undertaken using Mann-Whitney U test. Furthermore, within-group comparisons for omega-3 index were performed using Wilcoxon signed-rank test. Omega-3 index statistics were corrected for multiple hypotheses comparisons (\( p < 0.025 \) was considered to be statistically significant). A probability level of \( p < 0.05 \) was considered to be statistically significant. Effect sizes assessing meaningfulness of differences within- and between-group design were calculated and expressed using the following equation: \( r = \frac{Z}{\sqrt{N}} \) where \( Z = Z\)-score and \( N = \) amount of participants.

An effect size of \( r = 0.1 \) is considered a “small” effect, around 0.3 a “medium” effect, and 0.5 and above a “large” effect. Effect sizes assessing between × within group design were calculated and expressed as \( \eta^2 \) where percentage of the total variance can be accounted for by group membership [90], using the following equation: \( \eta^2 = \frac{SS_{effect}}{SS_{effect} + SS_{error}} \) where \( SS = \) sum of squares.

5.3 Results

A total of 58 participants were randomly assigned to one of the two groups: (1) fish oil and exergame training or (2) olive oil and exergame training. Forty-three participants completed the whole study procedure. One participant was excluded from analysis because the supplementation intake was 39%. The study flow chart is presented in Fig 1. The analysis does not consider intention-to-treat analysis because of a clear description of the reason(s) for drop-out (CONSORT 2010 guidelines [91]). Table 1 summarizes demographic characteristics, screening values, and intervention details of the participants. The significant difference for the GDS value was not considered for the analysis, since all the values were in the normal range indicating no signs of depression. All the participants reached a minimal amount of 70% (21 training sessions) training participation.
FIGURE 5.1 | Study flow chart. NS = nutritional supplementation.

TABLE 5.1 | Demographic characteristics, screening values, and intervention details.

<table>
<thead>
<tr>
<th></th>
<th>Fish oil and Exergame (N=22)</th>
<th>Olive oil and Exergame (N=20)</th>
<th>z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender [F/M]</td>
<td>[10/ 12]</td>
<td>[13/ 7]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
<td>67 (65.75; 72.50)</td>
<td>67.50 (65.25; 75.75)</td>
<td>-0.537</td>
<td>0.592</td>
<td>-0.08</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>76.5 (62.5; 85.25)</td>
<td>74.50 (61.75; 81.50)</td>
<td>-0.554</td>
<td>0.579</td>
<td>-0.09</td>
</tr>
<tr>
<td>Height [m]</td>
<td>1.70 (1.62; 1.80)</td>
<td>1.69 (1.60; 1.76)</td>
<td>-1.148</td>
<td>0.251</td>
<td>-0.18</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.66 (22.80; 27.32)</td>
<td>25.49 (23.47; 27.93)</td>
<td>-0.302</td>
<td>0.762</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mini Mental Status</td>
<td>28.5 (27.75; 29.25)</td>
<td>28 (28; 29)</td>
<td>-0.495</td>
<td>0.620</td>
<td>-0.08</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>1 (0; 3)</td>
<td>0 (0; 1)</td>
<td>-2.209</td>
<td><strong>0.027</strong></td>
<td>-0.34</td>
</tr>
<tr>
<td>Short-FES-I</td>
<td>8 (7; 9.25)</td>
<td>7 (7; 8.75)</td>
<td>-1.203</td>
<td>0.229</td>
<td>-0.19</td>
</tr>
<tr>
<td>Resting motor threshold</td>
<td>42 (40; 45)</td>
<td>43.5 (38.5; 50)</td>
<td>-0.540</td>
<td>0.589</td>
<td>-0.13</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training participation (100% = 30 sessions)</td>
<td>27 (23; 29)</td>
<td>26 (22.25; 27)</td>
<td>-0.881</td>
<td>0.378</td>
<td>-0.14</td>
</tr>
<tr>
<td>Supplementation intake [%]</td>
<td>98.2 (94.5; 100)</td>
<td>96.4 (91.8; 99.5)</td>
<td>-0.840</td>
<td>0.401</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

Data are number of participants or median (interquartile range) values as indicated. p values are two-tailed. *p < 0.05. FES-I: falls efficacy scale international.
5.3.1 Motor Evoked Potentials

For the analysis, one participant had to be excluded due to a technical problem. The RCs including the pre and post MEPs for both groups are illustrated in Fig 2. Table 2 summarizes the time main effects and time × group interaction effects for RLP in Fp1 and Fp2. Significant time main effect was present for 110% RMT stimulation ($L(40) = 4.993, p = 0.023$). No significant time × group interaction effects were present.

**FIGURE 5.2 |** Pre- and post-measurement values of motor evoked potential (MEP) building the recruitment curve. Data are median values (interquartile ranges) as indicated. MEP values were normalized to the individual maximum (140%) pre-measurement RMT.

**TABLE 5.2 |** Time main effects and time × group interaction effects of repeated measures Puri & Sen-analysis of ranked data for motor evoked potentials.

<table>
<thead>
<tr>
<th>Stimulation intensity (%RMT)</th>
<th>Main effect (time, pre vs. post)</th>
<th>Interaction effect (time × intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>$L$</td>
</tr>
<tr>
<td>90</td>
<td>0.021</td>
<td>0.831</td>
</tr>
<tr>
<td>100</td>
<td>0.015</td>
<td>0.611</td>
</tr>
<tr>
<td>110</td>
<td>0.125</td>
<td>4.993</td>
</tr>
<tr>
<td>120</td>
<td>0.091</td>
<td>3.623</td>
</tr>
<tr>
<td>130</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>140</td>
<td>0.018</td>
<td>0.730</td>
</tr>
</tbody>
</table>

$N = 41$; fish oil and exergame group $N = 21$ and olive oil and exergame group $N = 20$. *p $< 0.05$, p-values are two-tailed. $\eta^2$: effect size.
5.3.2 Response-Locked Potentials

For the analysis of Fp1, ten participants had to be removed because no RLP was present. For the analysis of Fp2, data from two participants had to be removed because of channel artifacts and data from seven participants had to be removed because no RLP was present. The RLP of Fp1 and Fp2 showed an equal composition with a positive peak before the response onset followed by a negative peak after the response onset (Fig 3). The total number of epochs, used for analysis, were for the fish oil and exergame group pre (Fp1: N = 1838, Fp2: N = 1868), post (Fp1: N = 1626, Fp2: N = 1650) and for the olive oil and exergame group pre (Fp1: N = 1499, Fp2: N = 1500), post (Fp1: N = 1542, Fp2: N = 1463). Table 3 summarizes the time main effects and time × group interaction effects for RLP in Fp1 and Fp2. Significant time main effects were present for Fp1 (L(31) = 3.922, p = 0.046) and for Fp2 (L(32) = 6.054, p = 0.011) for the positive peak before the response onset as well as for the first negative peak after the response onset Fp1 (L(31) = 3.816, p = 0.049) and for Fp2 (L(32) = 4.393, p = 0.034). No significant time × group interaction effects were present; neither for Fp1 nor for Fp2.

![FIGURE 5.3 | Pre- and post-measurement response-locked potentials for the prefrontal cortex. The curves represent the mean of both groups and of the channel Fp1 and Fp2 together.](image-url)
TABLE 5.3 | Time main effects and time × group interaction effects of repeated measures Puri & Sen-analysis of ranked data for response-locked potentials.

<table>
<thead>
<tr>
<th></th>
<th>Main effect (time, pre vs. post)</th>
<th>Interaction effect (time × intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>$L$</td>
</tr>
<tr>
<td>Positive peak before response onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fp1</td>
<td>0.127</td>
<td>3.922</td>
</tr>
<tr>
<td>Fp2</td>
<td>0.189</td>
<td>6.054</td>
</tr>
<tr>
<td>Negative peak after response onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fp1</td>
<td>0.123</td>
<td>3.816</td>
</tr>
<tr>
<td>Fp2</td>
<td>0.137</td>
<td>4.393</td>
</tr>
</tbody>
</table>

For Fp1 $N = 32$; fish oil and exergame group $n = 17$ and olive oil and exergame group $n = 15$. For Fp2 $N = 33$; fish oil and exergame group $n = 18$ and olive oil and exergame group $n = 15$. *$p < 0.05$, $p$-values are two-tailed. $\eta_G^2$: effect size.

5.3.3 Executive Functions

For working memory and divided attention test, the reaction time, errors, and omissions of both groups (pre and post) are presented in Table 4. Table 5 summarizes the time main effects and time × group interaction effects for working memory and divided attention test. Significant time main effects were present for divided attention test including errors ($L(41) = 13.700$, $p < 0.001$). No significant time × group interaction effects were present.
TABLE 5.4 | Pre- and post-measurement values of working memory and divided attention test.

<table>
<thead>
<tr>
<th></th>
<th>Fish oil and Exergame (N = 22)</th>
<th>Olive oil and Exergame (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Working memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT [ms]</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>688.50 (587.75; 811)</td>
<td>725 (599; 758)</td>
</tr>
<tr>
<td>Errors</td>
<td>1 (0; 2.25)</td>
<td>1 (0; 3)</td>
</tr>
<tr>
<td>Omissions</td>
<td>1 (0; 3.25)</td>
<td>1.5 (0; 3)</td>
</tr>
<tr>
<td><strong>Divided attention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT auditory [ms]</td>
<td>649.50 (554.50; 712)</td>
<td>684 (611.25; 729)</td>
</tr>
<tr>
<td>RT visual [ms]</td>
<td>950 (838.75; 988.75)</td>
<td>927.5 (831.5; 1015.25)</td>
</tr>
<tr>
<td>Errors</td>
<td>2 (1; 5.5)</td>
<td>1 (0; 1.25)</td>
</tr>
<tr>
<td>Omissions</td>
<td>1.5 (1; 4)</td>
<td>1.5 (0; 3)</td>
</tr>
</tbody>
</table>

|                          |                               |                                 |
| **Working memory**       |                               |                                 |
| RT [ms]                  | 658.5 (589.75; 863)           | 748 (611.50; 838.25)            |
| Errors                   | 2.5 (1; 5)                    | 2 (1; 5.5)                      |
| Omissions                | 3 (2; 5)                      | 2 (1; 4.5)                      |
| **Divided attention**    |                               |                                 |
| RT auditory [ms]         | 674.50 (615.50; 759)          | 691 (629; 764.50)               |
| RT visual [ms]           | 895.5 (830.75; 1000.75)       | 936 (825.25; 1018)              |
| Errors                   | 2 (0; 5.5)                    | 1 (0; 2)                        |
| Omissions                | 2.5 (2; 4.75)                 | 2 (1; 3)                        |

*RT = reaction time.*

TABLE 5.5 | Time main effects and time × group interaction effects of repeated measures Puri & Sen-analysis of ranked data for working memory and divided attention test.

<table>
<thead>
<tr>
<th></th>
<th>Main effect (time, pre vs. post)</th>
<th>Interaction effect (time × intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>L</td>
</tr>
<tr>
<td><strong>Working memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time</td>
<td>0.040</td>
<td>1.659</td>
</tr>
<tr>
<td>Errors</td>
<td>0.003</td>
<td>0.137</td>
</tr>
<tr>
<td>Omissions</td>
<td>0.027</td>
<td>1.095</td>
</tr>
<tr>
<td><strong>Divided attention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT auditory</td>
<td>0.067</td>
<td>2.727</td>
</tr>
<tr>
<td>RT visual</td>
<td>0.014</td>
<td>0.586</td>
</tr>
<tr>
<td>Errors</td>
<td>0.334</td>
<td>13.700</td>
</tr>
<tr>
<td>Omissions</td>
<td>0.054</td>
<td>2.227</td>
</tr>
</tbody>
</table>

*RT = reaction time, N = 42; fish oil and exergame n = 22 and olive oil and exergame n = 20 *p < 0.05, p-values are two-tailed. ƞ²_G²: effect size.*
5.3.4 Spatio-Temporal Gait Parameters

For the toe clearance, dual-task walking and DTC analysis, one participant had to be excluded due to a technical problem. Spatio-temporal gait parameters including speed, cadence, stride length, and toe clearance of both groups (pre and post) are presented in Table 6. Table 7 summarizes the time main effects and time × group interaction effects for spatio-temporal gait parameters. Significant time effects were present for speed including dual-task walking ($L(41) = 6.182, p = 0.011$) and DTC ($L(41) = 6.603, p = 0.008$), for cadence including dual-task walking ($L(41) = 4.179, p = 0.039$) and DTC ($L(41) = 5.078, p = 0.022$), for stride length including dual-task walking ($L(41) = 7.250, p = 0.006$) and DTC ($L(41) = 5.036, p = 0.023$), and for toe clearance including single- ($L(41) = 6.551, p = 0.009$) and dual-task walking ($L(40) = 7.252, p = 0.006$). No significant time × group interaction effects were present.
TABLE 5.6 | Pre- and post-measurement values of spatio-temporal gait parameters.

<table>
<thead>
<tr>
<th></th>
<th>Fish oil and Exergame (N = 22)</th>
<th>Olive oil and Exergame (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Speed [m/s]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task walking</td>
<td>1.34 (1.29; 1.42)</td>
<td>1.33 (1.25; 1.45)</td>
</tr>
<tr>
<td>Dual-task walking</td>
<td>1.15 (0.96; 1.24)</td>
<td>1.12 (0.94; 1.23)</td>
</tr>
<tr>
<td>Dual-task cost [%]</td>
<td>14.01 (7.52; 24.72)</td>
<td>13.51 (9.91; 23.61)</td>
</tr>
<tr>
<td><strong>Cadence [steps/min]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task walking</td>
<td>112.82 (109.41; 118.39)</td>
<td>116.93 (109.24; 121.82)</td>
</tr>
<tr>
<td>Dual-task walking</td>
<td>102.50 (97.03; 112.58)</td>
<td>104.85 (88.10; 114.56)</td>
</tr>
<tr>
<td>Dual-task cost [%]</td>
<td>7.16 (2.68; 12.18)</td>
<td>10.71 (4.56; 16.59)</td>
</tr>
<tr>
<td><strong>Stride length [m]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task walking</td>
<td>1.42 (1.33; 1.48)</td>
<td>1.40 (1.27; 1.44)</td>
</tr>
<tr>
<td>Dual-task walking</td>
<td>1.31 (1.23; 1.42)</td>
<td>1.29 (1.18; 1.37)</td>
</tr>
<tr>
<td>Dual-task cost [%]</td>
<td>7.40 (3.93; 11.67)</td>
<td>6.70 (4.12; 9.72)</td>
</tr>
<tr>
<td><strong>Minimal toe clearance [m]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-task walking</td>
<td>0.025 (0.021; 0.028)</td>
<td>0.026 (0.019; 0.031)</td>
</tr>
<tr>
<td>Dual-task walking</td>
<td>0.025 (0.021; 0.028)</td>
<td>0.024 (0.021; 0.029)</td>
</tr>
<tr>
<td>Dual-task cost [%]</td>
<td>0.48 (-9.49; 6.07)</td>
<td>8.64 (-3.28; 14.53)</td>
</tr>
</tbody>
</table>

\(^1\)N = 19.
### TABLE 5.7 | Time main effects and time × group interaction effects of repeated measures Puri & Sen-analysis of ranked data for spatio-temporal gait parameters.

<table>
<thead>
<tr>
<th></th>
<th>Main effect (time, pre vs. post)</th>
<th>Interaction effect (time × intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>L</td>
</tr>
<tr>
<td><strong>Speed</strong></td>
<td></td>
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</tr>
<tr>
<td>Single-task</td>
<td>0.002</td>
<td>0.068</td>
</tr>
<tr>
<td>Dual-task</td>
<td>0.151</td>
<td>6.182</td>
</tr>
<tr>
<td>DTC</td>
<td>0.161</td>
<td>6.603</td>
</tr>
<tr>
<td><strong>Cadence</strong></td>
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<tr>
<td>Single-task</td>
<td>0.013</td>
<td>0.521</td>
</tr>
<tr>
<td>Dual-task</td>
<td>0.102</td>
<td>4.179</td>
</tr>
<tr>
<td>DTC</td>
<td>0.124</td>
<td>5.078</td>
</tr>
<tr>
<td><strong>Stride length</strong></td>
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<tr>
<td>Single-task</td>
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<td>0.085</td>
</tr>
<tr>
<td>Dual-task</td>
<td>0.177</td>
<td>7.250</td>
</tr>
<tr>
<td>DTC</td>
<td>0.123</td>
<td>5.036</td>
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<tr>
<td><strong>Minimal toe clearance</strong></td>
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<tr>
<td>Single-task</td>
<td>0.160</td>
<td>6.551</td>
</tr>
<tr>
<td>Dual-task$^1$</td>
<td>0.177</td>
<td>7.252</td>
</tr>
<tr>
<td>DTC$^1$</td>
<td>0.004</td>
<td>0.154</td>
</tr>
</tbody>
</table>

*N = 42; fish oil and exergame n = 22 and olive oil and exergame n = 20. *p < 0.05, p-values are two-tailed. $\eta_G^2$: effect size. $^1N = 41$. DTC = dual-task cost.

#### 5.3.5 Blood Fatty Acids Level – Omega-3 Index

Omega-3 index of both groups (pre, middle, and post) are presented in Fig 4. Significant time × group interaction effect ($L(41) = 27.349, p < 0.001, \eta_G^2 = 0.3$) was present in favor of the fish oil intake group, while no significant time main effect ($L(41) = 0.185, p = 0.915, \eta_G^2 = 0.0$) was present. The fish oil intake group showed a significant and meaningful increase ($z = -4.107, p < 0.001, r = -0.88$) after 16 weeks followed by a continued stable omega-3 index at this higher level. Within the olive oil intake group, no difference was present from pre- to middle-measurement, while from middle- to post-measurement the omega-3 index significantly and, based on the effects size, meaningfully decreased ($z = -2.837, p = 0.005, r = -0.63$).
FIGURE 5.4 | Omega-3 index for the three measurement time points (N = 42). The fish oil group (N = 22) had a significant increase of the index from pre- to middle- (after 16 weeks) measurement. The olive oil group (N = 20) had a significant decrease from middle- to post-measurement. An optimal protective level of the omega-3 index is suggested as 8% or higher, an intermediate-risk zone being 4-8%, and an undesirable level as being less than 4% [290]. *p < 0.05, p-values are two-tailed.

5.4 Discussion

The aim of this study was to investigate the effect of exergame training combined with omega-3 FA supplementation on the structure and function of the elderly brain. We hypothesized that the combination of exergame training and omega-3 FA would differently affect neuronal system levels in the elderly brain compared to exergame training alone. The results of our study showed no significant time × group interaction effects in any of the structural or functional parameters.

However, the omega-3 index showed a significant time × group interaction effect in favor of the fish oil intake group indicating the fish oil intake caused higher EPA and DHA levels in the blood. Additionally, during the period of exergame training, the olive oil intake group showed a significant decrease of their omega-3 index while the fish oil intake group remained on a stable, albeit higher then baseline, level. This process might indicate that because of the physical exertion required by exergame training concurrent requirement of omega-3 FA was present. This caused the omega-3 index decreased level in the olive oil intake group, while the level remained stable on a high level in the fish oil intake group due to the supplementation. Although, this seems to indicate interplay between these two intervention components occurred, the observed time main effects in our measures are mainly due to the exergame component of the intervention, while the omega-3 FA might have acted as a supportive component. This
would be in line with a recently published pilot study where the combined approach of aerobic exercise and cognitive stimulation with omega-3 FA showed an effect on gray matter volume as opposed to omega-3 FA intake in combination with stretching and toning [93]. Hence, the motor-cognitive training seems to be the main component evoking the positive effect in our sample of healthy older adults. Because of omega-3 FA being available in all participants at the start of the training, it cannot be completely ruled out that reserves were tapped by the body and used as supportive component for the exergame training.

5.4.1 Omega-3 Index

For the first 16 weeks (pre- to middle-measurement), the fish oil intake group showed a significant increase of their omega-3 index (mean ± standard deviation) from 4.79% ± 0.95% to 10.36% ± 1.72%. The 16 weeks’ period was chosen with the aim of reaching a steady state [59-61]. The olive oil intake group remained on the low baseline level from 5.57% ± 1.47% to 5.70% ± 1.38% in this same period indicating olive oil was an appropriate placebo. An optimal cardio protective level of the omega-3 index is suggested as 8% or higher, an intermediate-risk zone being 4-8%, and an undesirable level as being less than 4% [92]. As is known from previous research, omega-3 FA reduces vascular risk factors in normal aging adults [46]. Connected to the aging brain, omega-3 FA are important for perfusion in the brain and may therefore protect against brain deterioration [46-48]. Furthermore, omega-3 FA are important for energy metabolism and for the function and integrity of the neuronal plasma membranes and therefore, may improve cognition and decrease (neuro)inflammation [46, 47]. During the 10 weeks of exergame training (middle- to post-measurement), the olive oil group showed a significant decrease of their omega-3 index from 5.70% ± 1.38% to 5.25% ± 1.43%, however, remained in the intermediate risk zone. The fish oil intake group remained on the same level from 10.36% ± 1.72% to 9.91% ± 1.98% during this training period. Interestingly, this result might hint at the interplay of these two factors in the sense that the physical activity related to the exergame training required omega-3 FA. Therefore, the olive oil intake group showed a significant decrease because their reserves were tapped. In the fish oil intake group, the omega-3 FA remained on a constant high level due to the daily supplementation of omega-3 FA. We assume that omega-3 FA were used in both groups to support the energy metabolism that was stimulated by the exergame training. Previous findings revealed that omega-3 FA are important for the energy metabolism [47]. Furthermore, exergame training might stimulate neuroplastic changes and thus omega-3 FA provided building material to the brain [41]. Thus, omega-3 FA might support the effects of exergame training. Nevertheless, within the fish oil intake group the omega-3 index was distributed over a bigger range when comparing middle- to post-measurement. This might be indicative for the amount of used omega-3 FA, which is individually different for the participants; while the supplemented omega-3 FA amount was insufficient in some participants, albeit overall the omega-3 index remained constant. It can be hypothesized that a longer exergame training period would be able to lead to undesirable and insufficient levels of omega-3 FA in the control group, such that the
participants would no longer be able tapping their omega-3 FA reserve while training. Further research into this topic is warranted where a longer time frame for the intervention should be adopted. Furthermore, it can be speculated that interplay investigations should focus on populations with an identified undesirable omega-3 index below 4% at the start of an intervention.

5.4.2 Motor Evoked Potentials

No time × group interaction effects were present for any of the MEPs. Considering time main effects, the results just showed a significant decrease of the 110% RMT MEP.

The only significant decrease might be not meaningful, since none of the other stimulation intensities significantly changed over time. Though, a measurable increased neuronal excitability (increased MEP) was expected induced by neuroplastic changes in the motor cortex. Exergame training might improve the neuronal communication between motor cortex and leg muscles due to the training of specific well-coordinated leg movements. Furthermore, exergame training might induce neuroplastic mechanism (e.g. neurogenesis, synaptogenesis, and angiogenesis) by the release of trophic factors during the exercise [94]. Moreover, exergame training combines physical and cognitive exercises and both trainings (alone or together) can induce structural and functional brain alterations [94, 95]. Besides, omega-3 FA might support the effect of the exergame training by its positive effects on brain structure, function, and cerebral blood flow [48]. For example, DHA acts as a neurotrophic factor by increasing the level of brain-derived neurotrophic factor [49]. However, no changes of the neuronal excitability were measured in this study. A first explanation for this lack of effect might be that the neuroplastic adaptations rather took place in other brain areas and the motor cortex, by itself, was just a too small region with measurable changes. Maguire et al. 1998 showed that navigation in a virtual environment activated hippocampus, caudate nuclei, frontal and parietal cortex, and cerebellum [96], indicating specificity of training. It might well be that the training routine was not enough related to the desired outcome [97]. Another study that used exergame training showed modulated prefrontal brain activity during walking [37]. In addition, a study using omega-3 FA supplementation showed increased gray matter volume within the hippocampus, precuneus, and temporal lobe in older healthy adults [50]. A study combining omega-3 FA, aerobic exercise, and cognitive stimulation indicated that the combination preserved or even increased grey matter in frontal, parietal, and cingulate cortex in mild cognitive impaired patients [93].

A second explanation might be the used measurement method. Brain imaging methods, e.g. MRI and positron emission tomography (PET), have the possibility to measure changes of grey and white matters as well as metabolic processes that might be better indicators for neuroplastic changes. These imaging methods would allow the measurement of several brain areas and would be able reaching deeper brain regions, e.g. hippocampus. To sum up, the results did not show any meaningful increases of neuronal excitability measured between the motor cortex and right leg muscle (M. tibialis anterior). Thus, this study could not find evidence for neuroplastic changes in the motor cortex and involved neuronal pathways.
5.4.3 Response-Locked Potentials

No time × group interaction effects were present for the RLP for Fp1 and Fp2. Considering time main effects, the positive peak before and the negative peak after the response onset showed a significant increase of the peak amplitude in Fp1 and Fp2.

The inhibition ability, measured by a Go/No-go paradigm, can be assigned to the EFs [98]. EFs, in turn, are anatomically linked with the frontal lobe, especially with the PFC [16, 17]. Better EF were associated with a larger PFC volume and a greater PFC thickness [18]. Furthermore, a study that used a Go/No-go paradigm showed an increasing contribution of frontal brain areas to cognitive response control during lifespan [99]. Thus, these observations seem to explain why increased RLP were found in channel Fp1 and Fp2.

The cognitive task used control processes and required visual stimuli processing followed by execution of a prepared motor response. So, RLP allow determination of neural processes involved in monitoring performance [100]. The main research using RLP focused on the commission of an error reaction (e.g. reaction to No-go-signals). Such studies found that active older adults showed reduced negative amplitude after response onset relative to their sedentary counterparts [74, 101]. However, within our study the analysis was based on Go-signals. Our results showed increased amplitude of the negative peak after response onset. It might be that physical activity induced a reduction in the negative peak amplitude after an error reaction (No-go-signals), while in our study an increase of the negative amplitude was visible for a non-error reaction (Go-signals). Hence, the focus of the participants might be enhanced on the relevant stimuli. A review proposed that chronic exercise and/or aerobic fitness are related to more efficient response monitoring and upregulation of cognitive control [100]. Moreover, the increased peak amplitude might indicate a better focus on and processing of Go-signals and, thus, better selective attention. Ineffective operation of selective attention results in the intrusion of task irrelevant information into working memory [102]. The entrance of irrelevant information into working memory seems, in turn, to be linked with increased processing time and reductions in recall and recognition of relevant information [102]. In addition, the results demonstrated increased amplitude for the positive peak that appeared before the response onset. This peak before the response onset might be indicative for a readiness potential [103]. To sum up, the physically-active exergame have positively influenced the focus on and processing of relevant stimuli, similar to a recent study showing that exergame training can improve performance of an inhibition task in elderly [38]. Further, omega-3 FA might support the positive effect of the exergame training by its effects on brain structure, function, and cerebral blood flow [48], however, shows no additional effect in healthy older adults with an omega-3 index above 4% at the start of intervention. The latencies were not considered in our study due to technical problems. At the moment, not a lot of studies exist that focus on Go-signals for RLP. Therefore, further studies are needed that focus on the influence of exergame training and omega-3 FA on brain activity.
5.4.4 Executive Functions

No time × group interaction effects were present for the working memory or the divided attention test. Considering time main effects, the participants demonstrated significant improvements within the divided attention task for the number of errors. No time main effects were present for the working memory test.

A recent study showed that exergame training improves divided attention in older adults [38]. Divided attention was included in this exergame training through the cognitive-motor training. Besides, the video games included specific stimuli that trained divided attention through the combination of two different stimuli (e.g. auditory and visual). This video game task was close to the divided attention test of the TAP that included the concurrent observation of visual and auditory stimuli. Thus, video game-based training efficacy might be ensured by video game components that were close to the cognitive outcome of interest [104]. However, most studies considered an improvement of the reaction time for a divided attention task [38], while our study found an improvement for the number of errors. The declining number of errors might be indicative for the participants enhanced ability to inhibit their reactions (inhibitory processes) to irrelevant stimuli and focus on relevant stimuli (selective attention) during the divided attention task [105]. Therefore, it might be that the reaction time improved, but due to improved error monitoring, the participants needed additional time and, therefore, the reaction time remained on the same level. Furthermore, the number of omissions was, from the beginning, on a good level. So, the range of improvement might have been too small to generate significant differences.

For the working memory task, the video games might have not been specific enough to generate positive results. As aforementioned, video game characteristics that are close to the cognitive outcome of interest might ensure video game-based training efficacy [104]. Different genres of video games may not have equal positive effects on the same cognitive aspects [106]. Furthermore, a recent meta-analysis concluded that combined cognitive and physical intervention was effective at improving cognitive function in healthy older adults while intervention effects varied across cognitive domains [107]. On the other hand, omega-3 FA DHA and EPA were associated with better working memory function [108]. However, a meta-analysis study found no benefits of omega-3 FA treatments on working memory [109]. Thus, lack of interplay might be explained because both factors seem to have no positive effects on working memory.

5.4.1 Spatio-Temporal Gait Parameters

No time × group interaction effects were present for any of the spatio-temporal gait parameters. Considering time main effects, the participants demonstrated significant improvements of gait parameters predominantly under dual-task walking and consequently for dual-task costs of walking.
These results are in line with previous studies that showed similar results for dual-task walking and dual-task related costs after a motor-cognitive training, or a so-called exergame training [25, 27, 38]. Divided attention or dual-tasking are associated, especially, with spatial and temporal dual-task cost characteristics of gait [13]. Moreover, dual-tasking is involved in real life walking situations and seems to be associated to fall events in elderly [110, 111]. Gait performance under dual-task conditions is positively influenced by exergame training due to the combined motor and cognitive exercise [23, 29, 112]. Furthermore, exergaming trains divided attention by concurrent observation of cognitive stimuli and performance of well-coordinated movements. Especially in this study, participants observed drifting symbols or figures and, at the same time, initiated outward steps on a pressure sensitive area. To initiate an outward step, participants needed to rapidly unload the leg, they were standing on, allowing taking a step. This process might challenge cognition, reaction time, and muscle power generation [113]. Consequently, exergame training does not only require well-coordinated leg movements, but also requires cognitive work, e.g., paying attention, making quick decisions, and integration of stimuli [114]. As a result, regular exergame training might improve dual-task interference and might support a transfer of the trained cognitive abilities on the concurrent performance of multiple tasks [115, 116]. In the same way, omega-3 FA might have positively influenced dual-task walking and DTC of walking. As mentioned before, spatial and temporal dual-task gait parameters are associated to the EF component divided attention. As is known from a previous study, omega-3 FA supplementation has positive effects on EFs in healthy older adults [50]. Furthermore, a systematic review revealed various brain areas being associated with various measures of gait variability [117], thus underpinning the argument of a too limited focus of assessment with our TMS approach. In brief, spatio-temporal gait parameters under dual-task condition and DTC of walking seem to be positively influenced by the exergame training and possibly by supportive effects of omega-3 FA.

5.4.2 Limitations

Some limitations of this study should be mentioned. The participants selected were quite fit elderly that probably received a too short training intervention time. A longer exergame training period might have led to such a low level of omega-3 FA in the control group that the participants no longer would be able to tap their omega-3 FA reserves to support the exergame training. This situation might then lead to significant time x group interaction effects. Furthermore, the presumable usage of omega-3 FA during exergame training cannot completely be related to the brain metabolism, structure or vascularization since also other body parts seem to use omega-3 FA. For the EEG measurements we used, some issues related to our measurement protocol might limit the measurement interpretation. The analysis showed a great range of latencies in the observed peaks. For example, the latencies (mean ± standard deviation) of the negative peak after response onset were for the fish oil and exergame group pre: Fp1 420.40ms ± 243.13ms, Fp2 385.60ms ± 213.71ms and post: Fp1 464.53ms ± 219.58ms, Fp2 501.26ms ± 238.12ms and for the olive oil and exergame group pre: Fp1 518.00ms ± 254.31ms, Fp2 514.00ms ± 250.53ms,
post: Fp1 423.76ms ± 231.23ms, Fp2 431.13ms ± 261.72ms. It cannot be ruled out that differences in EEG cap positioning during the different measurement events are, at least in part, responsible for this observed variability. The EEG cap positioning might have had an influence on the latency. Future studies should confirm or refute our results using a smaller range of the latencies an emphasizing reproducibility of accurate 10-20 system placement. Nevertheless, the RLP shape was evident for the included participants, while in a few participants the RLP appeared at a later time point. Furthermore, the randomized study design can be considered most optimal for controlling factors related to measurement issues. Accordingly, the reason for the shift in time might be due to a technical problem. Since the EEG activity was recorded using wireless signal transmission, it can be speculated that the signal transmission was slightly delayed in a few participants. Nevertheless, during the experiments other electrical devices were switched off to minimize interference. However, the analysis of the peak amplitudes and the display of the RLP shape were still possible, while the latency of the peaks can differ. TMS measurement was a limitation as it just measured neuronal excitability from the motor cortex to the right leg muscle (M. tibialis anterior). At the time of research protocol development, we were not aware of the systematic review of Tina and colleagues mapping relevant brain areas for gait variability [117]. With this knowledge, we recommend using brain imaging methods in future similar studies since magnetic resonance imaging and positron emission tomography allow measuring changes of grey and white matter volume and structure as well as assessing metabolic processes that might be better indicators for neuroplastic changes. Moreover, these imaging methods allow the measurement of several brain areas and deeper located brain regions, e.g. hippocampus.

5.5 Conclusion

On the first sight, the results of our study show no interplay, since no significant time × group interaction effects were measured in any of the structural or functional parameters. However, the omega-3 index showed a significant time × group interaction effect in favor of the fish oil intake group. So, the fish oil intake caused higher DHA and EPA levels in the blood. Interestingly, for the time of exergame training, the olive oil intake group showed a significant decrease of the omega-3 index while the fish oil intake group remained on a stable level. This process might indicate that exergame training required omega-3 FA and therefore the omega-3 index decreased in the olive oil intake group while the level remained stable in the fish oil intake group. Thus, interplay between these two factors might have happened enhancing time main effects. For time main effects, response-locked peak amplitudes showed a significant increase in prefrontal channels. Furthermore, the number of errors for the divided attention test significantly decreased. Moreover, gait parameters significantly improved predominantly under dual-task walking and, therefore, also dual-task cost of walking.
The observed time main effects, however, might be mainly due to the exergame component of the intervention, while the omega-3 FA might have acted as a supportive component. This assumption would be in line with a recently published pilot study where the combined approach of aerobic exercise and cognitive stimulation with omega-3 FA showed an effect on gray matter volume as opposed to omega-3 FA intake in combination with stretching and toning [93]. Thus, the motor-cognitive training seems to be the main component that evokes the positive effects. However, it cannot be ruled out that omega-3 FA acted as a supportive component for the training. An assumption might be that omega-3 FA were used to support the energy metabolism that was stimulated by the exergame training. As is known, omega-3 FA are important for the energy metabolism [47]. Furthermore, exergame training might stimulate neuroplastic changes and thus omega-3 FA provided building material to the brain and supported brain perfusion [41, 48]. In rats, DHA supplementation enhanced the effects of exercise on cognition and brain derived neurotrophic factor-related synaptic plasticity [49]. Hence, exergame training and omega-3 FA seem to have the potential for interplay. This supposition is in line with the aforementioned pilot study that combined omega-3 FA, aerobic exercise, and cognitive stimulation in mild cognitive impaired patients [93], proposing that omega-3 FA may allow training to exert maximal benefits. Future studies should expand the exergame training period that might generate time × group interaction effects as the omega-3 FA level of the intervention group without fish oil intake would drop to such a low level that not enough omega-3 FA would be available to support the exergame training effects. A too low level of omega-3 FA might be connected with limited training results or even worse with negatively affected training results. Furthermore, future studies should investigate neuroplastic brain level changes using brain imaging methods.

Author contribution: AS developed the research question under the lead of EDB, IHA, and VKM. The concept and design part was established by AS, CB, and DM while EDB, IHA, and VKM acted as methodological council. AS, CB, and DM did data acquisition, analysis, and interpretation of the results which was edited and improved by EDB. AS produced an early version of the manuscript. EDB, IHA, and VKM substantially revised the manuscript to bring it to its current version. All authors have read and approved the final manuscript.

Acknowledgements: The authors wish to thank San Omega GmbH for supplying the fish oil (NORSAN Omega-3 Total) and olive oil and Swissmedic Plus for the helpful support. The trial was supported by the ETH Foundation through ETH Research Grant ETH-17 13-2. The authors would like to thank Floriana Sonder and Mélanie Röthlisberger for instructing trainings and helping with data acquisition, and Manuela Omlin and Federico Gennaro for their helpful inputs. Furthermore, we would like to thank all participants for their kindness and enthusiasm during the training sessions and measurement period.

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. EDB was a co-founder of dividat, the spin-off company that developed the video games used in this study, and is associated to the company as an external advisor. No revenue was paid (or promised to be paid) directly to EDB or his institution over the 36 months prior to submission of the work.
Chapter 6

Epilogue
6.1 Overview

In the near future, the number of elderly people suffering from age-related bodily dysfunction will increase due to demographic changes [1]. The brain is one part of the body that is affected by age-related changes [15, 18, 19]. Brain deterioration seems to impair cognitive [15, 18-20, 23-27] and motor performance [49, 52, 54], leading to unstable gait patterns or, even worse, increased likelihood of falls amongst the elderly. Since cognitive and motor decline threatens the independence and quality of life of older adults, prevention and treatment of such decline have assumed increasing importance [55]. Exergame training by itself and in combination with omega-3 FA supplementation suggests promising interventions in support of healthy brain aging. Exergame training, a motor-cognitive training, may influence the brain through physical exercise (PE) that induces brain plasticity by means of cell proliferation and synaptic plasticity, while the cognitive part supports the survival of newborn neurons and their integration into pre-existing networks in the brain [68-70]. Furthermore, previous studies have shown that docosahexaenoic acid (DHA) supplementation enhances the effects of exercise on axonal growth, brain-derived neurotrophic factor (BDNF)-related synaptic plasticity, and cognition in rats [77, 78]. However, there is incomplete evidence about the effect of exergame training on the elderly brain. Moreover, no study has so far investigated the effects of exergame training combined with omega-3 fatty acids (FA) supplementation on brain structure and function in older adults.

This doctoral thesis discusses approaches that are worthy of consideration with a view to including exergame training as well as its combination with omega-3 FA to support healthy brain aging. Furthermore, the overall aim of this doctoral thesis has been to help to fill the gap in knowledge by identifying the effects of exergame training, by itself and in combination with omega-3 FA, on the structure and function of the elderly brain along with associated cognitive and motor abilities. The first step was to explore the current state of research about the effects of PE, alone and combined with nutritional supplementation, on brain structure, function, and metabolism in human and mammalian studies. Afterwards, a first experimental study was performed to examine the effects of exergame training compared to conventional balance training on prefrontal brain activity and associated cognitive and motor performance in the elderly. The findings of the systematic review and the first experimental study were taken into consideration for the second experimental study. Before the second study, a protocol paper was written to argue for the study design of the second experiment. The second experimental study combined exergame training with omega-3 FA supplementation. The objectives were to study the effects of a combined approach on brain structure and function in the elderly as well as associated cognitive and motor performance. Both experimental studies were performed in Zurich (Switzerland).
In the next section, the important findings of the literature investigation and of the two experimental studies are summarized. In a third section, the epilogue addresses more general aspects, including the exergame and its combinations with omega-3 FA supplementation. The fourth section outlines limitations and methodological issues. Future perspectives are presented in the last section.

### 6.2 Important Findings

This section summarizes the most important findings presented in Chapters 2, 3, and 5 according to the aims defined in Chapter 1. Detailed results can be found in the previous chapters.

**6.2.1 Effects of Physical Exercise Combined with Nutritional Supplements on Aging Brain Related Structures and Functions: A Systematic Review (Chapter 2)**

**Aim 1:** Examining whether the combination of physical exercise and nutritional supplementation has greater (additive) effects on brain structure, function, and/or metabolism than their separate administration in mammals and humans.

Within our systematic review, 3129 studies were identified after a search process, from which three human combinatory studies and twelve rodent combinatory studies were selected after screening. In healthy elderly humans, the three selected studies combined strength exercise with creatine or protein supplementation or aerobic exercise with vitamin E supplementation. However, none of these studies could show any additive effects on brain structure, function, or metabolism when PE was combined with nutritional supplementation. Thus, the combination of PE and nutritional supplementation did not lead to superior results compared to the sole-administration of either PE or nutritional supplementation. With reference to the sole-administration studies in humans, the main interventions that resulted in improved cognition or associated parameters were aerobic type of exercise, strength training, coordination training, calorie restriction, and DHA supplementation. On the other hand, exercise combined with DHA or selenium supplementation resulted in additive effects on learning, hippocampal BDNF levels, synaptic protein levels, and/or oxidative stress in four out of twelve combinatory studies with rodents. Our systematic review revealed that the application of targeted PE in combination with nutritional supplements aiming to influence the elderly human brain are still at a fledgling stage.
6.2.2 Adaptations of Prefrontal Brain Activity, Executive Functions, and Gait in Healthy Elderly Following Exergame and Balance Training: A Randomized-Controlled Study (Chapter 3)

**Aim 2:** Comparing exergame training with conventional balance training focusing on prefrontal brain activity and associated cognitive and motor performance in healthy older adults.

Twenty-seven healthy elderly participants (mean age 79.2 ± 7.2 years) completed the intervention (24 training sessions within 8 to 10 weeks). Our results illustrated that exergame training (N = 13), compared to balance training (N = 14), positively affected prefrontal brain activity, in particular by a significant decrease of theta relative power (RP). Within the exergame group, the Test for Attentional Performance (TAP) reaction times for working memory, divided attention, inhibition, and set-shifting were significantly faster after the training period. Within the balance group, only the TAP reaction time of set-shifting was significantly faster after the training period. Spatio-temporal gait parameters significantly improved within the exergame group primarily under dual-task condition and within the balance group primarily under single-task condition, respectively.

6.2.3 Interplay of Exergame Training and Omega-3 Fatty Acids on the Elderly Brain: A Randomized Double-Blind Placebo-Controlled Trial (Chapter 5)

**Aim 3:** Examining whether the combination of exergame training and omega-3 fatty acids affect brain structure and function differently than exergame training alone in healthy older adults.

Forty-three healthy elderly participants (mean age 69.4 ± 4.6 years) completed the intervention (nutritional supplementation over 26 weeks and 30 training sessions within the last 10 weeks). Our results showed no significant time × group interaction effects for any of the measured parameters. Time main effects were found for several assessed parameters amongst all participants. Regarding electroencephalography (EEG) measurement in prefrontal channels (Fp1 and Fp2), positive (before) and negative (after) response-locked peak amplitudes showed a significant increase. On the behavioral level, the number of errors for the divided attention task significantly decreased, while no effects were found for the working memory task. Gait parameters significantly improved, predominantly under dual-task walking condition and, therefore, also for dual-task cost of walking. However, no significant changes were found for the structural measurement. In addition, the omega-3 index (DHA and eicosapentaenoic acid (EPA) blood values) significantly increased within the fish oil intake group (N = 22), while the olive oil intake group (N = 20) showed no change for the first 16 weeks. From week 16 to week 26 (exergame training period), the olive oil intake group showed a significant decrease of the omega-3 index, while the fish oil intake group remained at its pre-attained level.
6.3 General Discussion and Implications

The sections named above dealt with the specific findings of each study. This section will focus on the general aspects of the main findings and will concern itself with the implementation of the results in the field of healthy brain aging supported by PE, especially exergame training, and its combination with nutritional supplementation, especially omega-3 FA supplementation. Detailed discussion of each study can be found in Chapters 2, 3, and 5.

6.3.1 The Effect of Physical Exercise Combined with Nutritional Supplementation on the Elderly Brain (Chapter 2)

The brain undergoes age-related neuronal changes that seem to affect cognitive and motor functioning and, therefore, daily life activities in the elderly. One of the most effective ways to maintain a healthy body and mind is PE [13]. In addition, previous research suggests that the effects of PE on the brain can be complemented by concurrent consumption of selected dietary elements [13, 76]. However, little is known about the effects on the elderly brain when PE is combined with the concurrent consumption of nutritional supplementation. Furthermore, it is not yet clear which combination might be most promising for the promotion of healthy brain aging. Therefore, our systematic review aimed to answer these questions.

Our systematic review illustrated that, at that time, no human study showed any additional effects when PE was combined with nutritional supplementation. However, initial results of interesting rat studies did show enhanced effects on brain structure, function, and metabolism during a combined intervention rather than sole-administrations. In the elderly human studies, the lack of affirmative results can be interpreted to mean that there is a mismatch in many studies between PE programs offered and the nutritional supplements given. Through our systematic review, it became apparent that isolated interventions of either PE or nutritional supplementation were able to affect the elderly human brain. The underlying effects investigated may help to identify nutritional components that could support PE by sharing the same neurobiological cascades. Therefore, it seems to be essential to consider not only combinations that were effective in rodent studies, but also sole-administration human studies that examined either the effects of PE or nutritional supplementation on the elderly brain. Nonetheless, researchers should bear in mind that other influencing factors, such as genetic components and living environment, are very individual in humans, whereas they are more or less identical in rodents.

To sum up, three aspects should be taken into account to evoke possible additive effects: (1) training principles should ensure quality and quantity of the PE component, (2) dose and duration of diet or nutritional supplementation should be appropriate, and (3) the selected nutritional component(s) and PE should act on the same neurobiological cascades. Moreover, our systematic review proposed the development, implementation, and evaluation of video game-based PE that encourages the combination
of aerobic and strength exercise together with cognitive training. Video games allow the implementation of cognitive stimuli and of FITT (Frequency, Intensity, Type, and Time) training principles to ensure that the dose and type of PE is planned to maximize the benefits for the recipients. For nutritional supplementation, a diet including omega-3 FA is assumed to have the potential to interact with PE by affecting synaptic plasticity and cognition. A previous study showed beneficial effects on brain function and structure by omega-3 FA supplementation in elderly humans [81]. Thus, a combination of video game-based PE and omega-3 FA supplementation may be a promising intervention design to support healthy brain aging.

6.3.2 The Effect of Exergame Training on the Elderly Brain (Chapter 3)

As proposed in our systematic review, video game-based PE should be developed, implemented, and evaluated in future studies. But, why use video game-based PE as an intervention setting with the elderly?

For aging humans, an exercise program that effectively addresses prefrontal brain structure and function, especially executive functions (EFs) and gait-related motor functions, may be important, since age-related reduction of EFs can impact gait and amplify the risk of falling [50]. Two recent reviews that focus on the interplay between physical functions and cognition concluded that it is important to combine motor and cognitive training in clinical practice to enable older adults to move more safely in their physical environment [64, 65]. Exergame training combines the exercise of cognitive and motor functions as the participants perform specific whole-body movements in response to video game-based stimuli. The aim of our first experimental study was to compare exergame training with conventional balance training, focusing on prefrontal brain activity and associated cognitive and motor abilities in healthy older adults.

Our experimental study is one of the first studies to illustrate that exergame training compared to balance training affects prefrontal brain activity by significantly decreasing theta RP. In the aging process, theta RP is expected to increase, leading to a so-called age-related “slowing” mechanism [21]. Exergame training may thus be able to ameliorate age-related “slowing”, which in turn is linked to superior cognitive functioning, improved motor performance, and enhanced sensory processing [21]. Moreover, exergame training may help to increase connections between different brain areas because of decreased theta RP, thus enabling better control and easier recruitment of specialized neural mechanisms [232, 236]. However, the results should be considered with caution as findings on the slowing effect are not consistently reported [21]. So far, these relations can be only hypothesized because it still remains unclear how age-related changes affect EEG (slow) oscillations and whether they appear to mediate control processes. It may well be that reduced frontal theta RP reflects an executive impediment in top-down control due to increasing task demands rather than due to an increase in brain connectivity [237].
Regarding the EFs, our results showed significantly improved working memory, divided attention, set-shifting, and inhibition within the exergame group, while within the balance group only set-shifting significantly improved. Exergame training might have been effective due to the characteristics of the video game being close to the cognitive outcomes of interest [240]. Moreover, the combination of PE and cognitive training leads to the improvement of general cognitive functions and memory in older adults [67]. Thus, PE should be executed in a cognitively challenging environment in order to effectively induce cognitive benefits [243, 244]. Exergames offer a specific concurrent combination of motor and cognitive training, enhanced by the fact that our video games included progressive levels of difficulty. Interestingly, cognitive benefits from exergaming increases with a dose of interactive mental challenge [245]. To summarize, our exergame training included specific EF training at levels matched to the user that could trigger beneficial effects on the measured EFs. On the other hand, our balance training consisted of highly variable exercises, which in fact is an important and effective method for training motor learning [209], and might have therefore helped participants learn to adapt to new and changing situations, an important aspect for flexibility [246]. However, no time × group interaction effects were found, as both interventions elicited beneficial effects on EFs. Nevertheless, a recent meta-analysis showed that exergame training significantly improved cognition, especially EFs, attention, and visuospatial skill, in clinical and non-clinical populations compared to a physically active control group [281].

Regarding gait performance, the balance group demonstrated significant within-group improvements primarily under single-task conditions. Balance training improves motor performance by eliciting changes on subcortical structures [205]. Similarly, one recent study showed improved gait speed after balance training [247]. On the other hand, the exergame group showed significant within-group improvements for gait parameters primarily under dual-task condition. Exergaming combines motor and cognitive training that therefore positively influence gait performance under dual-task conditions [7, 64, 65]. Moreover, divided attention is trained by concurrent observation of cognitive stimuli and performance of well-coordinated movements. The crucial point is that exergaming requires not only well-coordinated leg movements, but also cognitive work [202].

To conclude, our exergame training seems to counteract age-related prefrontal brain changes and associated reduction of EFs and instability of gait. Age-related reduction of EFs can impact gait and amplify the risk of falling [50]. Consequently, exergame training seems to offer additional and promising training effects to promote healthy brain aging. Therefore, exergame training may allow the elderly to perform activities of independent living more safely and to have a higher quality of life. Finally, exergame training should be considered as a piece of the puzzle in an overall training plan for the elderly consisting of aerobic, strength, and balance exercise. Nevertheless, future studies are needed to optimize the effects of exergame training.
6.3.3 The Effect of Exergame Training Combined with Omega-3 Fatty Acids on the Elderly Brain (Chapters 4 & 5)

The second experimental study integrated the knowledge about combinatorial interventions from our systematic review and about exergame training from our first experimental study. To pursue a promising combinatorial design, exergame training was combined with omega-3 FA supplementation.

Omega-3 FAs are important for the function and integrity of the neuronal plasma membranes, for energy metabolism, and for perfusion in the brain [74, 79]. Long-chain polyunsaturated FA (LCPUFA) intake seem to be important as LCPUFA improve cognition, decrease (neuro)inflammation, and reduce vascular risk factors in normal aging adults [79]. Previous randomized controlled studies showed that fish oil, including omega-3 FA, had positive effects on brain structure and function in healthy older adults [81, 82]. So, exergame training might benefit from the positive effects of omega-3 FA, which are important for energy metabolism and to provide building material for the brain [74, 76].

The results of the second experimental study, at first sight, appear to have shown no interplay effects, since no significant time × group interaction effects were assessed in any of the measured parameters. Time main effects were found for several assessed parameters amongst all participants. First, response-locked peak amplitudes showed a significant increase in prefrontal channels. The increased peak amplitudes might indicate a better focus on and processing of relevant stimuli and, thus, better selective attention. A review proposed that chronic exercise and/or aerobic fitness are related to more efficient response monitoring and upregulation of cognitive control [297]. Second, the number of errors for the divided attention test significantly decreased. The declining number of errors might show that the participants could inhibit their reaction (inhibitory processes) to irrelevant stimuli and focus on the relevant stimuli (selective attention) [301]. Divided attention was included in our exergame training through the simultaneous combination of motor-cognitive training as well as through the video games that included specific divided attention stimuli. For the working memory task, the video games might have not been specific enough to generate positive results. As already discussed in the first experimental study, video game characteristics that are close to the cognitive outcome of interest might ensure video game-based training efficacy [240]. Third, gait parameters significantly improved predominantly under dual-task walking and, therefore, also for the dual-task cost of walking. These results coincide with the results of the first experimental study where exergame training improved dual-task walking. Gait performance under dual-task conditions is positively influenced by exergame training due to the combined motor and cognitive exercise [7, 64, 65]. Therefore, regular exergame training might reduce dual-task interference and support a transfer of the trained cognitive abilities onto the concurrent performance of multiple tasks [248, 251]. Finally, no significant changes were found for the structural measurement of neuronal excitability between the motor cortex and the right TA muscle. One reason might be that the neuroplastic adaptations took place in diverse brain areas (e.g. hippocampus and frontal cortex) and the motor cortex, by itself, was just a too small region. A second reason might be the...
measurement method used, since brain imaging methods, e.g. magnetic resonance imaging (MRI) and positron imaging tomography (PET), are able to measure changes of gray and white matter as well as metabolic processes, which might better indicate neuroplastic changes.

On the other hand, the omega-3 index showed a significant time × group interaction effect in favor of the fish oil intake group. Thus, the fish oil intake caused significant higher DHA and EPA levels in the blood. Interestingly, during the time of exergame training, the olive oil intake group exhibited a significant decrease of the omega-3 index, while the fish oil intake group remained on the pre-attained level. This process might hint that the body required omega-3 FA during exergame training and, therefore, the reserves were tapped in the olive oil intake group. In the fish oil intake group, the omega-3 index remained at a constant high level due to the concurrent daily supplementation of omega-3 FA. Therefore, an interplay between exergaming and omega-3 FA might have happened during the training phase. This assumption is in common with the findings of a recent study that combined omega-3 FA, aerobic exercise, and cognitive stimulation in mild cognitive impaired (MCI) patients to prevent structural decline [291]. Omega-3 FA may allow training to exert maximal benefits. An assumption might be that omega-3 FA were used to support the energy metabolism that was stimulated by the exergame training. As is known, omega-3 FA are important for energy metabolism [74]. Furthermore, exergame training might stimulate neuroplastic changes, aiding omega-3 FA to provide building material to the brain and support brain perfusion [76, 80]. In rats, a study showed that DHA supplementation enhanced the effects of exercise on cognition and BDNF-related synaptic plasticity [77]. Nevertheless, the presumable usage of omega-3 FA during exergame training cannot completely be related to the brain metabolism, structure or vascularization since other bodily processes could also tap into omega-3 FA.

To summarize, exergame training, which was performed in both groups, seems to be the key factor evoking the positive time main effects in our sample of healthy older adults. Because of omega-3 FA being available in all participants at the start of the training, it cannot be completely ruled out that reserves were tapped by the body and used as supportive component for the exergame training. Hence, our second experimental study permitted an initial declaration about a possible interplay of exergame training and omega-3 FA supplementation and its effect on the elderly brain. In addition, these results support the results of our first experimental study as exergame training appears to be a promising way to improve prefrontal brain activity, EFs, and dual-task walking. Nonetheless, future studies are needed to expand the knowledge about potential interplay effects.
6.4 Limitations and Methodological Issues

The results of the studies reported in this doctoral thesis (*Chapters 2, 3, and 5*) should be interpreted within the context of the methodological limitations outlined below. This section includes the most important limitations as the detailed limitations of the systematic review and the two experimental studies have already been discussed.

First, the limitations within the systematic review will be outlined. One limitation relates to the focus on older humans and mammals. The precise correlation between the age of rodents and the age of humans is subject to debate, implying, when age is an important factor, that differences between animals and humans should be taken into consideration [199]. Another factor that limited the objective quantification of our results (meta-analysis) was the heterogeneity of the studies, including participants, interventional design, and outcomes. However, this fact also shows that there is a need for follow-up studies that use similar approaches as previous studies to try to replicate the interventions.

Second, the participants in both experimental studies were quite fit older adults. This fact might have limited the validity of the results as the elderly population tend to be physical inactive [308]. An unfit population might benefit more from the training as the baseline fitness starts on a lower level and, therefore, has a larger scope for improvement. Thus, future studies should consider including elderly participants who are on a lower fitness level. Furthermore, voluntary participation in the experimental studies might have caused a selection bias. Both factors might limit the generalizability of our results.

Third, some aspects of the intervention design may have influenced our results. Both experimental studies had a rather short intervention period. This could be a reason why little to no significant time × group interaction effects were measured. For our first experimental study, a longer training period might have strengthened the measurable effects elicited by exergame training, or improvements, not yet measurable, might have taken effect. In the second experimental study, a longer exergame training period might have led to such a low level of omega-3 FA in the control group that the participants would no longer have been able to tap their omega-3 FA reserves to support the exergame training. Consequently, future studies should take into account prolonged intervention periods.

Fourth, some assessment circumstances limited the results in both experimental studies. EEG results were restricted to the prefrontal brain activity. In our first experiment, the measurements were limited due to the low-density EEG device, while in our second experimental study response-locked potentials were identified just in the prefrontal channels. The PFC is an essential area for the topic of this doctoral thesis. However, the behavior of different brain areas could be interesting, too. Hence, future studies should consider different EEG protocols and analysis to expand knowledge, e.g. event-related potentials or resting state EEG using LORETA (low resolution electromagnetic tomography) analysis to estimate cortical connectivity. In addition, transcranial magnetic stimulation was limited as neuronal excitability measurement was restricted to the connection of motor cortex to the right leg muscle (M. tibialis
A recent systematic review mapped relevant brain areas for gait variability [307]. With this knowledge, the recommendation is to use neuroimaging methods in future studies. For example, MRI and PET allow the measure of gray and white matter volume and structural changes as well as the assessment of metabolic processes that might better indicate neuroplastic changes. Moreover, these imaging methods permit the measurement of several brain areas as well as regions located deeper in the brain, e.g., the hippocampus. Finally, measurements of gray matter volume might be more sensitive than behavioral assessments for detecting differences between a combined and a single intervention [291].

### 6.5 Implications for Future Research

The possible implementation of the study findings and future research directions have already been mentioned in previous chapters. Thus, the aim of the current section is to summarize possibilities and recommendations for improving future research, while keeping in mind the general discussion and limitations mentioned previously.

First, the limited availability of high-quality prospective studies that used a combined approach warrants further targeted research to investigate the effects of combined approaches on the elderly brain. Future studies should take into consideration the sole-administration human studies. These studies can help to identify promising combinations, as the components should interplay on the same neurobiological cascades. Furthermore, mammalian studies that combine exercise with nutritional supplementation can give indications for possible combinatory interventions in humans. Therefore, in mammalian studies, the combinatory interventions should identify possible components that interplay and, thus, support healthy brain aging in humans.

Second, aspects of exergame training should be adapted in future studies. A longer training period might strengthen the measured effects, while other effects may appear due to the prolonged training period. A home-based setting might simplify a longer study duration as participants can train at home and, therefore, the drop-out rate could be kept to a low level. Regarding the video games, upcoming studies should consider training principles, motivational aspects, abilities of recipients, and outcomes of interest. Finally, it would be interesting to perform an experimental study that incorporate exergame training into a program of aerobic, strength, and balance exercises.

Third, not only the exergame training but also the nutritional supplementation can be modified in future combinatory studies. As mentioned for the exergame training, interventions should be adapted to the characteristics of each recipient. Thus, regular and direct blood sample analysis might allow individual dose adaptations of the nutritional supplement during an intervention. In the case of this thesis, this approach could have helped to keep some of the participants taking the fish oil on the pre-attained level during the exergame training period. Furthermore, it can be speculated that interplay investigations should include a participant pool with an undesirable omega-3 index below 4% (risk level). These
participants might not be able to tap their omega-3 FA reserve to support training effects. Moreover, measurement of other blood-derived factors, e.g. inflammation factors [309], could support the identification of different bodily processes which might benefit from omega-3 FA reserves as well, minimizing the brain supply. Finally, researchers should keep in mind that the human body seems to need time to incorporate nutritional supplements. Therefore, future studies should set a long enough time frame to generate measurable effects.

Fourth, studies should investigate neuroplastic brain level changes due to exergame training or its combination with omega-3 FA supplementation using neuroimaging methods. Neuroimaging methods such as MRI and PET are able to measure changes of gray and white matter as well as metabolic processes that might better indicate neuroplastic changes. Moreover, these neuroimaging methods allow the measurement of various brain areas as well as regions located deeper in the brain, e.g. the hippocampus. Furthermore, adaptations of certain brain regions can be connected with behavioral level improvements. Another neuroimaging method that might be promising for future studies is functional near-infrared spectroscopy (fNIRS). fNIRS measures brain activities at rest but also in mobile settings, e.g. walking over a crosswalk. This mobile setup allows daily life activity to be monitored during and after study intervention, which promises a novel measurement approach.

As a last consideration, the proposed adaptations for future studies can be performed with healthy older adults to gain more information about how the aging brain reacts to exergame training and its combinations with omega-3 FA supplementation. However, the study population could also include MCI patients as they, too, may profit from the beneficial effects. A central element of successful cognitive rehabilitation for older adults should be the design of interventions that either reactivate disused or damaged brain regions, or that compensates for the decline in parts of the brain through the activation of compensatory neural reserves [201]. A recent published pilot study combined omega-3 FA, aerobic exercise, and cognitive stimulation in MCI patients [291]. The study found that the combination influences gray matter volume as opposed to omega-3 FA intake in combination with stretching and toning.

To conclude, this doctoral thesis was able to contribute to the evolving field of healthy brain aging and related cognitive and motor functioning. However, a lot of work is still needed to move this promising area forward. In the near future, the number of elderly people suffering from age-related dysfunctions will increase due to demographic changes [1]. Therefore, it is important that researchers continue to evaluate the influence of exergame training and its combination with omega-3 FA supplementation on healthy (brain) aging.


284. Schättin, A. and E.D. de Bruin, Combining Exergame Training with Omega-3 Fatty Acid Supplementation: Protocol for a Randomized Controlled Study Assessing the Effect on...
References


Acknowledgements
I am very grateful to all people who supported me during my PhD and contributed in different ways to the realization of this thesis.

First, I want to thank my former supervisor, PD Dr. Eling D. de Bruin. I am really grateful that you gave me the opportunity to do my PhD thesis at the IBWS and that you supported me during entire process. I was reassured by the fact that I could always count on your help, especially in difficult times when the projects did not run as planned. With your sense of humor, you eased even challenging situations. With your expertise, you helped me through every phase of my PhD from preparing my study proposals to writing my manuscripts.

I also respectfully acknowledge and thank Prof. Dr. Katrien de Bock and Dr. Isabelle Herter-Aeberli for accepting the role of co-referee and for the time and work they have invested in evaluating my thesis and in the doctoral exam. I would also like to thank Dr. med. Verena Klamroth-Marganska for her helpful contribution to the second experimental study.

Carrying out the studies was only possible thanks to the help of many people. Huge thanks go to all my past Master’s students – Jan Stutz, Rendel Arner, Simon Vogt, Martin Egloff, Corinne Baier, and Domenique Mai – for helping me with the recruitment, the measurements, the training sessions, and the analysis. Furthermore, I would like to thank Christina Pizio and Christina Chirila as well as Floriana Sonder and Mélanie Röthlisberger, who supported the study implementation during their internship at IBWS. Moreover, I would like to express my deepest gratitude to all participants for their effort, kindness and, enthusiasm while participating in these studies. Without them, the studies could not have been performed. Moreover, it was a great pleasure to see how much fun the seniors had during the video game-based training sessions.

Moreover, I would like to thank Joakim Graf from San Omega GmbH for sponsoring the nutritional supplementation and blood samples. I would also like to thank René Melliger from Swiss Medical Plus and Dr. med. Volker Schmiedel for their helpful support.

Additionally, I wish to express special thanks to the former and current group members of the IBWS for the pleasant working atmosphere and their helpful support. Regula, Yvonne, Roli, Andi, and Sarah, you always had a sympathetic ear for all matters, whether administrative or non-administrative. Rahel, I am very thankful for both the support that encouraged me to carry on and your helpful statistics tips. Moreover, I would like to thank to my former PhD colleagues Patrick, Eva and Seline for introducing me to life as a doctoral student, and for providing support and useful advice whenever needed. Further thanks go to my roommates Federico and Michael for the helpful scientific and non-scientific discussions. Federico, I want to thank you for your constructive feedback and support, especially for the EEG aspects. I was not always happy with it, but I always realized that my work as a researcher benefited from these inspiring discussions.
A special thank goes to Manuela. Manuela, at the beginning you were one of my roommates, but now you are my best friend. I am so happy that you entered my life and that we can support each other in both good times and bad. Further, you provided fresh impetus for our research group, which helped me to carry on with my PhD. I wish you all the best for your ongoing projects and hope you maintain a strong will in the difficult times.

My heartfelt thanks go to my whole family for being at my side whenever I needed them. My parents, my sister, and my parents-in-law with their persistence always believed in me and got me moving in the right direction. Your unconditioned love has and will support me through my whole life. Further, huge thanks go to all my friends for their support, for always being there, and for the hours of fun that accompanied beside my PhD. Last and most of all, I am deeply grateful to my boyfriend Tobi. Tobi, you really did manage to make me recover at the weekend so that I could continue with renewed energy. I know that I can always count on you. When I was sad, you gave me a hug and promised that everything will work out. Quite simply, you were always there for me with your unconditioned love – you and me.