Ethyl-eicosapentaenoic acid and carnosine supplementation´s effects on cognitive, behavioural and psychophysiological parameters on healthy young subjects - double-blind, placebo-controlled study

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Omega-3-rasvahapoilla on todettu olevan tiettyjä tärkeitä funktioita keskushermoston toiminnassa. Tutkimusten mukaan ne vaikuttavat mm. kognitiiviseen kehitykseen ja oppimiseen. Omega-3-rasvahapot ovat mukana synapsien tiedonsiirrossa, edistävät uusien synapsien kehittymistä ja lisäävät hermosolujen plastisuutta. Omega-3-rasvahappojen vaikutuksia keskushermostoon on tutkittu melko paljon, mutta tutkimus on painottunut kehityksellisten kysymysten tai erilaisten kliinisten ryhmien kuten masennuksen, ADHD:n ja skitsofrenian tarkasteluun. Näiden rasvahappojen vaikutuksia terveiden aikuisten aivotoimintaan on tutkittu hyvin vähän.

Tämän tutkimuksen tavoitteena oli selvittää etyyli-eikosapentaeenihappoa (E-EPA, Omega-3-rasvahappo) ja karnosiinia (proteiini) sisältävien lisäravinteiden vaikutuksia tiettyihin kognitiivisiin, psykofysiologisiin ja behavioraalisiin parametreihin. 24 tervettä vapaaehtoista 20–30-vuotiasta tutkimukseen valittua opiskelijaa jaettiin satunnaisesti, mutta sukupuolen suhteen tasan koe- ja kontrolliryhmään. Tutkittavat osallistuivat 45 päivää kestävään koejaksoon, jonka aikana koeryhmäläiset nauttivat E-EPA- ja karnosiinivalmisteita, ja kontrolliryhmäläiset vastaavia placebo-valmisteita. Koe toteutettiin kaksoissokkoasetelmalla ja koehenkilöt tutkittiin koejakson alussa ja lopussa. Erityisen kiinnostuneita tutkimuksessa oltiin lisäravinteiden mahdollisista vaikutuksista koehenkilöiden aivojen herätevasteisiin, reaktioaikoihin ja virheiden lukumääriin visuaalisen, esitystahdiltaan hitaan, jatkuvaa tarkkaavuuden ylläpitoa vaativan tehtävän aikana (Continuous Performance Task, CPT). Lisäksi koehenkilöt osallistuivat verikokeisiin ja täyttivät mielialaan (Profile of Mood States) ja uneen liittyvät kyselyt.

Veren rasvahappoanalyysien perusteella E-EPA- ja karnosiinivalmisteiden nauttiminen nosti seerumin EPA-pitoisuutta tilastollisesti erittäin merkitsevästi ja laski arakidonihappo/eikosapentaeenihappo-suhdetta (AA/EPA) merkitsevästi. Kyseisten monityydyttymättömien rasvahappojen matala suhde on todettu useiden tutkimusten mukaan terveydelle edulliseksi. Tästä huolimatta tilastollisesti merkitseviä eroja ryhmien välille ei löytynyt missään muissa mitatuissa parametreissä. Tämän tutkimuksen tulosten mukaan E-EPA- ja karnosiinilisäravinteet eivät vaikuta nuorten terveiden aikuisten aivojen herätevasteisiin (CNV ja P300), reaktioaikoihin tai virheiden lukumääriin jatkuvaa tarkkaavuutta ja valikoivaa reagoivuutta vaativassa tehtävässä. Lisäravinteilla ei myöskään ollut tilastollisesti merkitseviä vaikutuksia koehenkilöiden tarkkaavuuteen, impulsiivisuuteen, mielialaan tai unenlaatuun.

Avainsanat: Omega-3, EEG, herätevasteet, mieliala, reaktioaika, terveet aikuiset

Ethyl-eicosapentaenoic acid and carnosine supplementation´s effects on cognitive, behavioural and psychophysiological parameters on healthy young subjects - double-blind, placebo-controlled study

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Abstract

It has been documented that Omega-3 fatty acids have certain important roles in central nervous system (CNS) functions. Studies demonstrate that they improve cognitive development and memory related learning. Omega-3 fatty acids are also involved in synaptic transmission, they contribute to synaptogenesis and they increase neuroplasticy of nerve cells. Althought a lot of researches concerning Omega-3 fatty acids and CNS has been conducted, most of the studies have focused on developmental issues or on clinical situations such as depression, ADHD and schizophrenia. Very few basic experiments concerning healthy adults and their brain functioning in relation to Omega-3 fatty acids have been done so far.

The aim of this study was to examine the effects of ethyl-eicosapentaenoic acid (E-EPA, Omega-3 fatty acid) and carnosine (protein) supplementation on some cognitive, psychophysiological and behavioural parameters. 24 volunteer subjects, aged 20-30 years, were randomly, but balancing the gender divided into experimental and control groups. Subjects were tested in the beginning of the experiment and after 45 days. During this period, experiment group subjects were supplemented with E-EPA and carnosine products and control group subjects correspondingly with placebo products. The study was conducted in a double-blind fashion.

The main interest in the study was to examine dietary supplementation´s (E-EPA and carnosine´s) effects on subjects´ event-related potentials (ERPs), reaction times and number of errors they would make during a visual slow event rate Continuous Performance Task (CPT). The Profile of Mood States test, a sleeping questionnaire and blood´s fatty acid analysis were also conducted.

Blood analysis showed that after the E-EPA and carnosine supplementation, the EPA content increased and arachidonic acid/eicosapentaenoic acid ratio (AA/EPA) reduced significantly. Even though the low ratio of these polyunsaturated fatty acids (PUFAs) is beneficial according to many studies, there were no statistical significant differences between the groups in any other measured parameters. According to this study, E-EPA and carnosine supplementation does not have an influence on young healthy people´s ERPs (CNV and P300), reaction times or number of errors they make during CPT. Nutrition supplementation had also no effects on subjects´ attention, impulsivity, mood states or quality of sleep.

Keywords: Omega-3, EEG, ERP, mood state, reaction time, healthy adults

Introduction

Nutritional supplements not classified as drugs, especially the fish oil based ones that contain Omega-3 fatty acids, are a hot topic in the publicity at the moment. Omega-3 fatty acids are advertised to be almost a miracle substance that helps you to concentrate, gives you more energy and makes your memory, learning and mood state better. Is it really a fact that Omega-3 fatty acids can improve the attention, concentration and mood state status of healthy people? It has been reported that Omega-3 fatty acids have beneficial effects on the cardiovascular system and that they may decrease the risk of getting coronary heart disease CHD (Von Schacky & Harris, 2006). These results are, and have been, quite commonly accepted in Omega-3 fatty acid research field. Recently some researchers have yet come to the conclusion that even these effects are not so undisputable (Brouwer, Geelen & Katan, 2006). In the present study the main interest is in the more direct connection between Omega-3 fatty acids and central nervous system (CNS), not the cardiovascular system functions. It is, nevertheless, important to distinguish the difficulty (or, perhaps, the impossibility) of separating blood circulation and CNS from each other completely. For example, Das (2000) suggests a close interaction between the CNS, endocrine organs, cytokines, exercise and dietary Omega-3 fatty acids. This question is discussed more towards the end of the paper. This study will focus on the effects of ethyl-eicosapentaenoic acid (E-EPA) and carnosine supplementation on event-related potential (ERP) of the brain in the electroencephalographs (EEGs) of healthy 20-30-year-old adults. Special emphasis is given to the following ERP components: Contingent Negative Variation (CNV) and Positive 300 (P300). ERP components, reaction time, and number of errors were measured in slow stimulus rate Continuos Performance Task (CPT). Furthermore, blood fatty acid analyses, Profile of Mood State and sleeping questionnairs were conducted.

Omega-3 and Omega-6 fatty acids are long-chain polyunsaturated fatty acids (LC-PUFAs) which are normally found in the mammal CNS. Some Omega-6 fatty acids, such as arachidonic acid (AA), can be manufactured in the body using linoleic acid (LA) as a starting point. Whereas most of Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are manufactured in the body using alpha linolenic acid (ALA) as a starting point. Both Omega-3 and Omega-6 fatty acids influence on cellular activity by decreasing the level of cholesterol which hardens membranes (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002). Both of these are also required for normal membrane structure and functioning, and for normal signal transduction processes (Horrobin, Jenkins, Bennett, & Christie, 2002). Two types of PUFAs are so called “essential fatty acids” (EFAs) because they cannot be synthesized in the human body. Instead, they must be derived from the dietary resources. These are ALA and LA. They are precursors of the other longer-chain Omega-3 and Omega-6 fatty acids (Yehuda, Rabinovitz & Mostofsky, 2005). This study is focused mostly on the Omega-3 fatty acids and their functions, so Omega-6 fatty acid synthesis is not discussed here. According to Burdge & Calder (2005) human DHA and EPA synthesis from ALA is limited, therefore the types of fatty acids that are available to the composition of cell membranes depend a lot upon the diet. Brains, particularly the cerebral cortex, is rich in very long-chain Omega-3 fatty acids which are available mainly from a diet rich in fatty fish or some other seafood. The principle Omega-3 fatty acid in the brain is DHA, which comprises 10-20 % of all fatty acid compositions. ALA, EPA and docosapentaenoic acid comprise less

than 1 % and polyunsaturated Omega-6 fatty acid AA (arachidonic acid) approximately 10 % of total fatty acid composition (McNamara & Carlson, 2006). As regards the high concentration of these PUFAs in the nervous system, it is not surprising that investigators have focused on the role of these, and especially Omega-3 fatty acids, in studying brain functions. There are several different kinds of possible explanations on how Omega-3 fatty acids work in the brain. Yehuda et al. (2005a) have reported at least six different categories of PUFAs´ possible effects on brain functions. PUFAs can modifiy brain functions by affecting a) the cell membrane fluidity, b) the activity of membrane-bound enzymes, c) the number and affinity of receptors, d) the function of ion channels, e) the production and activity of neurotransmitters, and f) signal transduction, which controls the activity of neurotransmitters and neuronal growth factors (NGF). The critical factor in fatty acid efficacy does not, however, seem to be their absolute level in the body, but rather the ratio between various groups of fatty acids (Yehuda, Rabinovitz & Mostofsky, 1999; Yehuda, 2003). AA (Omega-6) competes directly with EPA (Omega-3) for incorporation into cell membranes and low AA/EPA ratio has been proposed as an index for the beneficial effects of Omega-3 (Yehuda et al., 2002). This has been described in animal and clinical experiments.

When studying the connection between fatty acids and CNS, it is possible to use either humans or animals. As usual, both situations have their advantages and disadvantages, and the option depends a lot on the exact questions that need to be answered. If the main interest is, for example, in the EFA deficiency during the development, or in the fatty acid compositions in the certain brain regions, it is usually animals that are used for ethical or practical reasons. Human studies are of course needed, since many researches focus on fatty acid supplementation and its effects for example on certain mood disorders. The following presentation of Omega-3 fatty acid and CNS-related studies is neither exclusive nor conclusive, but it aims to illustrate the research field and the importance of both human and animal studies.

A lot of basic research concerning Omega-3 fatty acids and their relationship to the brain has been done by using animals, mainly rats. Animal model building seems to be important in this research field because the ratio of saturated, monounsaturated, and polyunsaturated fatty acids is observed to be generally equal in post-mortem human frontal cortex and mammal cortex, including rats (Moriguchi, Loewke, Garrison, Catalan & Salem, 2001) and mice (Carrie, Clement, de Javel, Frances & Bourre, 2000). According to Wainwright (2002), Omega-3 fatty acids may play a role in cognitive development, and Omega-3 deficiency impairs the ability to respond to environmental stimulation in rats. Wainwright states that the provision of Omega-3 as well as Omega-6 fatty acids may be necessary for the normal growth and functional development to the developing brain. Li, Weisinger, Weisinger, Mathai, Armitage, Vingrys & Sinclair (2006) found that Omega-3 and Omega-6 imbalance early in life leads to persistent reductions in DHA levels in rat hypothalamus. This is not cured even after a long–term Omega-3 fatty acid repletion. Wu, Ying & Gomez-Pinilla (2004) have reported that Omega-3 fatty acid supplementation can facilitate the recovery from a traumatic brain injury by providing protection against reduced plasticity in the rat brain. Supplementation compensated for cognitive impairments, reduced oxidative damages and normalized the levels of Brain Derived Nerve Factor (BDNF) and other substances that effect synaptic transmission in the rat´s CNS. Omega-3 (E-EPA) supplementation can also attenuate inflammatory-induced impairments in spatial memory (Song & Horrobin, 2004) and significantly reduce stress and anxiety-like behaviour (Song, Li, Kang & Kadotomi, 2006) in rats. Song, Li, Leonard and Horrobin (2003) found that among three different fatty acids at 0,5 % concentration, EPA treatment alone is more effective in the modulation of stress hormone corticosterone, stress, and anxiety-like behaviour, wich were increased by proinflammatory cytokine interleukin-1β (IL-1β). Frances, Monier &

Bourre (1995) discovered that Omega-3 (ALA) deficient mice showed less efficient learning and poorer understanding of the situation/environment. According to Yehuda et al. (2005a), more general physical symptoms of EFA deficiency include fatigue, dermatological problems, immune problems, weakness, gastrointestinal disorders, heart and circulatory problems, growth retardation, and sterility. Additionally, they have documented that Omega-3 fatty acid deficiency reduces the dopamine vesicle density in the cortex and causes malfunction of the dopaminergic mesocorticolimbic pathway.

The following studies concern Omega-3 fatty acids, CNS and psychological variables in humans. Researchers have found many important roles for these fatty acids in the CNS functioning and also that the EFAs have potential in treatment of various neuropsychiatric disorders in humans. When studying humans and fatty acids from the psychological perspective, there are two main approaches. One approach is to try to find out whether there is a correlation between the examined variables and the fatty acid levels in the blood. The other approach is to use controlled intervention, like fatty acid supplementation, and then analyse the outcome. Outcome variables can be everything from reaction time to blood markers and psychological factors. In the psychology-related research field, roughly 50 % of the Omega-3 studies made with human subjects have focused on developmental issues. The other half of the studies have focused on pathological issues and/or their treatment.

Developmental psychological Omega-3 fatty acid researches have focused mainly on the relationship of the fatty acids and CNS maturation, and treatment of neurodevelopmental conditions, such as attention deficit/hyperactivity disorder (ADHD), dyslexia and dyspraxia, etc. It has been documented that the major Omega-3 fatty acid (DHA) accumulates in the brain during perinatal cortical expansion and maturation. In primates and humans, preterm delivery is associated with deficits in fetal cortical DHA accrual. Children/adults born preterm exhibit deficits in cortical gray matter maturation, neurocognitive deficits (particularly in attention), and increased risk for ADHD and schizophrenia (McNamara & Carlson, 2006). This association, Omega-3 fatty acids´ relation to dopamine neurotransmission shown in animals (Carrie et al., 2000), and intervention studies suggest that perinatal deficits in brain DHA accrual may present a preventable neurodevelopmental risk factor for the emergence of certain psychopathology. It has also been proposed that maternal intake of very long-chain (VLC) Omega-3 fatty acids during pregnancy and lactation may be favourable for later mental development of children. Helland, Smith, Saarem, Saugstad & Drevon (2003) reported that children´s mental processing scores at 4 years of age correlate significantly with maternal intake of Omega-3 fatty acids during pregnancy.

Division to developmental and pathological Omega-3 studies is somewhat artificial, because studies that focus on the neurodevelopmental conditions could belong to both of these groups. Although the aetiology of ADHD, dyslexia and dyspraxia are acknowledged to be both complex and multifactorial, the assumption behind many developmental fatty acid studies is that fatty acid levels reflect, at least partly, an underlying abnormality of fatty acid metabolism. Burgess, Stevens, Zhang & Peck (2000) illustrate the similarity between ADHD children and symptoms observed in EFA deficiency in animals and humans. According to Richardson & Puri (2000), the consistent findings of both clinical signs of fatty acid deficiency and blood biochemical indices of fatty acids abnormalities indicate that supplementation with LC-PUFAs might be helpful in the management of ADHD. Richardson (2004) suggests that Omega-3 fatty acids may also have positive effects on dyslexia, dyspraxia and the autistic spectrum disorder (ASD). Stevens, Zentall, Abate, Kuczek and Burgess (1996) found in their study that low concentration of Omega-3 fatty acids implies greater number of behavioural and sleep-related problems in 6 to 12 years old boys.

Neverheless, these studies are not the whole truth and many other researches fail to demostrate such beneficial effects.

Voigt, Llorente, Jensen, Fraley, Berretta & Heird (2001) studied ADHD children who had previously been using stimulant medication and received effective maintenance therapy. In this placebo-controlled double-blind study, the experiment group received 345 mg of DHA/day for 4 months. The control group received a corresponding amount of placebo products for the same period of time. Inattention and impulsivity was measured by tests in a laboratory and by parent evaluations. After the 4 months period, the plasma phospholipid DHA content was 2.6-fold in the experiment group. Nevertheless, no statistically significant improvement in ADHD symptoms was found in either group. Hirayama, Hamazaki & Terasawa (2004) studied ADHD children, from whom the majority was not under medication. This study was also conducted in a double-blind placebo-controlled manner. The experiment group children ate DHA-containing food for 2 months. Concurrently, the control group ate food that was indistinguishable by look, taste or smell, but did not contain fish oil. Researchers measured the attention deficit, hyperactivity and impulsivity of the subjects. They also measured visual perception, visual and auditory short-term memory, and studied the development of visual-motor integration, continuous performance and impatience. DHA-containing food supplementation did not have an effect on ADHD-related symptoms or on any aforementioned parameters. Placebo-controlled double-blind study carried out recently by NMI (Niilo Mäki Institute) and University of Jyväskylä studied the effects of E-EPA and carnosine, the same nutritional supplements used in the present study, but from a different perpective and using different population. Those results showed that 3 months of E-EPA and carnosine supplementation had no effects on dyslexic children´s attention, reading, writing or mathematic skills, even though a significant change in the blood AA/EPA ratio was visible (Kairaluoma, Närhi, Ahonen, Westerholm & Aro, submitted). Another substance used in this study, carnosine, is a protein that is normally found in the mammal CNS. It is also commonly used as a nutritional supplement. In animal studies it has been reported to improve, for example, orientation and learning after experimental brain ischemia by an increase in glutamate binding to n-metyl-d-aspartate (NMDA) receptors (Gallant, Kukley, Stvolinsky, Bulygina & Boldyrev, 2000).

Omega-3 studies that are related to pathological situations in adults have dealt with, for example, Alzheimer disease, autism, schizophrenia, hostility, anxiety, bipolar disorder and depression (Logan, 2003). Several epidemiological studies suggest covariation between seafood consumption and rates of mood disorders, such as depression and bipolar disorder. Biological marker studies indicate deficits in Omega-3 fatty acids in people with depressive disorders. Several treatment studies indicate the therapeutic benefits of Omega-3 fatty acid supplementation (Parker, Gibson, Brotchie, Heruc, Rees, & Hadzi-Pavlovic, 2006). Berger, Smesny & Amminger (2006) reported that purified EPA is a modestly effective augmentation treatment to antipsychotic medication in acute schizophrenia at dozes of 1-3 grams/day, but on the other hand, some other studies fail to demonstrate such an effect. Yao, Magan, Sonel, Gurklis, Sanders and Reddy (2004) demonstrated that EPA may be mediating its therapeutic effects in schizophrenia via modulation of the 5-HT(2) receptor complex. Another possible explanation to some of the beneficial effects of fish oils on diseases might relate to the change in the ratio of epinephrine and norepinephrine (NE) in the plasma. Hamazaki, Itomura, Huan et al. (2005) found that EPA and DHA supplementation lowered the plasma NE concentrations in normal volunteers even at the relative small total dose of 762 mg of EPA and DHA per day. Yehuda, Rabinovitz and Mostofsky (2005b) reported that a mixture of Omega-3 and -6 fatty acids can have an effect on the test anxiety, which is an incapacitating academic syndrome. These fatty acids have been documented to improve the behavioural variables associated with test anxiety, i.e. appetite, mood, mental concentration,

fatigue, academic organization and poor sleep, as well as lowering elevated cortisol levels, with a corresponding reduction of anxiety. A lot of promising results related to fatty acids, mental disorders, mood states and sleep have been presented, but further research is still needed.

Despite the fact that Omega-3 fatty acids have been studied widely, at least one clear gap in the research field can be found. Very few basic studies have so far concerned healthy adults, brain activation, and Omega-3 fatty acids. The author of the present study finds it important to study the effects of the nutrition supplements on the healthy population also, especially when Omega-3 fatty acids are advertised to improve the concentration, memory, learning and mood state status of the healthy people. The only study of this kind that was found was made in University of Siena, Italy, by Fontani, Corradeschi, Felici, Alfatti, Migliorini and Lodi (2005). They studied the effects of Omega-3 fatty acid supplementation on cognitive and physiological parameters in healthy 22-51-year-old subjects. A total of 49 healthy voluntary subjects were recruited from a local non-competitive athletic association. 33 (13 males and 20 females) of them received daily supplementation of Omega-3 (mainly DHA, EPA, ALA) for 35 days. 16 subjects (4 males and 12 females) received a daily supplementation of olive oil, considered as placebo. The study was conducted in a double-blind fashion. Both groups were tested in the beginning of the study and after 35 days of supplementation. Researchers measured, among other variables, event-related potentials (ERPs) by electroenchephalograph (EEG), and reaction times and errors made in different kinds of visual attention/reaction tasks. The test included four different kinds of tasks: 1) Alert (simple reactivity, no significant central processing), 2) Go/NoGo (impulsivity/response inhibition), 3) Choice (central processing) and 4) Sustained Attention. The Profile of Mood State test (POMS) and blood fatty acid analyses were also carried out. The blood analyses showed that the AA/EPA ratio was strongly decreased by the Omega-3 treatment. In the POMS analysis, the increase of vigour and decrease in other mood states (anger, anxiety, fatique, depression and confusion) was visible only in the experiment group. The reaction times were reduced after Omega-3 supplementation in the Go/NoGo and Sustained Attention tasks. The number of errors was also reduced in the experiment group. The ERP (CNV and P300) amplitudes changed significantly only in the Go/NoGo task and in the experiment group. These findings suggest that young healthy adults can benefit from the Omega-3 fatty acids supplementation.

The present study will focus on the effects of ethyl-eicosapentaenoic acid (E-EPA) and carnosine supplementation on some neuro-electrical parameters in EEG and on some behavioural and cognitive parameters. By EEG it is possible to measure the voltage changes on the surface of the skull. The changes are due to the huge amount of simultaneuos action potentials in certain regions in the brain. ERPs are voltage changes that are elicited or connected to some external or internal event. From the psychological perspective, especially the so-called slow ERP components, in which the latencies are more than 100 ms, are the most interesting. Slow ERP components are assumed to be “endogenous” and to reflect perceptual, cognive and motor processes that are related to the stimulus evaluation (Rockstroh, Elbert, Birbaumer & Lutzenberger, 1982).

In electrophysiological recordings of this study, the focus will be on two slow ERP components, the Slow Wave (SW) called Contingent Negative Variation (CNV) and Positive 300 (P300). CNV was first reported by Walter, Cooper, Aldridge, McCallum and Winter (1964). It develops during the period following a warning stimulus and prior to an event, reflecting processes of attention, expectancy, and preparation (Rockstroh et al. 1982). Two stimuli (S1-S2) paradigm is most traditionally used for generating the CNV. S1 is a warning or conditional stimulus triggering the CNV. S2 is an imperative stimulus, followed by the subject´s motor response. (Walter at al. 1964; Tecce, 1970.) Even though the

endogenous components´ scalp distributions are not very distinct, CNV tends to be largest over the frontal and central regions of the brain (Coles & Rugg, 1995). Two distinct phases of CNV are commonly distinguished. Early part of the CNV is called the orienting-wave (O-wave) and the terminal part is called the expectancy wave (E-wave) (Rohrbaugh & Gaillard, 1983). The O-wave is commonly associated with response orientation, while the role of the E-wave is not so clear (McCallum, 1988). It has been proposed that the E-wave would not be related to the warning but to the imperative stimulus, being similar to the readiness potential (Bereitschafts potential) and precede voluntary movements (Loveless, 1977). Even though CNV seems to be closely related especially to motor preparation, there is still a controversy between the motor and non-motor CNV in the research field. According to many studies motor activity is not essential for generating the CNV. This non-motor CNV reflects perhaps a more general preparation for planned action, which is not only related to motor readiness (Rockstroh et al. 1982; Gaillard & van Beijsterveldt, 1991).

P300 is a positive–going deflection within a latency between 250 and 350 ms (sometimes even 600 ms), that was first observed by Sutton, Baren, Zubin & John in 1965. This ERP component is measured by quantifying its amplitude (size) and latency (timing). Amplitude is defined as the voltage difference between a prestimulus baseline and the largest positive-going peak of the ERP waveform within the aforementioned latency range. This range can vary depending on the subject charasteristics, stimulus modality, task conditioning etc. Latency is defined as the time from stimulus onset to the point of maximum positive amplitude within the latency window (Polich, 1996). P300 component is one of the most studied ERP components and it is reported that several variables are necessary for evoking it. P300 is typically observed in study designs in which subject has to count or attend to infrequent stimuli within frequent ones, or count target stimuli within a sequence of target and non-target stimuli. Classical “oddball” design is maybe the most popular way to elicit P300. Probability and task relevance of the stimulus are proposed to be the major determinants of the P300. Its scalp distribution shows a centro-parietal maximum (Rockstroh et al. 1982). Even though the P300 component has been studied a lot, there is still a controversy about what cognitive events it reflects. It has also been suggested that several different P300 components exist and that these reflect different phenomena, at least partly. The most common way is to devide the P300 to P3a and P3b. P3a is an early peak that is called the novelty P3. P3b is the later canonical peak of P300 that is more related to information processing operations (Polich, 1996). Although division in two is the most popular in several studies, many more have been proposed in others (Tueting, 1978). The latency of P300 is proposed to be related to the stimulus evaluating processes. It is independent of response selection and execution processes (Donchin, Ritter & McCallum, 1978). The major theoretical interpretation of P300 amplitude is that it indicates brain activation stemming from “tasks that are required in the maintenance of working memory” when the mental mode of the stimulus environment is updated (Donchin & Coles, 1988). Thus, P300 is assumed to reflect neuroelectric activity related cognitive processes, such as attention allocation and activation of the immediate memory. Polich & Kok (1995) have found evidence that P300 is also influenced by biological processes, such as fluctuations in the arousal state of subjects.

According to all this background, ethyl-eicosapentaenoic acid and carnosine supplementation is proposed to have beneficial effects on healthy adults' CNS functions, and therefore, to the psychological factors also. In the present double-blind placebo-controlled experiment, these are studied by measuring psychophysiological (CNV and P300) and behavioural parameters (reaction times and errors) during visual slow event rate CPT. Subjective questionnaires are carried out in order to evaluate mood state and sleep quality. Blood analyses are conducted to

see the possible effects on the biological level. All parameters are examined in the beginning and at the end of the nutrition supplementation.

Hypotheses of this study are the following. E-EPA and carnosine supplementation is supposed to lower the plasma AA/EPA-ratio and to improve the attention, concentration, reactivity and accuracy status of the Experiment group subjects. This is assumed to be indicated in the slow evoked potentials and in the behavioural parameters. CNV and P300 amplitudes are assumed to increase and P300 latencies to get shorter after the E-EPA and carnosine supplementation. The Experiment group subjects are supposed to make fewer errors, and their reaction times are assumed to get shorter after the nutrition supplementation. Subjects´ mood states and quality of sleep are also presumed to elevate in the Experiment group. No alterations are supposed to be visible in any of the measured parameters in the Control group.

Method

Subjects

A total of 24 volunteers in the ages of 20-30 from the University of Jyväskylä participated in this study. Participants were recruited through the University`s mailing list. Subjects who had fish allergies, smoked more than eight cigarettes, or drunken more than two portions of alcohol daily, were excluded from the sample by informing this exclusion criteria in the recruiting mail. When enough participants were found, they were interviewed in order to ensure that their basic state of health is fine, and that they do not use any dietary supplements at the moment or have not done so during the last three months. Finally, 12 female and 12 male subjects were included in the experiment. The subjects' mean age was 23.8 years (SD 2.1), and all subjects excercised sports for about 5 hours per week in average. The subjects completed a background form and they were informed about all the procedures involved in the study. The subjects were divided into two groups randomly, however balancing the gender ratio so that an equal number of both sexes were present in both groups. Half of the subjects were in the *Experiment group* (mean age was 23.9 and SD 2.2 years**)** and the other half in the *Control group* (mean 23.7 and SD 2.0 years). Two groups were very equal as regards all the background variables (includig age, weight, height and the amount of weekly sports exercise). A printout of subjects' own EEG was presented as a reward for participating in the study. Participants were informed about the aim and the double-blind placebo design of the study. After analysing the data the experiment and control group codes were opened. After this, the participants were informed about the group they belonged to. After completing the testing period, the control group subjects had an opportunity to receive the actual dietary supplements. Licence for the study was applied from the Ethical Committee of the University of Jyväskylä.

Design and procedure

Experiment group subjects were given two different kinds of nutritional supplements during a 45-day period: E-EPA 500 mg capsules that contained ethyl-eicosapentanoicacid (E-EPA) and Bio-Carnosine 400 mg (pills) that contained carnosine. These supplements are not classified as drugs; instead, they are dietary supplements which are not unfamiliar to the

human body. The used products are well-tested and have been commercially available in shops for many years. The daily dosage of E-EPA in the present study was 1000 mg and carnosine dosage was 800 mg. Concurrently, the *Control group* subjects recieved an equivalent amount of placebo pills that should not be distinguishable from the real dietary supplements by look, smell or tast. Placebo pills contained substances that, according to the manufacturing medicine company, have practically a zero-effect to the human body. E-EPA placebo contained medium chain triglyseride–oil and carnosine placebo micro-cristallaneous cellulose, silica dioxide and magnesium salts of fatty acids.

Three different kinds of methods were used to acquire information about the phenomenon, namely blood analysis, beavioural and psychophysiological parameters in reaction time (RT) task, and subjective questionnaires. The main interest in this study was in the evoked potentials (EP) that were measured from the EEG. EEG, reaction time and number of errors were measured during the RT task. Questionnaires were subjective forms that participants had to fill in during the first and during the last three days of the dietary supplementation. The purpose of Profile of Mood States (POMS) questionnaire (McNair, Lorr and Droppleman, 1981) was to measure possible alternations in mood states. The other form measured the quality of sleep and it was created for this study. All subjects were tested before and after the 45-day dietary supplementation.

Blood analyses

Blood samples were taken from every subject in the beginning and at the end of the studying period in the laboratory of hospital Mehiläinen in Jyväskylä. Blood analyses were conducted in the Mineral Laboratory Ltd in Helsinki. First blood samples were taken in the beginning of the experiment 1-2 days prior to the date when the subject started to consume the dietary supplements. The same procedure was conducted at the end of the intervention, 2-5 days after the date when the subjects had consumed the last capsules/pills. The blood samples were frozen and sent to Mineral Laboratory. An extensive blood´s fatty acid analysis of different kind of fatty acid amounts and proportions in the subjects´ plasma was carried out. This paper concentrates only in those few blood markers that were considered as the most important. The focus was on the amount of EPA and the ratio of AA/EPA in the serum. The increase of EPA in the blood of the experiment group subjects would show that the substance has absorbed to the body. Nevertheless, the beneficial effects of Omega-3 fatty acids are not linearly equivalent to the amount of EPA in the blood. A better indicator of beneficial effects of Omega-3 fatty acids is a low AA/EPA ratio.

Reaction time task

The main interest in this study was in the reaction time (RT) task and variables measured during the task. The same reaction time task was presented to the subjects in the beginning and at the end of the nutritional supplementation. The subjects performed the task in a sound attenuated and electrically shielded room. Subjects were seated at the front of the desk to which the response button was attached. The television screen was in front of the subject at a distance of 130 cm. The experimental room was connected to the laboratory room with a bi-directional communication system.

Subjects were asked to perform the visual slow event rate Continuos Performance Task (CPT). They were given instructions which emphasized speed and

accuracy. The subjects were informed to adopt a relaxed position and to avoid any unneccesary movements. The subjects were instructed to react by pressing the button to one stimulus and not to react to the other. This CPT is based on the traditional S1-S2 anticipating paradigm. The difference to the traditional anticipating paradigms was that the trial length was varied. S1 was a plus sign (+) in the middle of the screen with either a fixed duration or 6500 ms (every second trial) or variable duration of 5500, 6500, 7500 or 8500 ms in pseudo-randomized order. Unlike the S1, the duration of S2 was always a constant 500 ms. S2 was either an asterisk (*, p=.75) or a circle (o, p=.25). The subjects were instructed to react to the asterisk by a fast button press with the forefinger of the dominant hand and to inhibit the response in the case of a circle. The task was preceded by a 5 minute training session under experimenter supervision for the full comprehension of the rules. Figure 1 illustrates the CPT design.

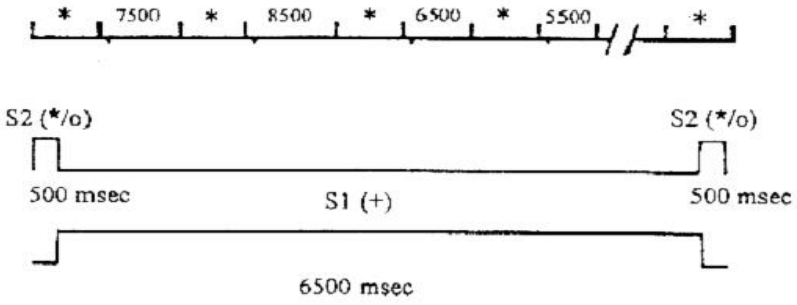

Figure 1. Graphical illustration of the visual slow event rate CPT design. The figure is adopted and modified from Kivijärvi and Saunamäki (1999).

In the so-called Go/NoGo-design, it is possible to measure reaction times, errors and subject´s response inhibition/impulsiveness. The RT task was purposefully designed as uninteresting and time-consuming. The stimuli were simple and slow event rate. The duration of the test was 30 minutes, so it stressed the subjects' sustained attention quite significantly. During the reaction test, the subjects´ reaction times and also the number of errors they made were measured. It was possible to make two different kinds of errors: pressing the button when not needed (commission) and not pressing when needed (omission). In the analysis part of this paper, no differentation was made according to the error types. Instead, all errors were under the same category.

All the measurements were done during the daytime, between 8 AM and 5 PM. Circadian variation in the attention within the subject remained constant because the measurement time was controlled. Every subject participated in the CPT nearly at the same time of the day (+- 1 hour) in the beginning and at the end of the study. The subjects were also pooled, so that nearly an equal number of subjects who were measured in the morning belonged to both study groups. These procedures were conducted also to ensure that the subjects´ arousal state will not have an effect on the measured P300 evoked potentials or any other measured parameters.

Electrophysiological recordings

Controlling the experiment, presenting and timing the stimuli and storing the behavioural responses were conducted by an Amiga 2000 computer. The EEG recording system was based on the Bio-logic Brain Atlas-system. Data acquisition of electrophysiological responses was conducted with Techmar´s Labmaster 12-bit, 16-channel AD converter and DSAMP software run on a 233 MHz Pentium PC.

During the reaction time task, EEG was measured from the surface of the skull. The EEG was recorded by using an EEG-cap (ECI) according to the international 10-20 system. Recordings were made from the five channels: frontal (fz), central (c3, cz and c4) and parietal (pz). The electroogulograph (EOG) was obtained with electrodes positioned at the lower corner of the right eye and upper corner of the left eye. Linked mastoids were used for the references for recordings. The impedance level of ECI, EOG and mastoid electrodes was always below 10 kΩ and mostly within 0,5-4 kΩ. Bandpass was DC -70 Hz and sampling rate in the recordings was 200 Hz. The measuring procedure started 2500 ms before S1 and ended 3000 ms after the onset of S2, resulting in a total trial length of 12 000 ms.

Mood

The subjects estimated their mood states by filling in the POMS-questionnaire. They filled in the questionnaire during the first three days of nutrition supplementation, and again during the last three days. The subjects were informed to fill in the questionnaire at the same time every day. They also were asked to mention if they had something significantly deviant from normal going on in their life which might affect to their mood state. Four different factors were derived from the POMS, namely vigour, aggression/anger, depression/fatigue and anxiety. First three factors were linear, that the higher numbers the subjects gave to themselves, the more strongly the subjects would exhibit the behaviour of the factor in question. The last factor which measured anxiety was an exception, as it was built up inversely.

Sleep estimation

The subjects estimated several different factors related to their sleep quality, habits and amount. Only the quality of the sleep in relation to normal is discussed in the present paper. The subjects estimated the quality of sleep during the first and last three nights of the nutrition supplementation. The scale was ordinal and it had five steps from 1 to 5. Number 1 meant that “Sleep was a lot worse than normally” and number 5 that “Sleep was a lot better than normally”. Number 3 presented the average quality of sleep. Mean value of the responses from the first three nights in the beginning of study was calculated and used as an index to sleep before the nutrition supplementation. A similar index was calculated from the last three nights.

Data Reduction and Analysis

The raw EEG data were managed by using the DSAMP-program. EOG and movement-related artifacts were investigated visually trial by trial. Different kinds of exclusion criteria for artifact reduction were tested. These operations did not prove out to “clean” or change the averaged EEG-curve significantly. Therefore, no artifact reduction was decided to be used in

the final analysis. From the 240 trials per task, only 120 trials containing fixed duration S1-S2-pairs were averaged. During the second CPT measurement, technical problems occurred with one experiment group subject. There was a problem with the electrode in the C3 location and only the first 68 of total 120 trials could be included in the final analysis. Otherwise, all raw EEG data from every subject were included in the analysis.

CNV data were first divided into five equal sequence time windows (500 ms each) according to Sintonen (2004). The mean amplitude of every window was calculated so that all five channels (fz, c3, cz, c4 and pz) had five averaged window values each. Figure 2 shows how averaged data of each subject was divided into the time windows: W1; 500-1000 ms, W2; 2000-2500 ms, W3; 3500-4000 ms, W4; 5000-5500 ms and W5; 6500-7000 ms. The first time window started when the S2 (asterisk or circle) of the previous trial ended and the last window ended when the new S2 (asterisk or circle) of the present trial appeared.

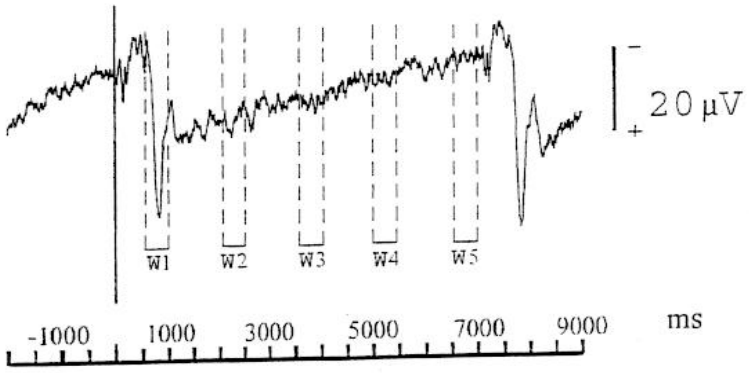

Figure 2. Averaged CNV waves were devided into the five time windows. Adopted from Sintonen (2004).

The difference window was created in order to study the change of the CNV in relation to time within one trial. This difference window (W52) was calculated by substracting the original value of time window W2 from that of W5. This index shows the change in CNV from the 2000 ms point to the end of the trial. A total of 5 CNV indexes were obtained because of the five measuring channels fz, c3, cz, c4 and pz were used. The more negative the values are the larger the CNVs are. In order to ensure that possible change in the P300 would not affect the CNV index, W1 time window was not used in the CNV calculations. On the other hand, the positive peak of the first time window (W1) was used as an index for the amplitude of P300.

SPSS for Windows 12.0 was used in the statistical analysis. A multivariate analysis of variance (MANOVA) design for repeated measures was conducted between subject factors *Task* (two levels: *Start* and *End*) and *Group* (two levels: *Experiment group* and *Control group*). The subject factor included CNV (CNV indexes: fzCNV, c3CNV, czCNV, c4CNV and pzCNV), P300 amplitudes (value of time window W1, in five channels), number of errors, reaction times, POMS factors and sleep index.

Results

Blood analysis
After 45 days of E-EPA and carnosine supplementation, blood serum´s EPA composition was 2.4-fold in the *Experiment group*. MANOVA revealed statistically significant Task and Group interaction ($F(1,22)= 26.7$, $p<.000$). There was no significant change in the blood status of the *Control group*. Figure 3 below illustrates these results.

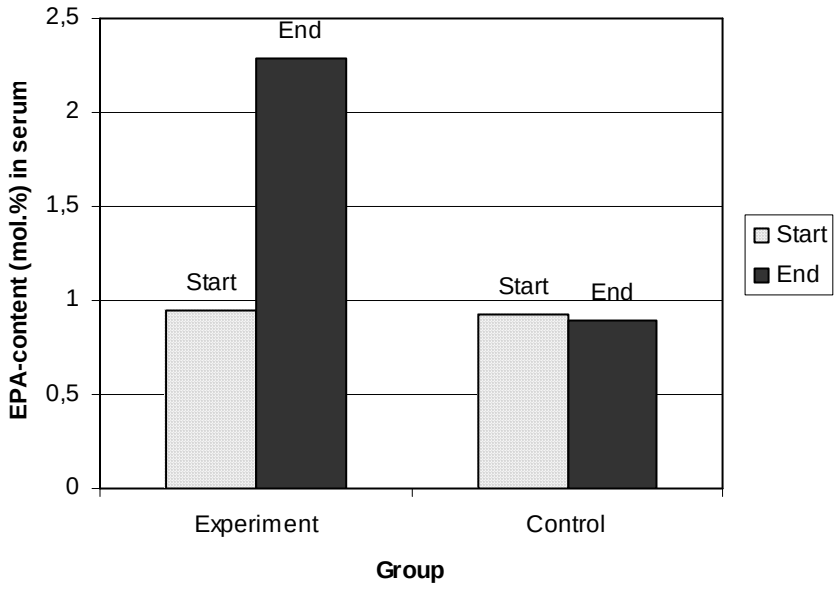

Figure 3. EPA-content (mol.%) in serum elevated 2.4-fold high in the Experiment group after nutrition supplementation.

Elevated EPA composition in the *Experiment group* indicates that the intervention period was long enough for the nutrients´ absorption into the body. A better indicator for the beneficial effects of Omega-3 fatty acids is the AA/EPA-ratio. Figure 4 illustrates the change in this ratio in both groups. AA/EPA-ratio was reduced statistically significantly only in the *Experiment group*. MANOVA revealed significant interaction between the *Task* and *Group* ($F(1,22)= 8.9$, $p<.01$).

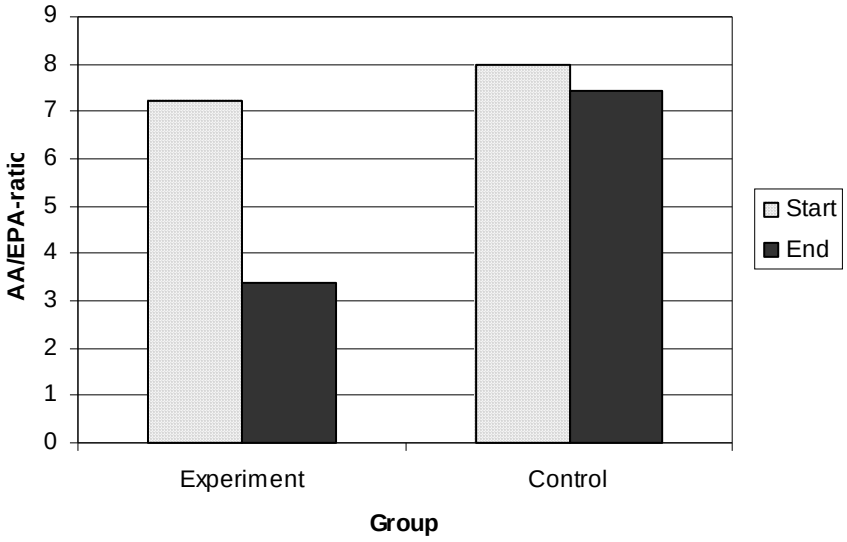

Figure 4. Intervention reduced the AA/EPA-ratio significantly in the Experiment group. (P-value <.01).

Reaction time task (CPT):
Examination of Evoked Potentials, CNV and P300

Event-related potentials (CNV and P300) that were measured during the CPT are presented next. In the present study, balancing the groups was achieved also in the level of ERP starting, which contributes to the reliability of the psychophysiological results. Figure 5 shows how similar the studied groups were in the beginning and at the end of the study as regards the slow potential CNV.

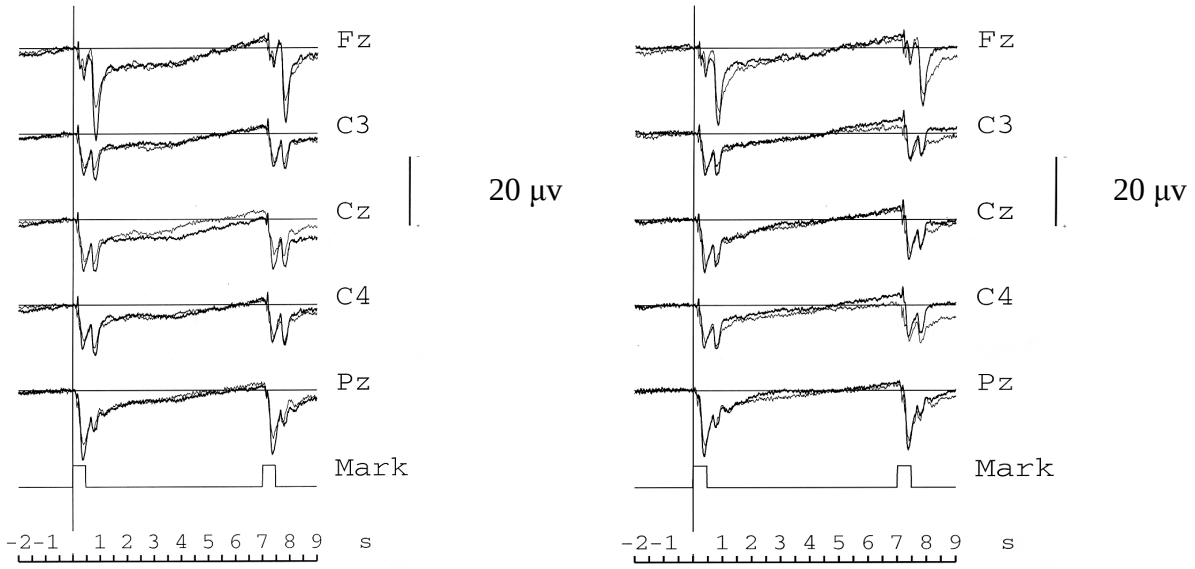

Figure 5. Grand averaged CNV-waves. Start measurement on the left and End measurement on the right. Thinner line represents the Experiment group in both situations. (Marker bars on the right side of the both illustrations are 20 µv high.)

This study focused on the group level differencies and no analyses within single subject are presented here. MANOVA revealed no statistically significant *Task* and *Group* interaction in CNV-amplitudes in any of the measured channels. Neither *Group* nor *Task* alone affected the values of CNV. Figure 6 illustrates the location of the five measuring points and also how the CNV curves remained astonishingly unaltered also within the subjects.

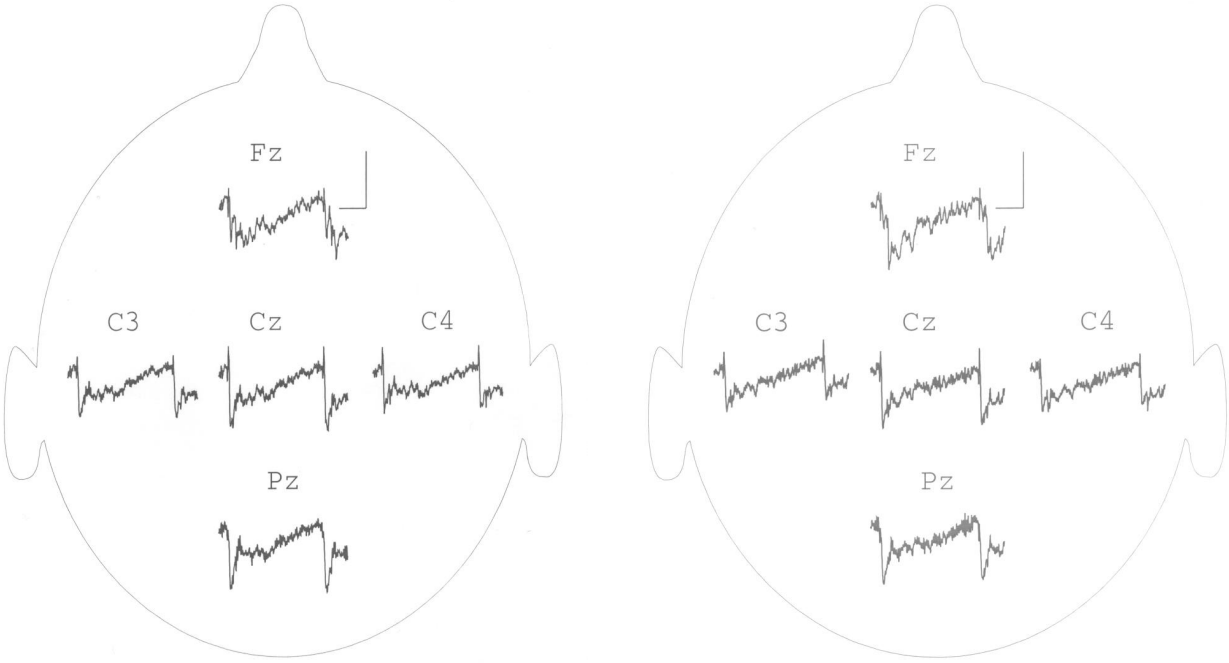

Figure 6. Averaged CNV curves of one experiment group subject illustrate the location of the five EEG measurement points on the skull. Head plot on the left represents the first measurement and the second measurement is on the right. Averaged CNV curves from single subjects remained amazingly unaltered. Marker bars in the right upper corner of both pictures are 10 µV high and 2000 ms long.

When studying the diffrences in P300 amplitudes, MANOVA again revealed no statistically significant main or interaction effect for *Task* or *Group* in any measured channel. The only P300 amplitude parameter which nearly reached the statistically significant value was the main effect for *Task* in the parietal region (Pz) ($F(1,22)= 4.0$, $p=.057$). As Figure 7 visually demonstrates, the P300 latency remained practically unaltered and no further statistical analysis was needed to confirm this. Two separate positive peaks can be identified from the pictures. The first peak appears just before the 500 ms point on the average, and it is assumed to reflect the noticing and simple interpretation of the stimulus. The second peak can be seen closer to the 1000 ms point, and this might reflect the motor activity and processing that is related to pressing the button.

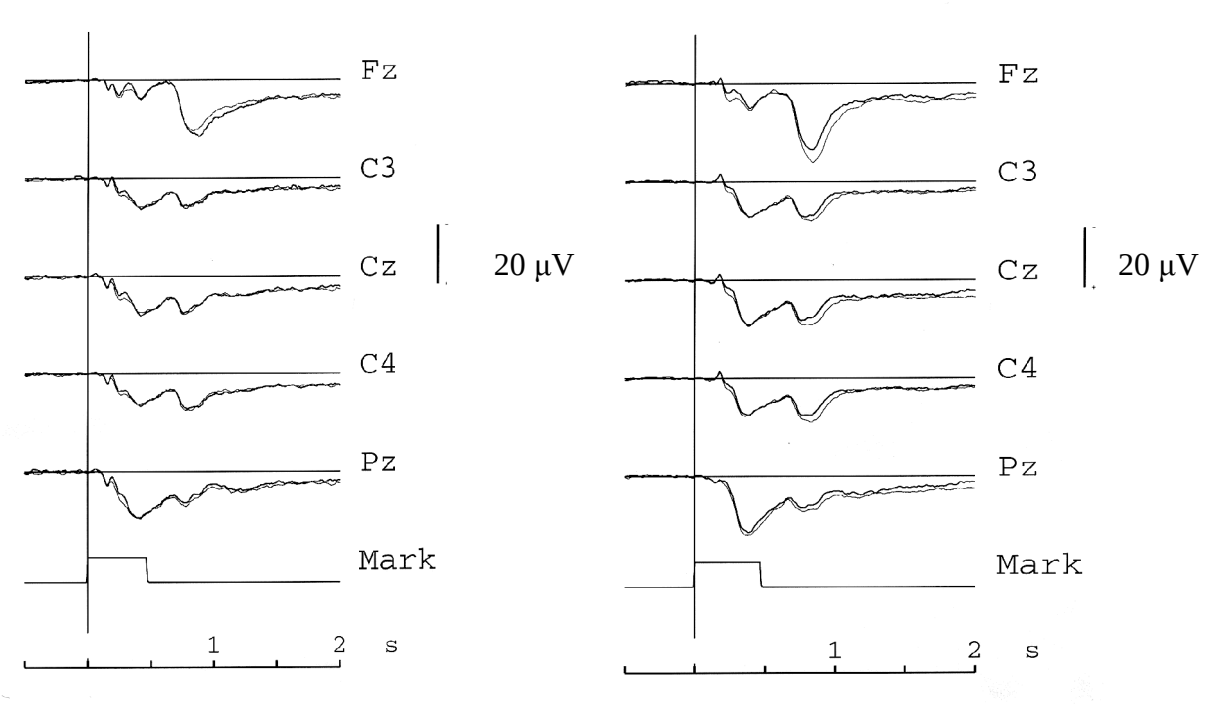

Figure 7. Grand averaged P300 waves. The thinner line represents the first measurement point. The Experiment group´s P300s are on the left and the Control group´s on the right. Marker bars are again 20 µv high.

Behavioural results

E-EPA and carnosine supplementation had no effect on subjects´ reaction times. MANOVA results show no Task and Group interaction ($F(1,22)= .198$, $p=.660$). The *Experiment group* subjects had slightly longer reaction times in the beginning and at the end of the experiment. MANOVA revealed a nearly significant difference between the groups ($F(1,22)= 4.11$, $p=.055$). This difference was, nevertheless, very small: about 20 ms. These results are presented in Figure 8.

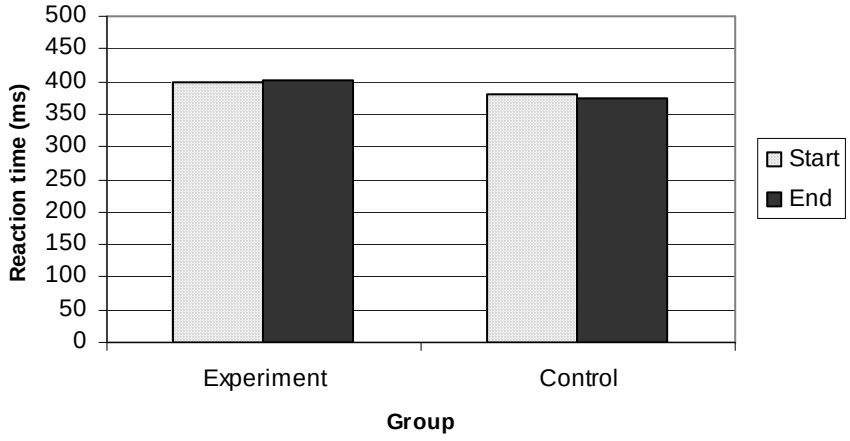

Figure 8. Averaged reaction times in milliseconds (ms). Reaction times remained practically unaltered in both groups.

When studying the error performance, it seemed at first that the subjects in the *Experiment group* were making a lot more errors on the average. This applied to both measurement times. MANOVA revealed, however, no significant group difference. After checking each participant's data separately, it eventually appeared that two Experiment group subjects were doing much more errors than the average in both measurements, and this had distorted the results. When these two subjects were excluded from the analysis, the error performance stabilized. Decline in the number of errors was greater in the *Experiment group*. According to MANOVA, no significant interaction between *Group* and *Task* was found. In both groups, the subjects made fewer errors in the second measurement. This could be interpreted as a normal learning effect. Figure 9a illustrates the error performance in both groups before, and Figure 9b after two subjects were excluded from the analyses.

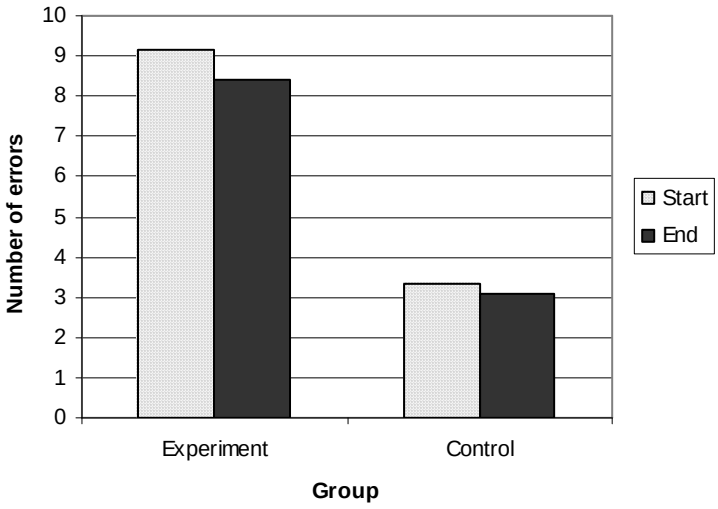

Figure 9a. Averaged error performance (n=24)

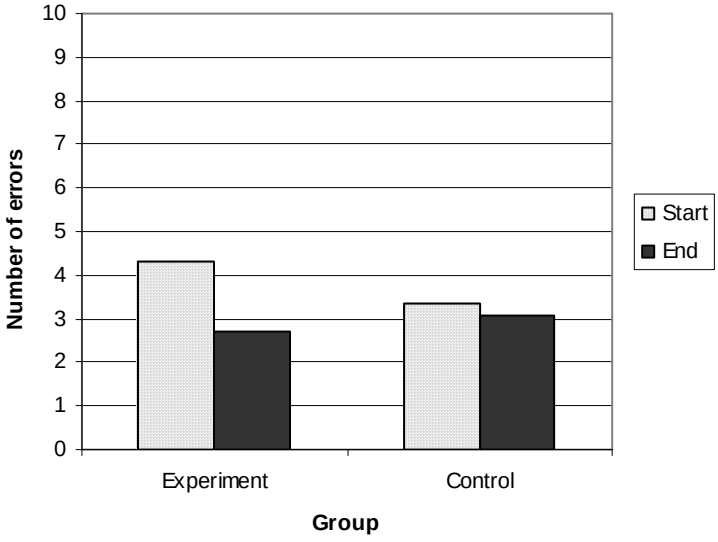

Figure 9b. Averaged error performance after two subjects from the Experiment group were excluded (n=22).

Mood State

Mood state factors were also analysed with the MANOVA design. Results revealed no significant main or interacton effect for the *Group* and/or *Task*. Only value which was nearly significant, was the main effect for *Task* in the vigour-factor ($F(1,22)= 4.03$, $p=.057$). Summarizing the mood state results, very little, not statistically significant, difference could be discerned. In general, alteration between the measurement times was greater in the *Control group*. Figure 11 illustrates these results. Four different factors presented in the figure are vigour, aggression/anger, depression/fatique and anxiety.

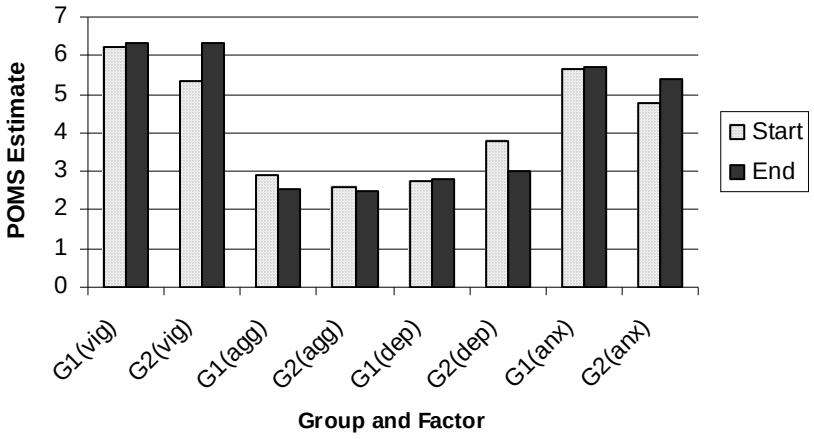

Figure 11. Profile of Mood States results in graphical form. Changes in mood states were very minor in both groups and in more visible in the Control group. G1 represents the Experiment and G2 the Control group. Factors are vigour (vig), aggression/anger (agg), depression/fatique (dep) and anxiety (anx).

Estimates of sleep quality

Results of the sleep factors were very coherent with the results of the other analysed data. No significant difference was found in the quality of sleep between the two measurements in MANOVA. Figure 12 shows that groups did not differentiate much from each other either. Subjects in both groups evaluated that they slept very much on the average in the beginning and at the end of the study. The changes between the measurement times were minor in both groups.

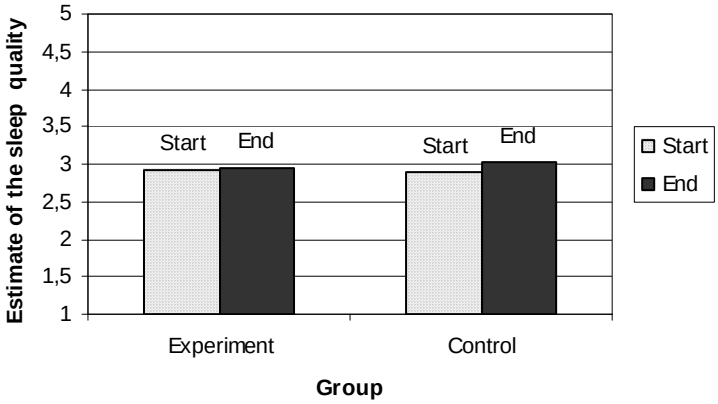

Figure 12. Subjects estimated the quality of their sleep during three days in the beginning and at the end of the study. They were able to rate the quality of the sleep in a scale from 1 to 5. (1= sleep was a lot worse than normally, 5= sleep was a lot better than normally. Number 3 represents the average sleep quality.)

Discussion and conclusions

The aim of the present study was to examine the effects of E-EPA and carnosine supplementation on healthy, young subjects´ cognitive, psychophysiological and behavioural parameters. Studying these nutrients with young, healhy adults is well-justified because many Omega-3 fatty acid products are advertised to have an effect also on healthy people's cognitive and attention status. As presumed, E-EPA and carnosine supplementation increased the EPA content and lowered the AA/EPA ratio in subject´s serum significantly. There was no alteration in the control group´s blood markers. Change in the fatty acid compositions in the serum was assumed, among other effects, to reflect improvement in attention and concentration status of the Experiment group subjects. Against assumption, this was not visible in the level of the slow ERPs. Nutrition supplementation did not have a statistically significant effect on the subjects´ CNV or P300 potentials. In the light of the behavioural data, the attention and concentration statuses of the subjects did not improve. Reaction times remained practically unchainged and error making decreased uniformly in both groups. One might suggest that this minor change reflects a normal learning effect. No significant change was perceived in the subjects´ mood states or quality of sleep in either group. The results of the subjective evaluation tasks imply that the placebo design was successful and that it was certain that the subjects could not quess the group which they belonged to. However, before the last measurements, a couple of subjects informed that the capsules had tasted fishy. This aroused suspicion that the subjects could guess their group. Damico, Stoll, Marangell and Cohen (2002) studied the reliability of fish oil versus pure olive oil placebo-design. Surprisingly, according to their results, the difference in the experience of taste was not connected with the correct guesses about the group. One must note that the subjects were not informed about the contents of the placebo product. Thus, they could not guess the taste either.

Results of the present study are very clear, but they are partially contradictory to what Fontani et al. (2005) found. They documented that Omega-3 fatty acid supplementation improved the attention and reaction status of healthy, young subjects. The AA/EPA ratio in subjects' blood was reduced strongly, whereas reaction times and error making decreased. Beneficial effects of the nutrients were visible also in the mood state and in the level of the ERP components. In the present study, a similar change in AA/EPA ratio was visible, but no statistically significant difference between the Experiment and Control groups was found in any other measured parameter. On the other hand, present results are comparable to many other Omega-3 studies that have identified change in the level of blood markers, but not in the psychological or behavioural level (Voigt et al. 2001; Hirayama et al. 2004, and Kairaluoma et al., submitted). In the beginning of this paper, the relationship between the blood circulation and CNS was introduced briefly. This is a rather important question, because it is quite difficult to know whether possible change in some psychological parameters, for example in elevated mood state, is due to enhanced blood circulation in certain regions in the brain or to some other more or less direct mechanisms in the cellular level in CNS. Omega-3 fatty acids are documented to affect in both ways (Von Schacky & Harris, 2006 and Yehuda, Rabinovitz & Mostofsky, 2005a). Minami, Kimura and Endo (1997) found that dietary DHA increased hipocampal acetylcholine levels. Hence, Das (2000) suggested that EPA/DHA supplementation increases the acetylcholine levels in the brain, which will lead to an increase

in the parasympathetic tone, and therefore, to an increase in heart rate variability (HRV) and protection from ventricular arrythmias. If the results of the present experiment would have been positive, these opportunities should have been taken into account.

Fontani and others (2005) made a pioneering experiment which focused on the effects of Omega-3 fatty acids on healthy adults´ cognitive, psychophysiological and behavioural parameters. The present study was built up in the light of that study, so that the results would be better comparable. In this paper, the focus was on those reaction time tasks and parameters (Sustained attention and Go/NoGo) that were found most relevant and statistically significant in the study of Fontani and others (2005). Most of the measurement methods were corresponding in both studies so this should not have affected the results. Comparable blood analysis, mood state evaluation, electro-physiological and behavioural recordings were conducted.

There can be many reasons for the fact that the results of the present study and the work of the Fontani et al. (2005) were contradictory. The major difference between these two studies was that the effective substances were not exactly the same. Fontani and others used the combination of many Omega-3 fatty acids (mainly ALA, EPA and DHA) and the present study focused only on one Omega-3 fatty acid (E-EPA) and one protein (carnosine). One must note that when talking about Omega-3 fatty acids, we are not talking about a single coherent group of fats. Freemantle, Vandal, Tremblay-Mercier, Tremblay, Blachere, Begin et al. (2006) have proposed that main Omega-3 fatty acids (ALA, EPA and DHA) might have distinct but complementary roles in brain functions. Even though DHA is the major fatty acid in this group, and its level seems to be crucially connected to many factors in the CNS, such as brain clucose uptake, it might be that also ALA and EPA have their own functions, not only by their conversation to DHA. For example, ALA is reported to be an efficient ketogenic fatty acid and EPA promotes fatty acid oxidation. In the light of these suggestions the advantage of the present study was that it focused on only one Omega-3 fatty acid. But, at the same time there are limitations with the comparability with the experiment of Fontani et al. (2005). There were two effective substances in the present study as well. Thus, if the results would have been positive, more experiments would have been needed to study the E-EPA and carnosine separately.

Second reason for the contradictory results of the studies might be the difference in the studied populations. In the present study, the studied population consisted of 20-30-year-old Finnish students mainly from the University of Jyväskylä. In the Fontani et al. (2005) experiment, the age distribution was larger (20-51 years) and population was recruited from a local non-competitive athletic association in Italy. The author of the present paper suggests cautiously that Omega-3 fatty acids might be more beneficial to older people, since, for example, according to Wu, Ying & Gomez-Pinilla (2004) and Yehuda, Rabinovitz & Mostofsky (2005), Omega-3 fatty acids have a repairing effect in the nerve cells, and usually older people have more degeneration in the nerve cells. Nevertheless, it seems quite unlikely that small difference in the age of the studied healthy populations would be the only reason for the difference between the results.

Third reason could be the reliability and validity-related factors in the study designs. In this light, the present study is more reliable in several ways. In this study the intervention period was ten days longer (45 days) and EEG was measured from several points on the skull, compared to one location (Cz) in the experiment of Fontani et al. Some researchers could still argue that the 45 days intervention time is too short for the Omega-3 fatty acids incorporation, but this claim can be overruled by the blood marker results. According to the present paper´s serum fatty acid analyses, the 45 days intervention was long enough, because EPA-content were elevated 2.4 times higher in the Experiment group. It is unlikely that the effect of the fatty acid supplementation is seen in the level of blood markers

but not yet in the psychophysiological or behavioural factors. This possibility cannot be, however, completely excluded. Balancing the Experiment and Control group also succeeded better in this study. Equal numbers of both sexes were present in both groups. The only subject-related factor that favours Fontani et al.´s study is that the total number of participants was higher in their study. They had a total of 49 subjects, 33 (13 males and 20 females) in the experiment and 16 subjects (4 males and 12 females) in the control group.

So far, it has been discussed why the results of the present study were different to the Fontani´s and his collagues´ findings. But we have not received the whole answer for the question of why the results of the present paper were negative. It could be considered that the intervention time could have been even longer, and the studied population larger for the results to be more reliable. What about the measurement equipment and their reliablity and validity? Questionnairy papers were simple to fill in and subjects were well-informed. Was three days in the beginning and at the end of the supplementation too short of a time for subjective evaluation? According to the answers, this does not seem to be the case. More variation was seen in the Control group answers. Reaction times and number of errors are very reliable variables in CPT, and it is widely accepted that these are related to attention and reactivity. What about the psychophysiological measurements, how sensitive are they and what do they measure? According to the previous studies made in the same laboratory utilizing the same equipment and study design as the present study, it has been proved that even a quite small reward is strong enough to change, for example, the CNV significantly. For instance, it has been proved that children who had no visible CNV in the first non-motivated situation, showed a clear negative shift after they had an opportunity to earn money if they succeeded well in an otherwise similar test (Kivijärvi & Saunamäki, 1999, Järveläinen & Niemelä, 2000 and Taipalus, 2005). These results imply that CNV is a very sensitive gauge of the attention status and even a minor change in attention or concentration should become visible. In the design used in the present study, motivation of the subjects was not varied because it was unlikely that the effects of E-EPA and carnosine would be different in the more or less motivated situations. In fact, the background assumption was that if the study design would have included the motivational fators, it might have resulted in more noise to the pure effects of Omega-3 fatty acid supplementation. The assumption was that measuring the subjects in a so-called “neutral state”, would be the most accurate way to perceive even the smallest of changes in the parameters. The results of the ERP analyses imply that the attention, concentration and stimulus processing status of the subjects did not alter significantly in either group. Debating over the different kinds of possible meanings of the measured endogenous ERP components (CNV and P300) is by far out of the scope of this paper, but we can accept at least the fact that they present one way to investigate the brain cells´ actionpotentials. In the present study, E-EPA and carnosine did not change this electrical activity in the brain during the visual slow event rate CPT.

According to this study the 45 days E-EPA and carnosine supplementation does not have an effect on the psychophysiological, behavioural or cognitive parameters on young, healthy adults. As discussed earlier, the results of many Omega-3 researches have been at least partly contradictory and a very small part of the results seems to be uniform. Again, it is very important to remember that there are many different Omega-3 fatty acids, and it might not be wise to discuss them as a single coherent group. The present paper is a good example of this. It has been documented that Omega-3 fatty acids may have beneficial effects in many different kinds of medical conditions, but with very many exceptions. The author of this study suggests that two major questions are challenging the field of Omega-3 fatty acid research at the moment: 1) What combinations and amounts of different Omega-3 (and Omega-6) fatty acids would be the most beneficial? 2) What proportion (if any) and of which clinical/age group can benefit from the different kinds of Omega-3 supplementations, and how can these

groups be identified? These questions remain to be partly unsolved, but the present study has given valuable information about the effects of a single Omega-3 fatty acid (E-EPA) in the population of young, healthy adults. Even though the results of this study are clear, they should be generalised with caution. More well-conducted studies with different kinds of Omega-3 fatty acids alone and in combinations are needed. In the light of the present paper young, healthy, adults will not benefit from the quite short E-EPA and carnosine supplementation as regards to attention, concentration, reactivity, mood state and quality of sleep. The results of the present study are in line with the study made in NMI (Kairaluoma et al., submitted) with the same nutritional supplements. These results can, nevertheless, help to aim the upcoming experiments to a new direction. In the future, it would be interesting to study the effects of E-EPA to older people and to different kinds of clinical groups' CNS functions. Because the Omega-3 fatty acids can be effective in both cellular and circulatory level, measuring the heart rate variabily (HRV) might also yield some new information about the phenomenon. Further research on the effects of larger combinations of Omega-3 fatty acids (ALA, EPA and DHA) on young, healthy adults' CNS functions is also needed.

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