European Journal of Clinical Investigation (2005) **35**, 691–699

Cognitive and physiological effects of Omega-3 polyunsaturated fatty acid supplementation in healthy subjects

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Abstract

Background It has been reported that Omega-3 fatty acids may play a role in nervous system activity and that they improve cognitive development and reference memory-related learning, increase neuroplasticity of nerve membranes, contribute to synaptogenesis and are involved in synaptic transmission. The aim of this study was to examine the effects of Omega-3 supplementation on some cognitive and physiological parameters in healthy subjects.

Materials and methods Subjects were tested at the beginning of the experiment and after 35 days. In this period they were supplemented with Omega-3 polyunsaturated fatty acids. A group was supplemented with olive oil (placebo). Tests involving different types of attention were used, i.e. Alert, Go/No-Go, Choice and Sustained Attention. For each test, the reaction time, the event-related potentials by electroencephalogram (EEG) and the electromyography (EMG) of the forefinger flexor muscle were recorded. The Profile of Mood States test (POMS) was also administered.

Results Blood analyses showed that after Omega-3 supplementation the arachidonic acid/eicosapentaenoic acid ratio (AA/EPA) was strongly reduced. The mood profile was improved after Omega-3 with increased vigour and reduced anger, anxiety and depression states. This was associated with an effect on reactivity with a reduction of reaction time in the Go/No-Go and Sustained Attention tests. The latency of EMG activation was concomitantly reduced in the same tests plus Choice. An EEG frequency shift towards the theta and alpha band were recorded in all the tests after Omega-3.

Conclusion Omega-3 supplementation is associated with an improvement of attentional and physiological functions, particularly those involving complex cortical processing. These findings are discussed in terms of the influence of Omega-3 on the central nervous system.

Keywords EEG, EMG, event-related potentials, mood states, Omega-3, reaction time.
Eur J Clin Invest 2005; 35 (11): 691–699

Introduction

Polyunsaturated fatty acids (PUFAs) include the family of Omega-6 and Omega-3 fatty acids. Some Omega-6 fatty acids, such as arachidonic acid (AA), can be manufactured in the body using linoleic acid as a starting point and other Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are manufactured in the body using alpha linolenic acid as a starting point [1]. In the cell membranes there are phospholipids which also contain fatty acids. In the nervous system PUFAs can be released from membrane phospholipids when neurones are stimulated with neurotransmitters and can be metabolized in the brain giving rise to a series of active products, the eicosanoids, a group of oxygenated C20 compounds, which includes prostaglandins, thromboxanes, leukotrienes and a variety of hydroxy and hydroperoxy fatty acids. These products may act in the intracellular environment as neuronal secondary messengers and may be released in the extra-cellular space and interact with G-protein-coupled receptors on neurones and glial cells, thus influencing neuromodulation and synaptic plasticity [2]. The PUFAs also have influence on cell migration and apoptosis [3,4] and contribute

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Received 21 July 2005; accepted 13 September 2005

to synaptogenesis [5] and are involved in cholinergic, serotoninergic and catecholaminergic synaptic transmission [5–7].

Both Omega-6 and Omega-3 PUFAs are able to influence cellular activity. They preserve membrane fluidity by decreasing the level of cholesterol which hardens membranes [1], and both Omega-3 and Omega-6 fatty acids are required for normal membrane structure and function and for normal signal transduction processes [8]. Besides influencing membrane fluidity, they can modify the activity of membrane bound enzymes, the number and affinity of receptors, the function of ion channels, the production and activity of neurotransmitters and signal transduction [9].

The types of fatty acids that are available to the composition of cell membranes depend upon diet. The retina and brain, particularly the cerebral cortex, are rich in Omega-3 fatty acids [5,6] which occur mainly in diets rich in fish oil and marine animals [10,11], and the role of Omega-3 in visual and cognitive development has also been described [12–15]. Moreover, it has been reported that maternal intake of Omega-3 during pregnancy and lactation may favour the later mental development of children [15]. However, the critical factor in fatty acids efficacy does not seem to be their absolute level, but rather the ratio between various groups of fatty acids, and it is known that the relative amounts of Omega-6 and Omega-3 PUFAs in the cell membrane are responsible for affecting cellular function [16,17].

As AA competes directly with EPA for incorporation into cell membranes a low AA/EPA ratio has been proposed as an index of the beneficial effects of Omega-3 [18,19], which have been described in animal and clinical experimentations. Omega-3 fatty acids are considered an important anti-inflammatory factor able to reduce pro-inflammatory cytokines [10]. High blood levels of Omega-3 fatty acids and low levels of Omega-6 fatty acids have been associated with inhibitory effects on tumorigenesis and various inflammatory diseases and with lower mortality from cardiovascular diseases in a variety of populations [10,20,21]. Animal studies have shown that Omega-3 fatty acids may play a role in cognitive development and Omega-3 fatty acid deficiency impairs the ability to respond to environmental stimulation in rats, which suggests that the provision of Omega-3 as well as Omega-6 fatty acids to the developing brain may be necessary for normal growth and functional development [22]. An Omega-3 deficiency in rat brain has been associated with reduced biosynthesis of catecholamine and decreased learning ability, with a lower synaptic vesicle density in the hippocampus [23,24], whereas chronic administration of Omega-3 helps to improve reference memory-related learning [25] probably owing to an increased neuroplasticity of nerve membranes [26]. In clinical studies it has been reported that cognitive performance improves with Omega-3 [27] and different mechanisms have been proposed to explain this effect, e.g. increased hippocampal acetylcholine levels [28], anti-inflammatory effects of Omega-3, decreased risk of cardiovascular disease or increased neuroplasticity [26].

On the basis of these mechanisms, positive effects of Omega-3 on dementia, schizophrenia and other central nervous system diseases have been reported [29,30]. The description of effects on depression, although controversial [31–34], has led to the conclusion that Omega-3 can affect not only cognitive functions, but also mood and emotional states and may act as a mood stabilizer [34,35]. On the basis of the above reported data, of PUFAs, Omega-3 seems to be crucial in the induction of beneficial effects in some neurological diseases [36,37] in addition to the chronic fatigue syndrome [38]. Both DHA and EPA appear to be necessary to show these effects and this has been demonstrated for depressive disorders [39]. However, some controversial effects observed in depressive and schizophrenic patients may be related to DHA and EPA different functions.

The numerous studies on the relationship between Omega-3 fatty acids and the central nervous system activity mainly involve pathological situations [5]. It remains to be proven if Omega-3 fatty acids can change or improve the status of healthy young people, as Omega-3 supplementation in healthy subjects has not been widely analyzed. In a previous experiment the team investigated blood profiles, body fat and mood state in healthy subjects on different diets and observed that Omega-3 supplementation was able to vary these parameters [40]. On the basis of the above reported influences of Omega-3 on neuronal activity, this study investigated the possible effect of Omega-3 on cognitive functions in healthy subjects receiving Omega-3 fatty acid supplementation and performing a series of attentional tests [41]. The tests were accompanied by neuro-physiological recordings to evaluate the possible modification of some neuro-electrical parameters. In particular, we considered the event-related potentials CNV and P3. The Contingent Negative Variation (CNV) is a slow negative wave elicited by the association of two successive stimuli (warning and imperative stimulus) followed by a response; where CNV has been related to attention and expectancy [42,43]. The P3, which occurred after the onset of the specific stimulus, has been interpreted as a signal of stimulus evaluation [43,44], processing capacities and attentional capabilities [45]. Other information about emotionality, reactivity, central stimulus processing and other neuro-physiological control mechanisms has been obtained from psychometric tests and EEG and EMG analyses. In the present study these experimental procedures were used to evaluate the possible effects of Omega-3 on neuro-psychological functions in healthy subjects.

Materials and methods

The experiment was carried out on 33 healthy voluntary subjects comprising 13 males and 20 females in the age group 22 to 51 years (mean 33 ± 7 years). The subjects were all tested and received a daily supplementation of Omega-3 for 35 days. On day 35 they were tested again using the same procedure. Similarly, a group of 16 subjects (four males and 12 females, mean age 33 ± 3 years) was tested with the same experimental procedure and received a daily a supplementation of olive oil, considered as placebo (P), for 35 days. Subjects were recruited from members of the local non–competitive athletic associations.

Before the experiment, all subjects signed an informed consent form and completed a questionnaire concerning their habits, health, diet, sleep, smoking, use of drugs, alcohol and caffeine, sport activity and work. All subjects were familiar with a computer (they spent more than 1 h per day operating a computer, with no significant differences between the groups), but they were not skilled in video-games or other computer-agility performances which could affect their reaction times. Only subjects in good health, free of drugs and medications and with negative psychiatric and endocrine histories were enrolled in the experiment. Moreover, they must have performed non-competitive athletic activities for 4 h weekly (aerobic activity, range 3–6 h). Criteria for exclusion from the study were heavy smoking (more than eight cigarettes per day), drinking (more than two glasses of spirits per day) and caffeine consumption (more than two cups of coffee per day). The experiment design complied with the current laws of Italy. The study protocol was approved by the Ethical Review Board of the University of Siena.

Experimental procedure

Throughout the study period the 33 subjects of the Omega-3 group consumed eight capsules (4 g) of fish oil (FO; 2·8 g of Omega-3 PUFAs, EPA + DHA in a ratio of 2:1 and 1·60 g of EPA, 0·80 g of DHA, 0·40 g of other types of Omega-3 PUFAs: alpha linolenic, stearidonic, eicosatetraenoic and docosapentaenoic acid), while the 16 subjects of P group consumed eight capsules (4 g) of olive oil (P) per day. Enervit SpA (Milan, Italy) prepared the Omega-3 fish oil and olive oil capsules and were indistinguishable by packaging, shape or taste. The amount of EPA per capsule was 200 mg and that of DHA per capsule was 100 mg. The Omega-3 fish oil was of a high purity, a low degree of oxidation, an absolute organoleptic neutrality and an absence of regurgitation effects. The capsules were taken before meals. The composition of the olive oil was: 6·4% linoleic acid, 77·5% oleic acid, 11·9% palmitic acid, 1·9% stearic acid, 195 mg kg^{-1} tocopherols, 195 mg kg^{-1} polyphenols. The subjects were randomly assigned to either the Omega-3 or P groups and consumed Omega-3 or P in a blind manner.

On day 1 and day 35, all subjects underwent a 08:00 h medical examination and on day 1 each subject met the dietician and received a personalized diet to avoid excesses. The subjects filled in a diary card to record their psychological and mood state and other general information used by the investigators to assess if any protocol violation occurred during the study (e.g. deviations from diet, use of drugs). No adverse events related or unrelated to fish oil supplementation were reported during the study.

Blood samples were taken on day 1 and day 35 to analyze the specific parameters: arachidonic acid/eicosapentaenoic acid ratio (AA/EPA), cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and glycaemia. The blood samples were analyzed according to standard methods.

The subjects then completed the Profile of Mood States (POMS) questionnaire and performed a series of attentional tests with concomitant recording of physiological activities, i.e. electroencephalogram (EEG), electromyography (EMG) and electrocardiogram (ECG).

POMS

On day 1 and day 35, before the beginning of the attentional test session, the subjects filled in the Profile of Mood States (POMS) psychometric scale [46] to assess their psychological state. Mood states are temporary and subjects had to report the mood state of the last 5 days. The POMS consisted of five negative mood scales: anger (lowest possible value: 40 – highest possible value: 99), anxiety (34–85), fatigue (88–37), confusion (32–92), depression (41–91) and one positive scale: vigour (24–75). The scaling of the lowest and highest possible values are reported in the tables, in which raw scores are changed in standard scores [46]. The above reported values refer to the Italian adaptation of the scale.

Attention tests

The experiment consisted of four attention tests according to the Zimmermann & Fimm Attention Test procedure [41]. Subjects were tested in homogeneous environmental and physiological conditions. Each subject sat in a comfortable reclining chair, one metre in front of a computer screen and with the forefinger of the dominant hand on the key of a modified computer keyboard (SuperLab Pro, Cedrus Corporation, San Pedro, CA) [42,47]. There was a 5-min interval between the various tests which were performed in the following order.

1 Alert (AL): this test involved the measurement of a simple reaction to a stimulus that was not considered to

Table 1 Mean reaction time (ms) ± standard deviation recorded during attentional tests: comparisons between values before and after Omega-3 supplementation (subjects $n = 33$)

Test	Before Omega-3	After Omega-3	t^*	$P <$
Alert	262·77 ± 45·9	261·84 ± 36·6	0·56	NS
Go/No-Go	524·12 ± 73·3	509·30 ± 66·1	2·61	0·01
Choice	649·92 ± 90·3	641·59 ± 80·5	1·02	NS
Sustained Attention	680·87 ± 89·7	636·60 ± 111·9	3·87	0·0005

*Paired samples *t*-test.

Table 2 Physiological recordings: mean latency of EMG activation (ms) ± standard deviation of the forefinger flexor muscle after the stimulus. Attentional tests: comparisons between the recording before and after Omega-3 supplementation (subjects $n = 33$)

Test	Before Omega-3	After Omega-3	t^*	$P <$
Alert	195·27 ± 36·9	188·91 ± 29·4	1·29	NS
Go/No-Go	408·73 ± 68·3	381·21 ± 57·2	3·99	0·0003
Choice	428·96 ± 80·6	404·30 ± 69·6	2·61	0·01
Sustained Attention	476·60 ± 89·8	423·40 ± 88·6	4·56	0·0001

*Paired samples *t*-test.

require significant central analysis. The test was presented as 80 trials with each of 6-s duration, with an intertrial interval of 2 s, which started with a warning stimulus (sound) and after 3 s a letter 'X' appeared on the computer monitor to which the subject had to respond by pressing a key as soon as possible. The reaction time was recorded.

2 Go/No-Go (GO): this test analyzed the specific ability of the subject to repress an unsuitable response and to react only in the presence of some stimuli and not in the presence of others, which required significant central intervention. The number of trials was 80 and each of duration 6 s, with an intertrial interval of 2 s, which started with a warning stimulus (sound) and after 3 s one of five squares of different colour (red, green, yellow, blue or black) appeared randomly on the computer screen. The key had to be pressed only if red or green squares appeared. Reaction times and errors were recorded. Go trials (in which were presented target stimuli) were 60% and No-Go trials (in which were presented stimuli not requiring a response) were 40% of the total number. They were divided and separately analyzed.

3 Choice (CH): this test assessed the subject's ability to react to different stimuli. This ability required substantial central analysis of the stimulus properties. The number of trials was 80 each of duration 6 s with an intertrial interval of 2 s, which started with a warning stimulus (sound) and after 3 s one of three differently coloured squares (red, green or yellow) was presented and the subject had to press one of three different buttons that related to a specific square.

4 Sustained Attention (SA): this test analyzed the subject's ability to react in the presence of stimuli activating a complex go/no-go paradigm. The number of trials was 80 each of duration 6 s with an intertrial interval of 2 s, which started with a warning stimulus (sound) and after 3 s a figure was presented on the monitor screen. There was a series of figures presented sequentially and the subject was required to recognize if a particular figure was equal to the previous one either in colour (red, green, yellow), in shape (triangle, circle or square) or in size (large, medium, small). If any of the criteria matched the subject pressed the single button (covering all combinations).

Several data were collected and analyzed for each attentional test where the reaction time (RT = time in milliseconds, ms, from the stimulus to the response of pressing of the key); the number of errors performed during the test; the RT variability, indicated by the Variability Index [VI = SD/(1000/mean RT)] [42]. Data from the various tests were collected and averaged and compared by standard statistical analyses.

5 A relaxation period (R) of 8 min was also recorded at the end of the attentional tests. The subject rested on a bed in the absence of any external stimulus with their eyes closed in a relaxed state.

Physiological recordings

Several physiological signals were recorded contemporaneously during each attention test and during the relaxation period. The electroencephalogram (EEG) was recorded with Ag/AgCl disc electrodes affixed to the scalp of the subjects at the midline of the central area, point zero (vertex) (Cz), with collodion and referred to linked mastoids. Impedance was kept below 5 kΩ. Additional electrodes were positioned superior and lateral to both eyes in order to monitor eye-related potentials. Data were digitized at a sampling rate of 500 Hz and passed through a 0–100-Hz bandpass filter (24 dB octave^{-1} roll-off). The electromyography (EMG) of the forefinger flexor muscle was recorded with surface electrodes. Heart rate was recorded with surface electrodes positioned on the standard arm and leg positions.

Data collection and analysis

The EEG was recorded and digitized. The recording period lasted for the duration of the entire test and different markers in separate channels of the recording system signalled the warning sound, the stimulus and the response for each trial and the pre and poststimulus periods were divided and separately analyzed. Occurrences after the response were not considered, but a 500-ms prestimulus baseline was considered. Waveforms were recorded, processed and averaged through a BIOPAC system (Biopac Systems Inc., Santa Barbara, CA). The following signals were analyzed.

1 Event-related potentials: averaged waveforms were divided into two periods: (i) the period preceding the stimulus in which a waveform similar to the Contingent Negative

of Variation (CNV) was recognized, and (ii) the recording of the period following the stimulus of the P3 positive peak. The amplitude and latency of the negative wave preceding the stimulus and the P3 positive peak were taken into account. CNV was referred to a prestimulus baseline of 250 ms; the last segment of 500 ms (End Wave) was used to measure the mean amplitude and the negative peak was considered as the CNV peak value. The P3 potential positive peak was generally found in the latency range between 200–700 ms. The latency of P3 was calculated as the interval between the stimulus onset and the peak of P3. The amplitude of P3 was determined as the voltage difference between P3 and a prestimulus baseline of 250 ms.

2 EEG frequency: the power spectra of the EEG activity recorded during the attentional tests and relaxation period were obtained by Fast Fourier Transform (FFT). The considered frequencies were divided into four bands: theta (4–8 Hz), alpha (9–12 Hz), beta 1 (13–20 Hz) and beta 2 (21–32 Hz). The percentage of the area of the power spectrum within each band was calculated for each test period.

3 EMG: the EMG of the forefinger flexor muscle was divided into two periods: the period preceding EMG activation (EMG latency) and the period from EMG activation to key pressure (movement speed).

4 ECG: the R-R interval of the ECG was used to study the heart rate during the attentional tests and relaxation period.

Statistical analysis

Data from the various tests and biological measures were collected, averaged and then compared by a paired samples *t*-test for comparison within the same group and a *t*-test for independent values when comparing between different groups. Descriptive statistical analyses were used to define the characteristics of the population studied. The associations between the primary measures of interest and cofactors (age and gender) were addressed by ANOVA statistical model. Correlations were measured by Pearson's *r*-test.

Results

No association was found between the variables studied and age or gender of subjects. Analysis of the blood samples showed that AA/EPA was strongly affected by Omega-3 treatment. The groups Omega-3 and P did not differ at day 1 (mean values: 16·39 ± 8·32 vs. 16·17 ± 10·63). However, comparisons between day 1 and day 35 in each group, after P and after Omega-3 supplementation, revealed a strong decrease of AA/EPA after Omega-3 [mean values, before Omega-3 (14·26 ± 8·87) and after Omega-3 (4·29 ± 2·60); $t = 6{\cdot}89$; $P < 0{\cdot}0001$], while no significant differences were observed after P. Other blood parameters did not show significant variations.

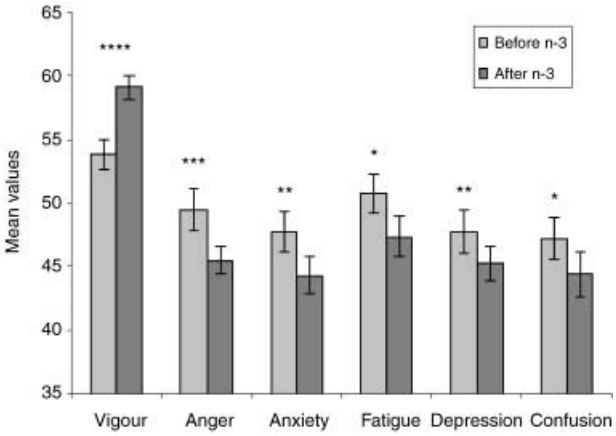

Figure 1 Profile of Mood States (POMS). Comparison before and after Omega-3 supplementation. Mean ± standard error (subjects $n = 33$). Paired samples *t*-test: $^{****}P < 0{\cdot}0001$, $^{***}P < 0{\cdot}001$, $^{**}P < 0{\cdot}01$, $^{*}P < 0{\cdot}04$.

POMS

Supplementation with Omega-3 PUFAs was associated with a clear variation of the profile of mood state. The POMS analysis showed an increase of vigour and a decrease of the other mood states (anger, anxiety, fatigue, depression, confusion) (Fig. 1). This change was not observed after P: vigour and the other mood states did not differ from the period preceding the olive oil supplementation.

Reaction time

The mean reaction times recorded during the attentional tests are shown in Table 1. The RT decreased only after Omega-3 supplementation. This reduction occurred in Go/No-Go and Sustained Attention test, but no significant effects were observed in the Alert and Choice tests. The effect was particularly evident in the Sustained Attention test and the reduction of RT appeared to have been distributed over the entire test period (Fig. 2) with a concomitant reduction of variability after Omega-3 supplementation. There was also a significant reduction in the number of errors from a mean of four to two errors/test after Omega-3 ($P < 0{\cdot}04$).

Physiological recordings

The decrease of RT after Omega-3 supplementation could have been owing to a reduction of the latency of EMG activation of the forefinger flexor muscle engaged in pressing the computer key (time from onset of the stimulus to beginning of EMG activation = EMG latency), or to an increase in the speed of contraction of the same muscle (time from the beginning of EMG activation to RT). While the latter

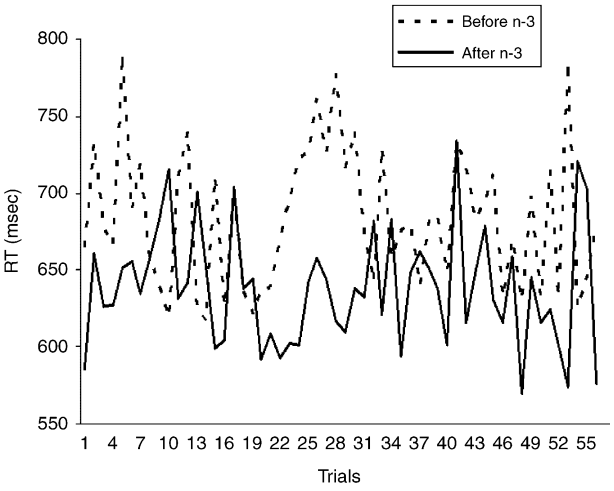

Figure 2 Reaction time: distribution of the mean values of each trial recorded during the Sustained Attention test before and after Omega-3 supplementation (subjects $n = 33$). Mean values: before Omega-3 = 683·30, after Omega-3 = 638·39; $t = 5·368$; $P < 0·0001$. Variability index: before Omega-3 = 143·50, after Omega-3 = 125·24; $t = 2·72$; $P < 0·01$.

measurement did not show any significant variation the EMG latency was reduced in Go/No-Go, Choice and Sustained Attention tests after Omega-3 supplementation (Table 2).

The event-related potentials showed no significant variations during Alert, Choice and Sustained Attention tests, while a change in the wave amplitude occurred in the Go/No-Go test after Omega-3 supplementation. There was an increase in amplitude for the negative wave preceding the stimulus (CNV) and for P3, the positive peak after the stimulus. The negative peak of the prestimulus wave passed through –30 μV to –60 μV after Omega-3 ($P < 0·0003$). The P3 positive peak recorded before and after Omega-3 supplementation showed an increase in the amplitude in both the Go trial [peak amplitude mean values: before Omega-3 (43·77 ± 42·80 μV) and after Omega-3 (84·27 ± 75·23 μV); $t = 2·46$; $P < 0·02$] and No-Go trial [peak amplitude mean values: before Omega-3 (37·40 ± 35·23 μV) and after Omega-3 (75·65 ± 69·28 μV); $t = 2·40$; $P < 0·02$].

The frequency distribution showed a shift towards low frequencies in all recordings after Omega-3 supplementation (Table 3); this effect was absent in the tests performed by the P group. In particular, after Omega-3 the percentage of the beta-2 band decreased significantly in all the tests and in the relaxation period. Its reduction was accompanied by a concomitant increase of the theta and alpha bands (Table 3).

The analysis of possible relationships between the variation of frequency percentage and other physiological parameters revealed a positive correlation between the theta band percentage in the Sustained Attention test and the vigour state recorded in POMS (Fig. 3). This correlation was absent before Omega-3 supplementation but present after the trials.

Discussion

The results of these experiments indicated a positive influence of Omega-3 on cognitive functions. While the decrease

Table 3 Physiological recordings: percentage of EEG frequency band distribution during the Alert, Go/No-Go, Choice, Sustained Attention tests and Relaxation period for the Theta, Alpha and Beta 2 bands (mean ± standard deviation) (subjects $n = 33$)

Test	Before Omega-3	After Omega-3	T^*	$P <$
Alert				
Beta 2 band (21–32 Hz)	20·01 ± 6·3	15·50 ± 4·0	3·42	0·001
Theta band (4–8 Hz)	26·67 ± 7·0	30·75 ± 7·2	2·50	0·01
Alpha band (9–12 Hz)	23·14 ± 4·1	26·57 ± 5·5	3·43	0·001
Go/No-Go				
Beta 2 band (21–32 Hz)	20·06 ± 7·3	15·54 ± 3·7	3·40	0·001
Theta band (4–8 Hz)	28·30 ± 7·2	32·11 ± 5·9	2·86	0·007
Alpha band (9–12 Hz)	23·55 ± 4·3	24·75 ± 4·5	1·30	NS
Choice				
Beta 2 band (21–32 Hz)	21·78 ± 5·4	15·68 ± 5·4	5·21	0·0001
Theta band (4–8 Hz)	28·48 ± 5·7	30·91 ± 6·9	1·78	NS
Alpha band (9–12 Hz)	22·69 ± 3·9	26·86 ± 6·3	4·48	0·0001
Sustained Attention				
Beta 2 band (21–32 Hz)	21·78 ± 4·8	15·42 ± 3·9	6·09	0·0001
Theta band (4–8 Hz)	27·70 ± 5·2	31·49 ± 8·6	2·74	0·009
Alpha band (9–12 Hz)	22·90 ± 3·3	26·50 ± 7·1	3·60	0·001
Relaxation period				
Beta 2 band (21–32 Hz)	21·25 ± 6·1	14·29 ± 3·5	5·34	0·0001
Theta band (4–8 Hz)	22·87 ± 4·3	25·45 ± 5·5	2·66	0·01
Alpha band (9–12 Hz)	27·47 ± 6·7	32·54 ± 8·0	3·55	0·001

*Paired samples *t*-test.

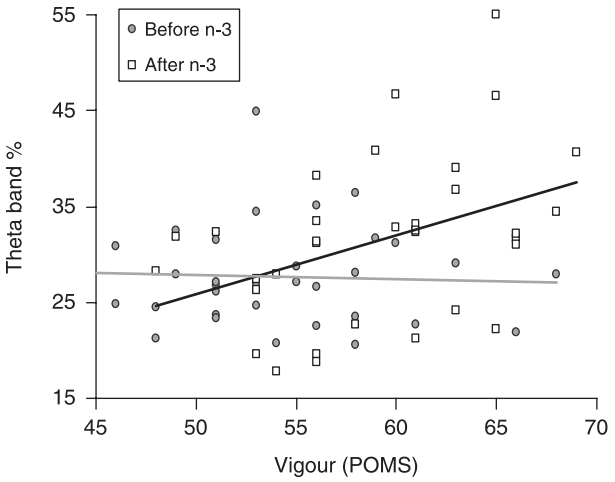

Figure 3 Correlation between theta band percentage and vigour state (POMS) in the Sustained Attention test before and after Omega-3 supplementation (subjects $n = 33$). Linear regression line: before Omega-3, $Y = -0{\cdot}045x + 30{\cdot}13$; Pearson's $r = -0{\cdot}055$ (not significant); after Omega-3, $Y = 0{\cdot}617x - 5{\cdot}01$; $r = 0{\cdot}403$; $P < 0{\cdot}02$.

of the AA/EPA ratio could be considered confirmation of the biological effect of Omega-3 [40], the changes in the reaction time and physiological parameters could be ascribed to the action of Omega-3 on the central nervous system. These effects involved reactivity, cognitive analysis of stimuli and emotional responses. The results of the POMS questionnaire showed an enhanced vigour state and a concomitant decrease of the negative mood states after Omega-3 supplementation. This effect confirmed with more details the earlier data [40] and can be considered a positive background for improved performance in the RT tests, as it has been reported that emotionality and the mood state in general can influence performance [47]. Reaction times are lower after Omega-3. This reduction was only present in those tests in which central processing of information was required (the effect was absent in the Alert test, a typical simple RT test); this was particularly true in tests requiring a Go/No-Go paradigm, while choice mechanisms did not seem to be influenced. A significant reduction of RT was observed in the GO and SA tests which both required a decision to react or not in the presence of specific stimuli.

These results are confirmed by the EMG data that showed the Omega-3-related reduction of RT was not owing to peripheral effects on muscles, such as improved speed of contraction, but to decreased EMG latency, which was probably influenced by a higher efficiency of central mechanisms. These central influences were explored by the analysis of event-related potentials and EEG frequency. The EEG was recorded only from the vertex (Cz) and the results have to be considered limited. It may have been that the localization of the event-related potentials on the scalp may differ dependent on the treatment. However, considering this limitation, the event-related potentials recorded from Cz confirmed that the Go/No-Go procedure seems to be crucial in this experimental approach, showing variations of amplitude of the main peaks recorded (CNV, P3) only in this test, both for Go and No-Go trials. The distribution of the EEG frequencies recorded from Cz changed in all the experimental tests after Omega-3 supplementation, with the main effects in GO, SA and the relaxation period. In particular, there was a reduction of the higher frequency band (beta-2) after Omega-3 supplementation and a concomitant increase of the low frequency bands (theta and alpha). However, the EEG data requires further confirmation and more detailed analyses in other regions of the brain to give a clearer understanding of a phenomenon which appears to be linked to Omega-3 supplementation.

These results, obtained from a population of healthy subjects, are in line with the reported effects of Omega-3 in pathological situations. Literature reports have associated Omega-3 supplementation with a reduced risk of impaired cognitive functions [27,28,48], dementia [30] and cognitive decline [49]. Moreover, Omega-3 fatty acids have been found to improve cognitive development [12–15], reference memory-related learning [25] and mood states [30]. The last effect supports this trials finding on the POMS changes after Omega-3, i.e. an increase of vigour and a general sense of well-being, and with other reports that Omega-3 PUFAs act as mood stabilizers [35].

This trial data and the above-mentioned studies strengthen the hypothesis of a direct action of Omega-3 fatty acids on the central nervous system. The possible mechanisms involved may be related to the fact that Omega-3 acts as a controller of neuronal excitability which influences protein kinases and thus protects the structure and function of the cell membrane [50]. Consequently, Omega-3 fatty acids can modulate many of the signal transduction mechanisms operating at the synaptic level [5]. Hence, they may influence several pathways with different neurotransmitters such as serotonin, noradrenalin, dopamine and acetylcholine, which may explain the reported effects on learning, mood stability and other important cognitive functions [5,23,24,28]. In previous experiments, the team has described the modulation of event-related potentials caused by training [42,43] or substances such as policosanol [51]. The effects of Omega-3 fatty acids appear to be more limited but in the same direction, being effective only in Go/No-Go paradigms, and increases amplitude in a test in which there is an important cortical intervention. The relationship between Omega-3 and EEG frequency variations can be considered in the same manner. A more detailed analysis and a global vision of the entire brain are necessary to clarify the extent and the characteristics of this frequency shift; however, this data indicates that it can be considered to be a general effect involving Go/No-Go responses and other tests. It is known that EEG recordings are related to intellectual abilities and it has been reported that alpha and theta oscillations reflect cognitive and memory performance [52,53]; in particular, the theta band reflects episodic memory processes, which leads to the conclusion that short-term memory demands are related to an increase in theta band power [54] while inaccurate attentional switching seems to be combined with an increase of the higher alpha band frequencies [55]. In

the development of cognitive skills, the attentional resources required by novel tasks are high and decrease with practice. This effect is accompanied by a reduction of RT and an increase in parietal alpha and frontal theta EEG spectral components, which increase with the time devoted to the task in parallel with performance gains. This probably occurs because as skill develops fewer cortical neurones are activated during the task performance [56,57]. Hence, the increase of lower frequencies observed after Omega-3 supplementation could be owing to a better selection of neurones involved in the task, which supports the fact that Omega-3 fatty acids improve neuronal efficiency. The high frequency bands (beta) are thought to be related to emotional activity and anger [58,59], thus their reduction after Omega-3 could be related to the concomitant reduction of anger and the increase of vigour observed in the POMS test. This relationship is supported by the positive correlation between the increase of the theta band and the increase of the vigour state as reported in Fig. 3.

In conclusion, the results of this experiment revealed an influence of Omega-3 on the activity of the central nervous system. This was shown by the improvement of reactivity, attention and cognitive performances in addition to the improvement of mood state and the modifications of some neuro-electrical parameters. These results have been obtained from a small study group and need further confirmation in a wider group of subjects and in particular for the possible influences of age and gender. The absence of association between the variable studied and age or gender, observed in the present experiment, may be owing to the limited number of subjects. However, considering these limitations, it may be assumed that the importance of these results is strengthened by the fact that they occur in subjects in good health and performing physical activity in whom Omega-3 fatty acids improve an already good condition of well-being.

Acknowledgements

The analyses of AA and EPA (AA/EPA ratio) were carried out at the Institute of Biochemistry, University of Milan, Italy, by Prof. Bruno Berra, Dr Gigliola Montorfano and Dr Anna M Rizzo.

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