Original research paper

The effect of the Mediterranean diet on plasma brain-derived neurotrophic factor (BDNF) levels: The PREDIMED-NAVARRA randomized trial

Almudena Sanchez, Cecilia Galbete, Miguel Angel Martinez-Gonzalez, Alfredo Martinez, Cristina Razquin, Jordi Salas-Salvadó, Ramon Estruch, Pilar Buil-Cosiales, Amelia Martí

[1] University of Las Palmas, Spain

Objectives: There are no human studies assessing the effect of nutritional interventions on plasma brain-derived neurotrophic factor (BDNF) concentrations. The aim of this study was to assess the role of a nutritional intervention based on a Mediterranean diet (MeDiet) on plasma BDNF levels.
Methods: PREvención con Dieta MEDiterránea (PREDIMED) is a randomized clinical trial designed to assess the effect of a Mediterranean diet (MeDiet) on the primary prevention of cardiovascular disease. For this analysis, 243 participants from the Navarra centre were randomly selected. Participants were assigned to one of three dietary interventions: control (low-fat) diet, MeDiet supplemented with virgin olive oil (MeDiet + VOO), or MeDiet supplemented with nuts (MeDiet + Nuts). Plasma BDNF levels were measured after 3 years of intervention. Multivariate-adjusted means of BDNF for each intervention were compared using generalized linear models. Logistic regression models were fit to assess the association between the dietary intervention and the likelihood to have low plasma BDNF values (<13 μg/ml, 10th percentile). Analyses were repeated after stratifying the sample according to baseline prevalence of different diseases.
Results: Higher but non-significant plasma BDNF levels were observed for participants assigned to both MeDiets. Participants assigned to MeDiet + Nuts showed a significant lower risk (odds ratios (OR) = 0.22; 95% confidence intervals (CI) = 0.05–0.90) of low plasma BDNF values (<13 μg/ml) as compared to the control group. Among participants with prevalent depression at baseline, significantly higher BDNF levels were found for those assigned to the MeDiet + Nuts.
Discussion: Adherence to a MeDiet was associated to an improvement in plasma BDNF concentrations in individuals with depression.

Keywords: Brain-derived neurotrophic factor, Depression, Clinical trial, Mediterranean

Introduction

Brain-derived neurotrophic factor (BDNF) is a dymeric protein belonging to the neurotrophin family. This peptide is synthesized by neuronal tissue and also by vascular endothelial, pancreatic, and both smooth and skeletal muscle cells.[1,2] Although BDNF is abundant in the hippocampus and frontal cortex of mammals, some studies have found adequate correlations between cortical levels and platelet or plasma concentrations of this peptide.[3] BDNF is related to several actions such as synaptic plasticity or neuronal survival and differentiation. Moreover, BDNF concentrations have been reported to be associated with different neurodegenerative or psychiatric disorders such as epilepsy, Alzheimer disease, Huntington's disease, autism, schizophrenia, and major depression.[4,5]

Animal studies have evaluated the effect of several dietary factors on brain or plasma BDNF levels. Positive associations have been reported between omega-3 fatty acids, vitamin E or flavonols intake and brain BDNF production, and expression in different animal models (healthy, parkinsonian, epileptic or trauma injured rats).[6,7] Contrarily, diets rich in saturated fatty acids or total fat have been related to lower brain BDNF levels, lower neuronal plasticity, and poorer cognitive ability.[8–11] However, there are no human studies analysing the effect of nutritional

[1] Correspondence to:

DOI 10.1179/1476830511Y.0000000011

interventions on BDNF plasma concentrations. Hence, the aim of the present study was to assess the effect of a nutritional intervention based on a Mediterranean diet (MeDiet) supplemented with either virgin olive oil or with nuts on plasma concentrations of BDNF in a subsample of participants from the PREvención con Dieta MEDiterránea (PREDIMED) study.

Methods

Study population

The PREDIMED study is a large, parallel-group, multicentre, randomized, controlled, 6-year clinical trial designed to ascertain the effects of the MeDiet on the primary prevention of cardiovascular disease (CVD). Its methodology has been published elsewhere.[12,13] The study population is composed of men aged 55–80 years and women aged 60–80 years with no previously documented history of CVD, but with high cardiovascular risk (either type 2 diabetes or at least three of the following major risk factors for CVD: smoking, overweight or obesity, hypertension, family history of early-onset CVD, hyperlipidaemia, or low HDL-cholesterol). The dietary intervention procedures have been shown to be able to obtain significantly different between-group changes in nutrient intake and in the overall dietary pattern.[13]

The present study was conducted to assess the effect of the dietary intervention on plasma BDNF levels after 3 years of intervention in a subgroup of 243 participants randomly selected from the PREDIMED-NAVARRA recruitment centre.

The institutional review board and the Ethics Committee approved the study protocol, and participants signed a written informed consent form.

Dietary intervention

Participants were assigned to receive one of three different dietary interventions: control (low-fat) diet, MeDiet supplemented with virgin olive oil (MeDiet + VOO), or MeDiet supplemented with nuts (MeDiet + Nuts). Participants allocated to the control group were advised to reduce all types of fat and were given written recommendations according to the American Heart Association guidelines. The groups assigned to MeDiet were advised to use extra virgin olive oil for cooking and dressing, to increase vegetable, nuts and fish consumption, to consume white meat instead of red or processed meat and for alcohol drinkers to follow a pattern of moderate red wine consumption. Each participant received a personal interview with a trained dietician and a group session conducted by the same dietician every 3 months during these 4 years. The two MeDiet groups received either free oil (1 l/week) or mixed nuts (30 g/day, as 15 g walnuts and 15 g almonds). No energy restrictions were prescribed for any intervention group. Other specific details of the intervention protocol have been previously published.[13]

Covariate assessment

The baseline examination included the assessment of cardiovascular risk factors, medical conditions including physician diagnoses of hypertension, diabetes, hypercholesterolaemia, or depression. Height and weight were directly measured by a trained nurse following a uniform protocol with the participant wearing light clothing and no shoes. The body mass index (BMI) was calculated as the weight (kg) divided by square of the height (m^2).

Weight was measured in each of the follow-up visits. For this analysis, weight change was calculated as weight after 3 years of follow-up minus weight at baseline and then categorized into two groups (weight gain vs. weight maintenance or lost).

Outcome: plasma BDNF determination

Plasma BDNF levels were collected after 3 years of follow-up for the overall sample. Moreover, baseline BDNF levels were also assessed for participants with prevalent depression. These levels were measured as described previously,[14] using a commercially available ELISA kit (Promega, WI, USA) with the range of sensitivity from 7.8 to 500 pg/Ml and inter-assay variation measured at 8.8% (low concentration), 2.9% (medium concentration), and 2.2% (high concentration). Briefly, blood samples were centrifuged at 3000 rpm for 30 minutes at 4°C. Plasma was carefully collected and was snap frozen on dry ice and subsequently stored at −80°C, until used for further analyses. The BDNF plate was coated with primary BDNF antibody overnight, the following day block and sample buffer was added to each well for 1 hour, and subsequently the standards and samples were added for 2 hours. Thereafter, anti-human BDNF pAb secondary antibody was added for 2 hours and the anti-Ig Y HRP conjugate was added for 1 hour. Then, TMB one solution was added to each well for 10 minutes and the reaction was stopped with hydrochloric acid. The plate was analysed within 30 minutes, at 450 nm. All reagents necessary were provided by the manufacturer. 2

Statistical analysis

Multivariate-adjusted means of BDNF and their 95% confidence intervals (CI) for each intervention group were calculated using generalized linear models with sex, age, prevalent hypertension, diabetes, hypercholesterolaemia, and depression at baseline, smoking (three categories), and previous weight change (after 3-year follow-up) as covariates. In addition, percentiles of plasma BDNF levels were calculated and

participants were classified as being below or above the value for the 10th percentile (13 μg/ml). Logistic regression models were fit to assess the association between the dietary intervention and the likelihood (odds) to have plasma BDNF values lower than the 10th percentile. Odds ratios (OR) and their 95% CI were calculated with the control group as the reference category.

Two-tailed P values <0.05 corrected for multiple comparisons using a *pos-hoc* procedure (Benjamini–Hochberg correction) were calculated.

To assess a possible effect modification by the presence of different diseases at baseline or by weight change during follow-up, product terms between the nutritional intervention and these variables were created and included in the final model. Moreover, the analyses were repeated after stratifying of the sample according to each of these characteristics: prevalence of each disease at baseline (no/yes) and weight change during follow-up (gain vs. lost or maintenance).

The SPSS software package for Windows version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Baseline characteristics of the participants of the subsample of the PREDIMED study according to the nutritional intervention are presented in Table 1. The groups were well balanced and only small, non-significant between-group differences were found. However, we adjusted all comparisons for baseline characteristics.

No significant differences were found for BDNF adjusted means according to the nutritional intervention. Multivariate-adjusted BDNF mean values were 23.37 μg/ml (95% CI: 15.73–31.01) for the control group, 24.66 μg/ml (16.68–32.64) for the MeDiet + VOO group, and 24.87 μg/ml (17.00–32.74) for the MeDiet + Nuts group ($P = 0.68$) (Table 2). However, after adjusting for several characteristics such as smoking or prevalence of diseases, participants assigned to MeDiet + Nuts showed significant lower risk to have very low plasma BDNF values as compared with participants allocated to the control group (OR = 0.22; 95% CI = 0.05–0.90). Table 3 shows the multivariate-adjusted OR and their 95% CI for the association between the dietary intervention and very low plasma BDNF concentrations (<13 μg/ml).

When the analyses were stratified according to the prevalence of different diseases at baseline, those participants with a diagnosis of depression at baseline showed significant between-group differences in mean plasma BDNF concentrations after 3 years of intervention according to the intervention group. After Benjamini–Hochberg *post-hoc* correction, mean plasma BDNF concentrations were significantly higher for those participants with prevalent depression allocated to the MeDiet + Nuts intervention as compared to participants in the control group (Table 4). In this subgroup analysis, additional adjustment for baseline BDNF plasma levels among initially depressed individuals did not change the reported results.

Discussion

We did not find higher overall plasma BDNF levels after a 3-year intervention in volunteers allocated to both MeDiets. However, in the group allocated to MeDiet plus free provision of nuts, there was a 78% lower risk of having low levels of plasma BDNF as compared with the control group. In addition, among depressed patients at baseline, significant differences in mean plasma BDNF values were observed for those assigned to the MeDiet supplemented with nuts compared to the control group. No differences in mean plasma BDNF concentrations were found for participants without depression or according to the presence of other diseases.

The role of different dietary factors on BDNF levels have been evaluated in several animals studies. Positive

Table 1 Main characteristics of the PREDIMED-NAVARRA subsample ($n = 243$) according to the nutritional intervention

	Control group, $n = 77$	MeDiet + VOO, $n = 91$	MeDiet + Nuts, $n = 75$
Age [mean (SD)]	68.0 (6.1)	68.1 (6.1)	67.4 (5.7)
Baseline weight [mean (SD)]	73.5 (12.4)	76.7 (12.3)	75.3 (10.4)
Baseline BMI [mean (SD)]	28.5 (3.4)	29.7 (3.6)	29.1 (2.7)
Sex (%)			
Women	51.9	53.8	48.0
Smoking (%)			
Never smokers	59.7	70.3	62.7
Current smokers	14.3	12.1	9.3
Prevalence of diseases (%)			
Hypertension	84.4	83.5	86.7
Diabetes	31.2	25.3	42.7
Hypercholesterolaemia	66.2	79.1	50.7
Depression	11.7	15.4	18.7
Weight gain after 3 years of follow-up (%)	49.3	47.3	49.3

SD: standard deviation; BMI: body mass index: MeDiet: Mediterranean diet.

Table 2 Multivariate* adjusted mean plasma BDNF concentrations (μg/ml) (95% CI) according to the intervention group

Intervention group			
Control group	MeDiet + VOO	MeDiet + Nuts	*P*
23.37 (15.73–31.01)	24.66 (16.68–32.64)	24.87 (17.00–32.74)	0.68

MeDiet: Mediterranean diet.
*Adjusted for sex, baseline age, smoking, prevalent hypertension, diabetes, hypercholesterolaemia, and depression, and weight change in 3 years (gain vs. maintenance or lost).

Table 3 Risk of very low plasma BDNF concentrations (<13 μg/ml, 10th percentile) after 3 years according to the randomized group Multivariate-adjusted OR and 95% CI

	n	OR (95% CI)*	*P*
Control group	77	1 (ref.)	
MeDiet + VOO	91	1.02 (0.38–2.76)	0.97
MeDiet + Nuts	75	0.22 (0.05–0.90)	0.04

MeDiet: Mediterranean diet.
*OR: odds ratios and 95% CI adjusted for sex, baseline age, smoking, prevalent hypertension, diabetes, hypercholesterolaemia, and depression and weight change in 3 years (gain vs. maintenance or lost).

associations with brain BDNF levels have been found for the intake of omega-3 fatty acids (both for α-linolenic and for docosohexaenoic fatty acids (DHA),[6,15] and even for the intake of vitamin E[8] or flavonols.[7] The antioxidant, anti-inflammatory, and endothelial effects of these components could explain, in part, these associations.[16–20] The mechanisms by which oxidative damage reduces BDNF expression are not entirely understood. The impairment of the *N*-methyl-D-aspartate channel function after energy depletion derived from oxidative stress[21] or the decrease of DNA-binding activities of some proteins such as the activator protein-1 and AMP-response element-binding protein (CREB) may be some of the responsible factors.[22] In fact, these antioxidant components upregulate BDNF levels and the phosphorylation of CREB pathway.[8,23,24]

A Mediterranean-type diet has been shown to prevent endothelial dysfunction and to be associated with lower circulating levels of pro-inflammatory cytokines.[12,25,26] Pro-inflammatory cytokines such as IL-6 and TNF-α could inhibit BDNF expression.[27] By this mechanism adherence to a MeDiet would be expected to be associated with higher plasma BDNF concentrations. Moreover, the endothelium is responsible for the synthesis and secretion of BDNF,[1] and therefore it would be also expected that the preservation of endothelial function by improved adherence to a healthy MeDiet pattern might lead to an optimum production of BDNF.

To our knowledge, this is the first human clinical trial analysing the association between nutritional factors and plasma BDNF levels. The MeDiet is a dietary pattern characterized by a high consumption of fruits and nuts, vegetables, legumes, olive oil, cereals and fish, low consumption of dairy products and meat, and a moderate intake of alcohol.[28] The composition of the MeDiet and specifically of one of the intervention groups supplemented with mixed nuts could explain the reported results. Nuts are rich in monounsaturated (almonds and hazelnuts) and

Table 4 Multivariate-adjusted* mean BDNF concentrations (μg/ml) (95% CI) according to the intervention group, prevalent diseases, and change in weight from baseline (weight gain vs. weight maintenance or lost)

	Intervention group			
	Control group	MeDiet + VOO	MeDiet + Nuts	*P*
Prevalent hypertension				
No (*n* = 37)	16.78 (9.44–24.11)	18.13 (10.44–25.81)	27.86 (21.22–34.50)	0.02
Yes (*n* = 206)	25.77 (18.18–33.35)	27.20 (19.22–35.19)	26.05 (18.23–33.87)	0.74
P value for interaction	0.45			
Prevalent diabetes				
No (*n* = 164)	22.14 (13.63–30.65)	24.47 (15.28–33.65)	25.02 (15.92–34.13)	0.43
Yes (*n* = 79)	30.54 (20.99–40.10)	26.89 (17.93–35.84)	28.34 (19.23–37.44)	0.51
P value for interaction	0.51			
Prevalent hypercholesterolaemia				
No (*n* = 78)	22.38 (14.64–30.13)	30.15 (21.07–39.22)	26.03 (17.71–34.35)	0.04
Yes (*n* = 161)	23.52 (14.22–32.82)	23.31 (13.69–32.94)	23.81 (14.08–33.54)	0.98
P value for interaction	0.56			
Prevalent depression				
No (*n* = 206)	24.62 (16.78–32.45)	25.81 (17.55–34.08)	24.12 (15.84–32.40)	0.71
Yes (*n* = 37)	22.72 (15.28–30.16)**	24.16 (16.19–32.13)**	32.37 (25.68–39.06)	0.007
P value for interaction	0.06			
Weight change				
Gain (*n* = 117)	23.21 (15.04–31.37)	25.29 (16.50–34.09)	24.88 (17.09–32.67)	0.72
Maintenance or lost (*n* = 124)	29.92 (22.06–37.78)	30.34 (22.64–38.04)	31.52 (23.57–39.47)	0.82
P value for interaction	0.80			

*Adjusted for sex, age, smoking, and the rest of the variables included in the table.
**Statistically significantly lower ($P < 0.05$) than the MeDiet + Nuts group (Benjamini–Hochberg post-test correction).

polyunsaturated fatty acids such as α-linolenic acid (walnuts), arginine, a precursor of nitric oxide, and several antioxidants,[29] fish is rich in DHA and eicosapentaenoic acid; olive oil is a good source of monounsaturated fatty acids, polyphenols with anti-inflammatory activity and vitamin E; and fruits, vegetables, and red wine are also rich in polyphenols and other elements with antioxidant and anti-inflammatory properties. However, the effect of the overall MeDiet pattern is thought to be greater than the sum of its individual parts because of synergistic interactions between its components.[30] Moreover, in a previous report of the PREDIMED trial, the intervention with a MeDiet enriched with nuts was shown to significantly reduce the prevalence of the metabolic syndrome.[31]

On the other hand, some animal studies have related some of these nutrients with improvement in neuropsychiatric symptomatology,[15,32] greater hippocampal volume,[32] and with increased expression not only of BDNF but also of synaptophysine and other key proteins such as CREB involved as well in synaptic plasticity.[8,15,24,32,33] These animal findings can provide clues to understand the results of several epidemiological studies that have related the intake of some nutrients with a lower risk or the amelioration of symptoms of neuro-psychiatric disorders such as Alzheimer disease, Parkinson, or depression. An increasing body of knowledge in nutritional epidemiology has been accumulated in recent years to support an inverse association between depression or neurodegenerative disorders and specific nutrients such as B vitamins or omega-3 fatty acids,[34–36] food items such as olive oil,[37] and specially an overall healthy dietary pattern like the MeDiet.[38–41] Contrarily, a Western dietary pattern has been associated with a higher risk of depression.[39,42,43] One of the possible mediating mechanisms exerted by these dietary factors on the risk of neuropsychiatric disorders can be the modulation of BDNF levels in relationship with the endothelial function or with their antioxidant and anti-inflammatory properties as we have reported here and elsewhere.[12,19,25]

BDNF affects synaptic function modifying the expression and phosphorilation of key proteins such as synapsin I and CREB.[44,45] Thus, BDNF modulates neurotransmitter release, growth, differentiation and survival of presynaptic structure, and axonal elongation.[46,47] So, low levels of this peptide could lead to an atrophy of specific brain areas such as the amygdala and the hippocampus as it has been observed among depressed patients.[48,49] In fact, the role of BDNF on psychiatric disorders and specifically on depression has been analysed by several epidemiological studies. Actually, post-mortem studies have found lower levels of hippocampal and cortical BDNF levels among suicide victims.[50] In the same way, BDNF expression in peripheral cells[51,52] and plasma BDNF levels are also decreased in depression. The results of recent meta-analyses have established that plasma BDNF levels are reduced in patients with depression.[53–55] Moreover, some studies have suggested an association between the presence of a polymorphism in the BDNF gene (Val66Met) and depression.[56,57]

So, in accordance with our results and the physiopathological mechanisms implied in depression occurrence, the effect of MeDiet supplemented with nuts on BDNF levels could be higher among depressed patients though mechanisms related with deficits in BDNF levels and an impaired function of the hippocampus in these subjects.

We acknowledge some limitations in our study. In the first place we are assuming that because of the randomized design of the trial, plasma BDNF baseline levels were well balanced in the three arms of the trial. In fact, the analyses comparing BDNF levels according to the dietary intervention among participants with prevalent depression were adjusted for baseline BDNF plasma levels and the estimations did not change. In the second place, we are aware that the significant findings restricted to subjects with baseline depression might be a chance for finding because of multiple comparisons. However, we have corrected our estimates for multiple comparisons using the Benjamini–Hochberg procedure. Thirdly, we do not have enough statistical power to properly assess interactions (product terms in the multivariable models) because the number of subjects in each subgroup was small. In any case, in spite of these limitations, and admitting the need for further larger studies, our results in the whole sample and for most subgroups showed higher point estimates, albeit non-statistically significant, for average levels of plasma BDNF in both MeDiet groups in comparison with the control group. Given that higher plasma BDNF levels are likely to contribute to prevent depression and cognitive decline, our findings would be consistent with previous epidemiological findings of an inverse association of MeDiet with depressive symptoms[37,58,59] and cognitive decline.[39,60] The strengths of our study are that this is the first assessment of the role of diet on plasma BDNF levels in humans, that our results are somewhat consistent with previous animal models, and above all that we used a randomized design with a long follow-up period.

In conclusion, adherence to a Mediterranean dietary pattern supplemented with nuts seems to improve plasma BDNF levels, especially among patients with depression. These findings open a new line of investigation about dietary adjunctive

therapeutic strategies in depression and the potential of healthy dietary habits to prevent depression and cognitive decline. Further studies are warranted to confirm these results.

Acknowledgements

CIBEROBN and RTIC 06/0045 are an initiative of the Instituto de Salud Carlos III, Science and Innovation Ministry, Spain. We thank all participants in the PREDIMED trial, the personnel of the primary care centres from Navarra, and other investigators of the PREDIMED group: Corella D, Covas MI, Fiol M, Ros E, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Warnberg J, Lamuela-Raventos RM, Saez G, Lapetra J, Serra-Majem L, Pinto L, Tur JA, Mitjavila MT, Portillo MP, Basora J, Fernandez-Crehuet J, Muñoz MA, Diez-Espino J, Toledo E, Tello S, Sanchez-Tainta A and Zazpe I.

The PREDIMED trial has received funding from the Department of Health of the Navarra Government (Spain) (grant PI45/2005), Linea Especial (LE 97) of the University of Navarra, and the Instituto de Salud Carlos III (FIS, PI042241, PI040233, PI050976, PI070240, and PI0801943).

References

1 Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, *et al.* Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett 2000; 470(2):113–7.
2 Matthews VB, Aström MB, Chan MH, Bruce CR, Krabbe KS, Prelovsek O, *et al.* Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia 2009;52(7):1409–18.
3 Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett 2002;328(3):261–4.
4 Fehér A, Juhász A, Rimanóczy A, Kálmán J, Janka Z. Association between BDNF Val66Met polymorphism and Alzheimer disease, dementia with Lewy bodies, and Pick disease. Alzheimer Dis Assoc Disord 2009;23(3):224–8.
5 Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, *et al.* Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 2006;30(8):1529–31.
6 Cysneiros RM, Ferrari D, Arida RM, Terra VC, de Almeida VC, Cavalheiro EA, *et al.* Qualitative analysis of hippocampal plastic changes in rats with epilepsy supplemented with oral omega-3 fatty acids. Epilepsy Behav 2010;17(1):33–8.
7 Hou Y, Aboukhatwa MA, Lei DL, Manaye K, Khan I, Luo Y. Anti-depressant natural flavonols modulate BDNF and beta amyloid in neurons and hippocampus of double TgAD mice. Neuropharmacology 2010;58(6):911–20.
8 Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci 2004;19(7):1699–1707.
9 Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, *et al.* Cognitive impairment following high fat diet consumption is associated with brain inflammation. J Neuroimmunol 2010;219(1–2):25–32.
10 Wu A, Molteni R, Ying Z, Gomez-Pinilla F. A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. Neuroscience 2003;119(2):365–75.
11 Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 2002;112(4):803–14.
12 Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérez V, Covas MI, *et al.* Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 2006;145:1–11.
13 Zazpe I, Sanchez-Tainta A, Estruch R, Lamuela-Raventos RM, Schröder H, Salas-Salvado J, *et al.* A large randomized individual and group intervention conducted by registered dietitians increased adherence to Mediterranean-type diets: the PREDIMED study. J Am Diet Assoc 2008;108(7):1134–44.
14 Aisa B, Gil-Bea FJ, Solas M, García-Allosa M, Chen CP, Lai MK, *et al.* Altered NCAM expression associated with the cholinergic system in Alzheimer's disease. J Alzheimers Dis 2010 [Epub ahead of print].
15 Wu A, Ying Z, Gomez-Pinilla F. Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. Neuroscience 2008;155(3):751–9.
16 Mori TA, Beilin LJ. Omega-3 fatty acids and inflammation. Curr Atheroscler Rep 2004;6:461–7.
17 Songur A, Sarsilmaz M, Sogut S, Ozyurt B, Ozyurt H, Zararsiz I, *et al.* Hypothalamic superoxide dismutase, xanthine oxidase, nitric oxide, and malondialdehyde in rats fed with fish omega-3 fatty acids. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:693–8.
18 Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. Annu Rev Nutr 2005;25:151–74.
19 Salas-Salvadó J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, *et al.* Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. Eur J Clin Nutr 2008;62:651–9.
20 Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 2007;190(1):181–6.
21 Lu C, Chan SL, Haughey N, Lee WT, Mattson MP. Selective and biphasic effect of the membrane lipid peroxidation product 4-hydroxy-2,3-nonenal on N-methyl-D-aspartate channels. J Neurochem 2001;78(3):577–89.
22 Iwata E, Asanuma M, Nishibayashi S, Kondo Y, Ogawa N. Different effects of oxidative stress on activation of transcription factors in primary cultured rat neuronal and glial cells. Brain Res Mol Brain Res 1997;50(1–2):213–20.
23 Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage and counteract learning disability after traumatic brain injury in rats. J Neurotrauma 2004;21:1457–67.
24 Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, *et al.* Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in C57BL/6 J mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. Neuroscience 2009;159(4): 1208–15.
25 Mena MP, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R, *et al.* Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. Am J Clin Nutr 2009;89(1):248–56.
26 Esposito K, Marfella R, Ciotola M, Di palo C, Giugliano F, D'Armiento M, *et al.* Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004; 292(12):1440–6.
27 Anisman H. Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J Psychiatry Neurosci 2009;34:4–20.
28 Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348(26):2599–608.
29 López-Uriarte P, Bulló M, Casas-Agustench P, Babio N, Salas-Salvadó J. Nuts and oxidation: a systematic review. Nutr Rev 2009;67(9):497–508.
30 Trichopoulos D, Lagiou P. Mediterranean diet and cardiovascular epidemiology. Eur J Epidemiol 2004;19(1):7–8.
31 Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M, Estruch R, *et al.* Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med 2008;168(22):2449–58.
32 Venna VR, Deplanque D, Allet C, Belarbi K, Hamdane M, *et al.* PUFA induce antidepressant-like effects in parallel to structural

and molecular changes in the hippocampus. Psychoneuroen 4
2009;34(2):199–211.
33 Blondeau N, Nguemeni C, Debruyne DN, Piens M, Wu X, Pan H, *et al.* Subchronic alpha-linolenic acid treatment enhances brain plasticity and exerts an antidepressant effect: a versatile potential therapy for stroke. Neuropsychopharmacol 2009; 34(12):2548–59.
34 Appleton KM, Rogers PJ, Ness AR. Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. Am J Clin Nutr 2010; 91(3):757–70.
35 Amminger GP, Schäfer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, *et al.* Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiatry 2010;67(2): 146–54.
36 Sánchez-Villegas A, Doreste J, Schlatter J, Pla J, Bes-Rastrollo M, Martínez-González MA. Association between folate, vitamin B(6) and vitamin B(12) intake and depression in the SUN cohort study. J Hum Nutr Diet 2009;22(2):122–33.
37 Kyrozis A, Psaltopoulou T, Stathopoulos P, Trichopoulos D, Vassilopoulos D, Trichopoulou A. Dietary lipids and geriatric depression scale score among elders: the EPIC-Greece cohort. J Psychiatr Res 2009;43(8):763–9.
38 Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A, Schlatter J, Lahortiga F, Serra Majem L, *et al.* Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. Arch Gen Psychiatry 2009;66(10): 1090–8.
39 Akbaraly TN, Brunner EJ, Ferrie JE, Marmot MG, Kivimaki M, Singh-Manoux A. Dietary pattern and depressive symptoms in middle age. Br J Psychiatry 2009;195(5):408–13.
40 Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66(2):216–25.
41 Gao X, Chen H, Fung TT, *et al.* Prospective study of dietary pattern and risk of Parkinson disease. Am J Clin Nutr 2007; 5
86(5):1486–94.
42 Jacka FN, Pasco JA, Mykletun A, Willians LJ, Hodge AM, O'Reilly SL, *et al.* Association of Western and traditional diets with depression and anxiety in women. Am J Psychiatry 2010; 167(3):305–11.
43 Nanri A, Kimura Y, Matsushita Y, Ohta M, Sato M, Michima N, *et al.* Dietary patterns and depressive symptoms among Japanese men and women. Eur J Clin Nutr 2010 [Epub ahead of print].
44 Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS. Synapsins as mediators of BDNF-enhanced neurotransmitter release. Nat Neurosci 2000;3(4):323–9.
45 Finkbeiner S. CREB couples neurotrophin signals to survival messages. Neuron 2000;25(1):11–14.
46 Ventimiglia R, Mather PE, Jones BE, Lindsay RM. The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 1995;7:213–22.
47 Wang T, Xie K, Lu B. Neurotrophins promote maturation of developing neuromuscular synapses. J Neurosci 1995;15(7 Pt 1): 4796–805.
48 Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. Am J Psychiatry 2004;161(11): 1957–66.
49 Hamilton JP, Siemer M, Gotlib IH. Amygdala volume in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. Mol Psychiatry 2008;13(11):993–1000.
50 Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Arch Gen Psychiatry 2003;60(8): 804–15.
51 Lee BH, Kim YK. BDNF mRNA expression of peripheral blood mononuclear cells was decreased in depressive patients who had or had not recently attempted suicide. J Affect Disord 2010 [Epub ahead of print].
52 Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biol Psychiatry 2007;61:911–22.
53 Bocchio-Chiavetto L, Bagnardi V, Zanardini R, Molteni R, Nielsen MG, Placentino A, *et al.* Serum and plasma BDNF levels in major depression: A replication study and meta-analyses. World J Biol Psychiatry 2010 [Epub ahead of print].
54 Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 2008;11(8):1169–80.
55 Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 2008;64(6): 527–32.
56 Lavebratt C, Aberg E, Sjöholm LK, Forsell Y. Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. J Affect Disord 2010 [Epub ahead of print].
57 Chen ZY, Jing D, Bath KG, Ieraki A, khan T, Siao CJ, *et al.* Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006;314(5796):140–3.
58 Muñoz MA, Fíto M, Marrugat J, Covas MI, Schröder H, REGICOR and HERMES investigators. Adherence to the Mediterranean diet is associated with better mental and physical health. Br J Nutr 2009;101(12):1821–7.
59 Gussinyer S, García-Reyna NI, Carrascosa A, Gussinyer M, Yeste D, Clemente M, *et al.* Anthropometric, dietetic and psychological changes after application of the 'Niñ@s en movimiento' program in childhood obesity. Med Clin (Barc) 2008; 131(7):245–9.
60 Féart C, Samieri C, Rondeau V, Amieva H, Portet F, Dartigues JF, *et al.* Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302(6):638–48.
61 Bousquet M, Gibrat C, Saint-Pierre M, Julien C, Calon F, Cicchetti F. Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. Prog Neuropsychopharmacol Biol Psychiatry 2009;33(8):1401–8. 6
62 Gartner A, Staiger V. Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiationinducing electrical stimulation patterns. Proc Natl Acad Sci USA 2002;99: 6386–91.
63 Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Zhang H, Pavuluri MN. Brain-derived neurotrophic factor gene and protein expression in pediatric and adult depressed subjects. Prog Neuropsychopharmacol Biol Psychiatry 2010;34(4):645–51.

Authors Queries

Journal: **Nutritional Neuroscience**
Paper: **NNS52**
Article title: **The effect of the Mediterranean diet on plasma brain-derived neurotrophic factor (BDNF) levels: The PREDIMED-NAVARRA randomized trial**

Dear Author
During the preparation of your manuscript for publication, the questions listed below have arisen.
Please attend to these matters and return this form with your proof. Many thanks for your assistance

Query Reference	Query	Remarks
1	Please check the affiliation of authors and provide full details of correspondence address and e-mail id.	
2	Please provide expansion of HRP and TMB.	
3	Please provide volume and page numbers in references 14, 43, 51, 53, 56.	
4	Please provide one more author name in reference 32, as per the style.	
5	Please provide three more authors name in reference 41, as per the style.	
6	References 61–63 is not cited in the text. Please cite in the text else delete from the list.	