



## Blood markers of fatty acids and vitamin D, cardiovascular measures, body mass index, and physical activity relate to longitudinal cortical thinning in normal aging

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### ABSTRACT

We hypothesized that higher levels of omega-3 fatty acids, vitamin D, and physical activity relate to cortical sparing, whereas higher levels of cholesterol, systolic blood pressure, and body mass index (BMI) relate to increased atrophy in the adult lifespan. Longitudinal measures of cortical thickness were derived from magnetic resonance imaging scans acquired (mean interval 3.6 years) from 203 healthy persons aged 23–87 years. At follow-up, measures of BMI, blood pressure, and physical activity were obtained. Blood levels of docosahexaenoic acid, eicosapentaenoic acid, vitamin D, and cholesterol were measured in a subsample ( $n = 92$ ). Effects were tested in cortical surface-based analyses, with sex, age, follow-up interval, and the interactions between each included as covariates. Higher levels of docosahexaenoic acid, vitamin D, and physical activity related to cortical sparing. Higher cholesterol and BMI related to increased cortical thinning. Effects were independent, did not interact with age, and the cholesterol effect was restricted to males. Eicosapentaenoic acid and blood pressure showed no effects. The observed effects show promise for potential factors to reduce cortical atrophy in normal aging.

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### 1. Introduction

There is increasing awareness that lifestyle factors may affect neuroanatomical and cognitive outcomes across the lifespan. Also healthy aging yields cortical thinning, reduced neuroanatomical volumes, and cognitive decline (Fjell et al., 2009, 2010, 2012; Nyberg et al., 2012; Raz et al., 2010; Walhovd et al., 2011), but there is wide variability in measures of brain integrity among individuals of similar age. Whereas genetic risk factors so far may explain a small portion of the variance (Erten-Lyons et al., 2013), several lifestyle factors have been related to neuroanatomical volumes in aging (Brooks et al., 2013; Erickson et al., 2008, 2013; Scarmeas et al., 2011; Smith et al., 2010). In the present study, we investigate how markers associated with: (1) nutrient intake, specifically eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA),

and vitamin D; (2) cardiovascular health, specifically cholesterol, and blood pressure; as well as (3) body mass index (BMI) and physical activity level, relate to longitudinal cortical atrophy in adults. These markers have not been collectively investigated in a longitudinal design to determine their common and unique influence on regional cortical thinning.

Nutritional differences have been linked to brain and cognitive outcomes at different stages of life (Helland et al., 2003; Henriksen et al., 2008; Nurk et al., 2007). Findings with regard to effects of omega-3 have been mixed. Some have found low levels to be a risk (Cederholm and Palmblad, 2010; Dangour et al., 2010; Kalmijn et al., 1997, 2004; Samieri et al., 2008), and higher levels to be protective for brain and cognition (Brenner, 2012; Samieri et al., 2012; Tan et al., 2012; Titova et al., 2012), while others have not observed an association (Devore et al., 2009; Engelhart et al., 2002). Recently, however, an omega-3 fatty acid intervention study in older adults reported improvements in brain structure and function (Witte et al., 2013). Whereas some authors have reported effects on whole brain or regionally unspecific associations (Tan et al., 2012;

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Titova et al., 2012), specific regional effects including the temporal lobe have also been found (Samieri et al., 2012; Witte et al., 2013), indicating a candidate effect site. Vitamin D deficiency has been related to cognitive function and higher risk of Alzheimer's disease (AD) (Balion et al., 2012) and magnetic resonance imaging (MRI)-indicators of cerebrovascular disease (Buell et al., 2010). However, data are lacking as to whether and which parts of the brain may be affected by vitamin D levels (Annweiler et al., 2012).

Cholesterol is assumed a mediator of brain changes in aging, but its role is uncertain, as the findings from different, mostly cross-sectional studies, are highly mixed (Kin et al., 2007; Kivipelto et al., 2002; Leritz et al., 2011; Mielke et al., 2005; Reitz et al., 2004; Solomon et al., 2009). High blood pressure is a well-known risk factor for cognitive decline and neurodegenerative disease, and has been linked to gray matter changes in normal aging (den Heijer et al., 2005; Korf et al., 2004; Leritz et al., 2011; Nagai et al., 2008; Raz et al., 2007a, 2007b), but there may be a complex and bidirectional relationship (Skoog et al., 1998). For BMI, the focus has been primarily on obesity, which is associated with increased risk for dementia (Kivipelto et al., 2005; Profenno et al., 2010), global brain volume reduction (Gunstad et al., 2008), and reduced gray matter volumes in limbic circuits, including frontal and temporal areas, and effects have also been found in parietal areas (Brooks et al., 2013; Kurth et al., 2012; Pannaciuoli et al., 2006). Longitudinal investigation covering a wide span of BMIs may illuminate whether only obesity is associated with structural differences, or atrophy associations can be found as a linear effect along the full range of BMI. The focus on limbic circuits may make frontal and temporal areas especially likely effect sites. Furthermore, higher physical activity levels have been associated with higher cognitive function and lower incidence of dementia (Chang et al., 2010; Erickson et al., 2012; Laurin et al., 2001). Besides effects on hippocampal volume (Erickson et al., 2011), studies have especially found effects of physical activity and exercise on frontal gray matter volumes (Colcombe et al., 2006; Weinstein et al., 2012). However, general physical activity level may in principle affect brain changes more widely (Erickson et al., 2013), for example via cardiovascular mechanisms. There is a need to investigate to what extent effects of physical activity interact with effects of BMI, or relate to effects of other lifestyle factors on cortical structure.

We hypothesize that some life style factors will be protective and yield cortical sparing, whereas others represent risks yielding accelerated atrophy in aging. Based on the previously reviewed research, likely effect sites for some factors can be suggested, whereas for others, only a direction of effect is hypothesized as: (1) higher levels of omega-3 fatty acids and vitamin D will be related to temporal lobe cortical sparing; (2) higher cholesterol and systolic blood pressure will be associated with more atrophy; (3) higher BMI will be associated with more frontal and temporal atrophy; and (4) higher level of physical activity will be associated with less atrophy, especially in frontal areas. The extent to which these effects may be unique or interdependent, and whether they will interact with age and sex, is unknown, and will also be investigated in a retrospective study of these markers in participants who had 2 brain scans on average 3.6 years apart.

## 2. Methods

### 2.1. Sample

The longitudinal sample was drawn from the ongoing project *Cognition and Plasticity through the Lifespan* at the University of Oslo (Fjell et al., 2008; Westlye et al., 2010), approved by the Regional Ethical Committee of Southern Norway. Written consent was obtained from all participants. Participants were recruited through

newspaper ads. At both time points (Tp1, Tp2), participants were screened with health interviews. Participants were required to be right handed, fluent Norwegian speakers, and have normal or corrected to normal vision and hearing. Exclusion criteria were history of injury or disease known to affect central nervous system (CNS) function, including neurologic or psychiatric illness or serious head trauma, being under psychiatric treatment, use of psychoactive drugs known to affect CNS functioning, and MRI contraindications. Participants were required to score  $\geq 26$  on the Mini Mental State Examination (MMSE; Folstein et al., 1975), have a Beck Depression Inventory (Beck and Steer, 1987) score  $\leq 16$ , and obtain a normal IQ or above ( $\geq 85$ ) on the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). At follow-up, an additional set of inclusion criteria was employed: MMSE change from Tp1 to Tp2  $< 10\%$ ; California Verbal Learning Test II—Alternative Version (CVLT II; Delis et al., 2000) immediate delay and long delay T-score  $> 30$ ; CVLT II immediate delay and long delay change from Tp1 to Tp2  $< 60\%$ . At both time points scans were evaluated by a neuroradiologist and required to be deemed free of significant injuries or pathologic conditions.

Two hundred eighty-one participants completed Tp1 assessment. For the follow-up study, 42 opted out, 18 could not be localized, 3 did not participate because of undisclosed health reasons, and 3 had MRI contraindications, yielding a total of 66 dropouts. Independent samples *t* tests revealed that dropouts had significantly lower full-scale intelligence quotient ( $t = -3.92$ ,  $p < 0.001$ ) and Beck Depression Inventory ( $t = -2.02$ ,  $p = 0.046$ ) scores but comparable CVLT and MMSE scores. Of the 215 participants who completed MRI and neuropsychological testing at both time points, 8 failed to meet additional inclusion criteria for the follow-up described previously. This resulted in a follow-up sample of 207 participants of whom 4 had no measures for BMI, blood pressure, or blood biomarkers (see the following), and were excluded from the final analyses. The final sample for the current analyses included  $n = 203$  in the age range 23–87 years, and for 92 (90 among the 203, plus 2 of the 207 satisfying criteria but lacking the BMI measure), blood biomarkers measures were available. Please see Table 1 for descriptive details for the full sample and subsample.

### 2.2. MRI acquisition and processing

Imaging data were collected using a 12-channel head coil on a 1.5 T Siemens Avanto scanner (Siemens Medical Solutions; Erlangen, Germany) at Rikshospitalet, Oslo University Hospital. The pulse sequence used for morphometric analyses were 2 repeated 160 slices sagittal T<sub>1</sub>-weighted magnetization prepared rapid gradient echo sequences with the following parameters: repetition time/echo time/time to inversion/flip angle = 2400 ms/3.61 ms/1000 ms/8°, matrix = 192 × 192, field of view = 240, voxel size = 1.25 × 1.25 × 1.20 mm per participant per visit. To increase the signal-to-noise ratio the 2 runs were averaged during pre-processing at both time points. Scanning time for each magnetization prepared rapid gradient echo sequence was 7 minutes 42 seconds.

The raw data were reviewed for quality, and automatically corrected for spatial distortion because of gradient nonlinearity (Jovicich et al., 2006) and B<sub>1</sub> field inhomogeneity (Sled et al., 1998). The two image volumes collected at each time point were co-registered, averaged to improve the signal-to-noise ratio, and resampled to isotropic 1-mm voxels. Images were first processed cross-sectionally (independently) for each time point with the FreeSurfer software package (version 5.1.0; Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, USA. <http://surfer.nmr.mgh.harvard.edu/>). This processing includes motion correction, removal of nonbrain tissue, automated Talairach transformation, intensity correction, volumetric segmentation (Fischl et al., 2002),

**Table 1**  
Sample descriptives

	Full sample n = 203 (120F)		Sample with blood markers n = 92 (54F)	
	Mean (SD)	Range	Mean (SD)	Range
Age (y)	53.8 (16.6)	23.5–87.8	63.3 (8.7)	44.486.3
Follow-up interval (y)	3.6 (0.5)	2.7–4.8	3.6 (0.4)	2.8–4.4
MMSE	29.2 (1.0)	26–30	29.0 (1.0)	27–30
IQ	119 (10)	90–146	120 (9.9)	96–146
Education	15.9 (2.6)	8–26	15.7 (2.4)	8–22
Cigarettes per day <sup>b</sup>	0.7 (2.3)	0–20	1.0 (3.7)	0–20
Alcohol units/week <sup>c</sup>	4.7 (4.1)	0–30	6.2 (4.9)	0–30
BMI (kg/m <sup>2</sup> )	25.5 (4.1)	17.5–43.6	25.9 (4.6)	17.5–43.6
BP systolic <sup>a</sup> (mm Hg)	134 (18.3)	102–189	141 (17.8)	111–180
BP diastolic <sup>a</sup> (mm Hg)	81 (11.5)	41–120	85 (10.5)	61–120
MET-min/week	2509 (1987)	50–12,186	2700 (1955)	50–9546
EPA (g/100 g FAME)			1.19 (0.65)	0.16–3.00
DHA (g/100 g FAME)			2.38 (0.98)	0.91–4.65
Vitamin D (nmol/L)			46.6 (13.2)	21.5–83.7
Cholesterol (mmol/L)			5.47 (1.00)	3.23–7.32

All variables except education were measured at follow up.

BMI was available for 88 in the sample with blood markers. MET, a measure of physical activity level, available for 160 with BMI, and 75 in the sample with blood markers. Key: BMI, body mass index; BP, blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; MET, multiples of the resting metabolic rate; MMSE, Mini Mental Status examination.

<sup>a</sup> BP available for 202 samples.

<sup>b</sup> BP available for 161 samples.

<sup>c</sup> BP available for 159 samples.

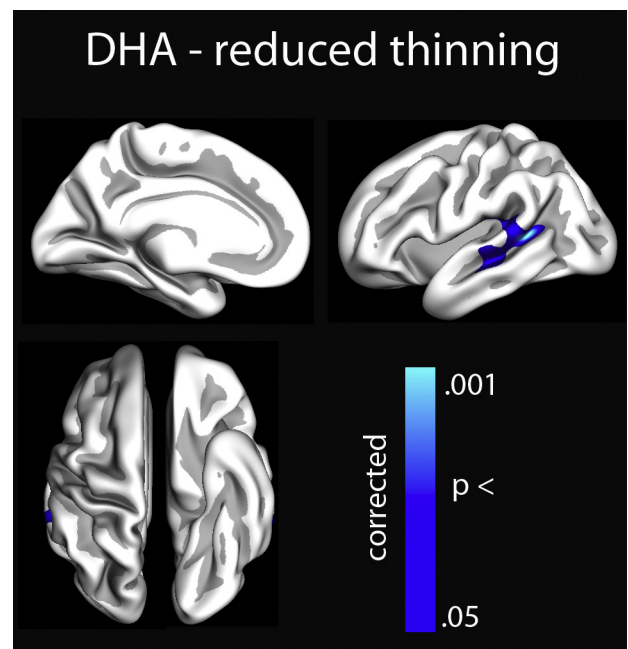
and cortical surface reconstruction (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999a, 1999b) and parcellation (Desikan et al., 2006; Fischl et al., 2004). All volumes were inspected for accuracy and minor manual edits were performed when needed by a trained operator on the baseline images, usually restricted to removal of nonbrain tissue included within the cortical boundary. To extract reliable longitudinal cortical thickness and volume estimates, the cross-sectionally processed images were subsequently run through the longitudinal stream in FreeSurfer (Reuter et al., 2012). Here, an unbiased within-subject template volume based on the 2 cross-sectional images is created for each participant, and processing of both time points are then initialized using common information from this template. This increases the sensitivity and robustness of the longitudinal analysis, and ensures inverse consistency (Reuter et al., 2010), meaning that the inverse transform is obtained when registering Tp2 to Tp1 as opposed to Tp1 to Tp2 (Reuter and Fischl, 2011), which is important in longitudinal analyses (Thompson and Holland, 2011). In addition, new probabilistic methods (temporal fusion) were applied to further reduce the variability across time points. Maps were re-sampled, mapped to a common surface, smoothed using a circularly symmetric Gaussian kernel with a full-width half-maximum of 15 mm (Fischl et al., 1999a, 1999b) and submitted to statistical analyses.

### 2.3. Blood biomarker levels, blood pressure, BMI and physical activity

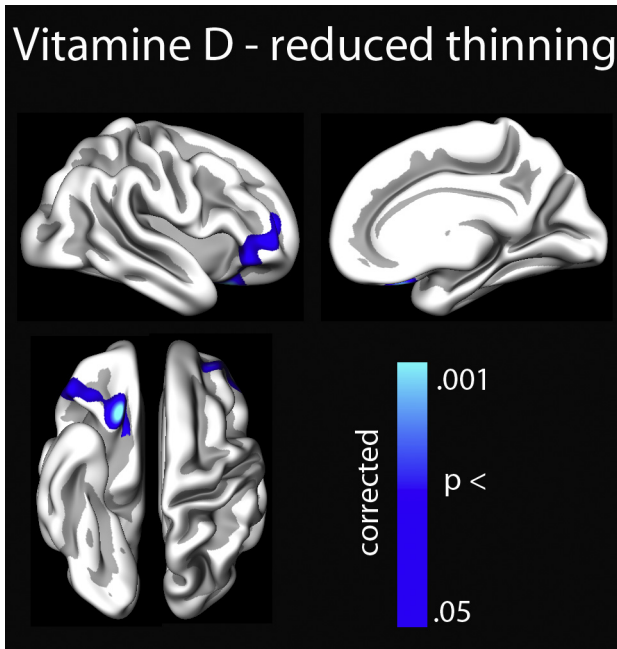
Blood measures were collected at the day of neuropsychological testing at Tp2, within 2 months of the MRI scan (mean interval = 9.6 days, SD = 12.2, range = 0–59 days). Blood biomarker levels were monitored in dried blood spots as developed by Vitas ([www.vitas.no](http://www.vitas.no)). The different devices of the collection kit have been carefully selected and tested to provide easy and robust collection of dried blood spots. The analytical assays are mainly based on different chromatographic techniques. For further details of measurement, see supplementary methods (Supplementary text 1), and for measurement units, see Table 1. Blood pressure was measured with AND UA-767 + 30 digital upper arm blood pressure monitor while participants were seated, before and after the neuropsychological testing, and the average of the 2 measurements was used for the

analyses. Participants' height and weight were measured, and BMI was calculated as weight (kg)/height × height (m).

Physical activity level was measured using the International Physical Activity Questionnaire (Hagstromer et al., 2006), self-administered short version in Norwegian. The International Physical Activity Questionnaire asks participants to report their physical activity for the previous 7 days in these domains: walking, moderate-intensity, and vigorous-intensity. Computation of the total score is based on summation of the duration (in minutes) and frequency (days) of all 3 types of activities. The volume of activity is computed by weighting each type of activity by its energy



**Fig. 1.** Higher docosahexaenoic acid (DHA) level was related to less thickness change in a cluster in the left middle and superior temporal cortex. Abbreviation: DHA, docosahexaenoic acid.



**Fig. 2.** Higher levels of vitamin D was related to less cortical thinning in right lateral prefrontal cortex.

requirements defined in METs (multiples of the resting metabolic rate) to yield a score in MET-minutes. Furthermore, participants can be categorized into 3 different populations based on their reported activity, namely low, moderate, and high physical activity.

#### 2.4. Statistical analyses

For descriptive purposes, age correlations, and partial correlations controlling for age and sex, were calculated among variables of interest and descriptive variables listed in Table 1. Thickness change was computed at each vertex by subtracting Tp1 thickness from Tp2 thickness, and dividing by the mean Tp1 and Tp2 thickness at that vertex. Separate general linear models with thickness change at each vertex across the brain surface as dependent variable, were run with the variables of interest in turn as independent variables. For all surface-based analyses, sex, age, follow-up interval, and the interactions between each were included as covariates. The results were tested against an empirical null distribution of maximum cluster size across 10,000 iterations using Z Monte Carlo simulations as implemented in FreeSurfer (Hagler et al., 2006; Hayasaka and Nichols, 2003) synthesized with a cluster-forming threshold of  $p < 0.05$  (two-sided), yielding clusters corrected for multiple comparisons across the surface. Mean annual percentage change in the significant clusters was extracted to allow post hoc analyses of factors contributing to the main effect of each target variable.

### 3. Results

#### 3.1. Correlations among variables

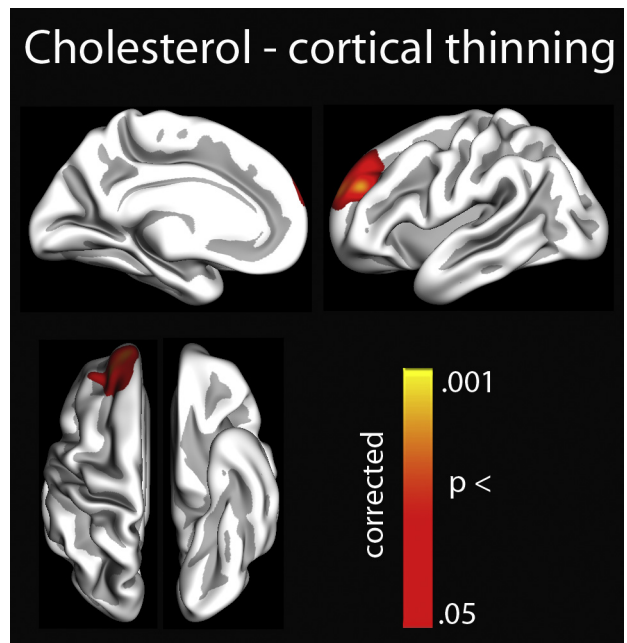
Age correlated significantly ( $p < 0.05$ ) with MMSE ( $-0.26$ ), BMI ( $0.16$ ), systolic blood pressure (BP) ( $0.53$ ), diastolic BP ( $0.27$ ), alcohol units/week ( $0.23$ ), EPA ( $0.40$ ), DHA ( $0.60$ ), and cholesterol ( $-0.44$ ). Partial correlations among the variables, controlling for age and sex revealed few significant ( $p < 0.05$ ) correlations: BMI correlated with diastolic BP ( $0.20$ ) and alcohol units/week ( $-0.16$ ), systolic BP

correlated with diastolic BP ( $0.53$ ), EPA ( $-0.22$ ), MET-minutes/week ( $-0.17$ ), and alcohol units/week ( $0.17$ ). EPA correlated with DHA ( $0.71$ ) and MET-minutes/week ( $0.25$ ). Cholesterol correlated with vitamin D ( $0.23$ ). Education correlated with MMSE ( $0.24$ ) and IQ ( $0.21$ ), and MMSE and IQ correlated ( $0.27$ ).

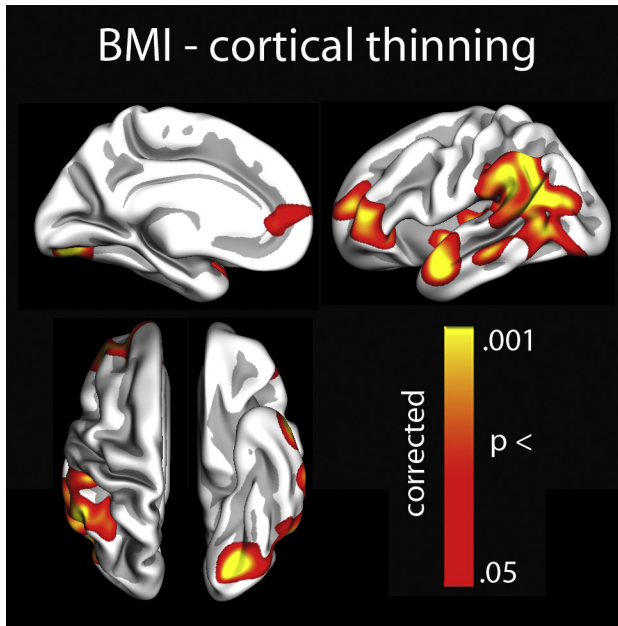
#### 3.2. DHA (22:6 n-3), EPA (20:5 n-3), and vitamin D (25-hydroxivitamin D)

As can be seen from Fig. 1, DHA level was related to thickness change in a cluster in the left middle and superior temporal cortex (cluster size  $2226 \text{ mm}^2$ , cluster-wise  $p < 0.05$ , peak effect Montreal Neurological Institute (MNI) coordinates XYZ  $-47.5, -47.8, 6.7$ ). A similar cluster of effects was seen in the right hemisphere lateral temporal cortex not surviving proper statistical corrections for multiple comparisons (see Supplementary Fig. 1), but suggesting that the effect is not strongly lateralized. No significant effects of EPA were found.

Higher levels of vitamin D were related to less cortical thinning in right lateral prefrontal cortex (cluster size  $2169 \text{ mm}^2$ , cluster-wise  $p < 0.05$ , peak effect MNI XYZ  $15.6, 22.0, -23.8$ ), see Fig. 2. The cortical thickness effect was followed up with region of interest (ROI)-analyses, where age, follow-up interval, and sex were used as covariates and additional variables were entered. First, season of blood sampling was added. Based on approximate values of vitamin D from other samples (Rapuri et al., 2002; Stamp and Round, 1974), participants were assigned the value 1 for lower-season (November, December, January, February, March, April) and 2 for higher-season (May, June, July, August, September, October). This variable turned out not to be significant ( $\beta = 0.08, p = 0.501$ ), and the unique effect of vitamin D remained virtually unchanged, (from  $\beta = -0.36, p = 0.001$  to  $\beta = -0.35, p = 0.001$ ). Next, vitamin D level was z-transformed and squared, and entered into the analysis, but the coefficient for the squared term was not significant ( $p > 0.35$ ), indicating a linear relationship with cortical change. Buell et al. (Buell et al., 2010) characterized vitamin D concentrations in elderly individuals as deficient when below  $10 \text{ ng/mL}$  (equivalent of  $25 \text{ nmol/L}$ ) and insufficient



**Fig. 3.** Higher levels of cholesterol were related to more cortical thinning in a cluster in the left hemisphere.



**Fig. 4.** Higher body mass index (BMI) was related to more cortical thickness reduction in the left hemisphere. Abbreviation: BMI, body mass index.

when at or below 20 ng/mL (equivalent of 50 nmol/L). We split the sample in 2 groups according to these criteria, 1 consisting of those within the normal or high range ( $n = 30$ ) and 1 including those with insufficiency and deficiency ( $n = 3$  and 59, respectively, total group  $n = 62$ ). Partial correlations between atrophy in the cluster and vitamin D was calculated. Correlations of atrophy and vitamin D concentrations did not reach significance for either of these smaller groups (insufficient/deficient  $r = -0.16$ , and normal  $r = -0.26$ ), and the difference between z-transformed correlation coefficients was not significant ( $p > 0.65$ ).

### 3.3. Cholesterol and blood pressure

Higher cholesterol levels were related to more cortical thinning in a cluster in the left hemisphere, covering the lateral section of the anterior superior frontal gyrus (cluster size 1735 mm<sup>2</sup>, cluster-wise  $p < 0.05$ , peak effect MNI XYZ  $-22.5, 46.5, 27.2$ ; see Fig. 3). Analyses of effects of blood pressure on cortical change, revealed no significant effects.

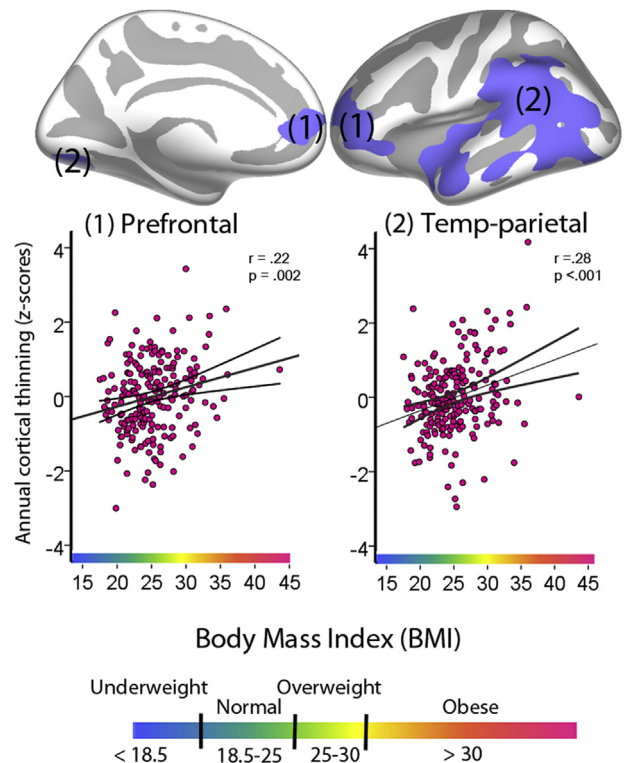
### 3.4. BMI and physical activity

Higher BMI was significantly related to cortical thickness reduction in the left hemisphere (see Fig. 4). One cluster of effects covered most of the lateral and anterior temporal cortex, the temporo-parietal junction and the inferior parietal cortex, and the lingual and fusiform gyrus (cluster size 12,914 mm<sup>2</sup>, cluster-wise  $p < 0.0001$ , peak effect MNI XYZ  $-49.7, -51.0, 30.1$ ). A second cluster was found in the lateral and medial prefrontal cortex (cluster size 3114 mm<sup>2</sup>, cluster-wise  $p < 0.0005$ , peak effect MNI XYZ  $-38.5, 45.4, 7.5$ ). No effects in the right hemisphere survived corrections. Uncorrected effects are shown in Supplementary Fig. 1. Cortical change values for each vertex in the 2 clusters were computed for each participant. Scatterplots illustrating the relationship between BMI and change in these areas are shown in Fig. 5. For all ROI-analyses, age, follow-up interval, and sex were used as covariates. First, we tested whether the effects of BMI on cortical thinning were greater for higher levels of BMI. BMI was z-transformed and squared, but the coefficient for

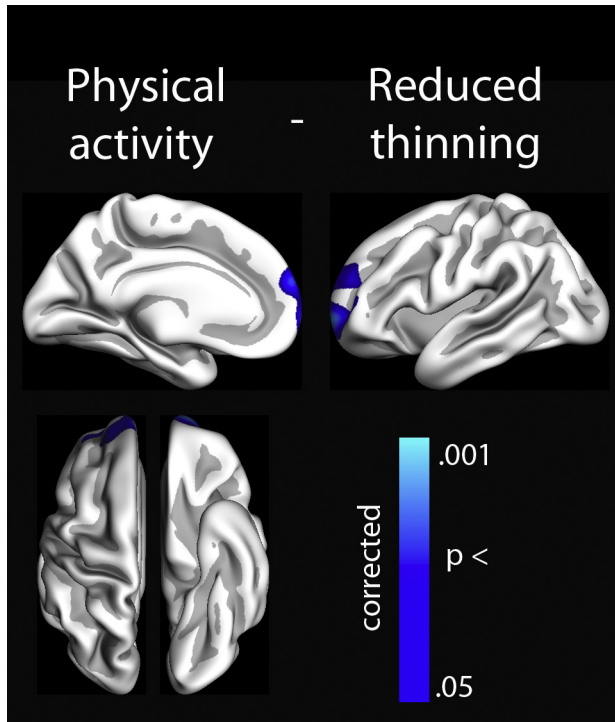
the squared term was not significant ( $p > 0.46$ ) for either of the clusters. We then split the sample in 2 groups, those within the normal or lower range (BMI  $< 25$ ,  $n = 107$ ) and those with overweight (BMI  $\geq 25$ ,  $n = 96$ ). Partial correlations between atrophy in each cluster and BMI were calculated. For the normal and low weight group, the correlations were not significant (prefrontal cluster  $r = -0.03$ ,  $p = 0.80$ ; lateral temporal cluster  $r = 0.02$ ,  $p = 0.86$ ). In contrast, significant correlations were observed in the overweight group (prefrontal cluster  $r = 0.28$ ,  $p < 0.01$ ; lateral temporal cluster  $r = 0.27$ ,  $p < 0.01$ ). A cluster in the left prefrontal cortex showed less thinning with higher physical activity levels operationalized as total MET-minutes per week (see Fig. 6).

### 3.5. Analyses of interactions with sex and age

Cortical change values for each vertex in the cluster where the previously mentioned variables had a significant effect on cortical thinning were computed for each participant. We tested whether sex affected the atrophy relationship by adding a sex interaction term as an additional covariate (sex  $\times$  DHA, sex  $\times$  vitamin D, sex  $\times$  cholesterol, sex  $\times$  BMI, sex  $\times$  physical activity). In none of the cases did the interaction term add significantly to the amount of explained variance, with the exception of cholesterol ( $\beta = 0.80$ ,  $p = 0.012$ ). Splitting the sample by sex revealed that a correlation between cholesterol and atrophy in the cluster (controlling for age and interval between scans) was found only in men ( $r = 0.46$ ,  $p = 0.005$ ), not in women ( $r = 0.08$ ,  $p = 0.589$ ). The same procedure was used for age (age  $\times$  DHA, age  $\times$  vitamin D, age  $\times$  cholesterol, age  $\times$  BMI, age  $\times$  physical activity). Neither of these interaction terms added significantly to the amount of explained variance.



**Fig. 5.** Scatterplots illustrating the relationship between body mass index (BMI) and change in the areas where significant relationships between BMI and cortical atrophy were identified (see Fig. 4). Pearson correlations values ( $r$ ) and  $p$ -values shown in the plots are for BMI (x-axes) and residualized cortical change scores after regressing out variance associated with age, sex, and interval between scans (y-axes). Abbreviation: BMI, body mass index.



**Fig. 6.** Higher physical activity level was related to less thinning in the right prefrontal cortex.

### 3.6. Validation analyses controlling for physical activity, education, blood pressure, smoking, alcohol, and BMI

The previously described ROI analyses for effects of DHA, vitamin D, and cholesterol level, as well as BMI, were rerun including MET-minutes per week. In no case did this variable explain unique variance in the identified effects. For DHA, vitamin D and BMI, the inclusion of MET-minutes did not render the observed relationships insignificant, but the effect of cholesterol was reduced to a trend ( $p = 0.09$ ) when restricting the sample to those having both measures of cholesterol and the measure of total MET-minutes per week ( $n = 76$ ). The inclusion of physical activity did not change this significance level. Education was added in all regression models on the ROI effects. In no case did education explain unique variance, or show a trend towards this. To test whether blood pressure, amount of smoking, and alcohol consumption affected the relationship between BMI and brain atrophy, partial correlations with these variables as additional covariates were run ( $n = 148$ ). Both clusters were still significantly related to BMI (prefrontal cluster  $r = 0.26$ ,  $p < 0.001$ ; lateral temporo-parietal cluster  $r = 0.40$ ,  $p < 10^{-5}$ ) after controlling for the effect of these variables. Finally, an analysis was run for the effect of physical activity including BMI as an additional covariate, but this did not exert a significant influence ( $p = 0.187$ ), and the effect of MET-minutes per week remained significant, independently of BMI ( $p = 0.006$ ).

## 4. Discussion

In the present study we identify a number of lifestyle-related protecting and negative influences on longitudinal cortical atrophy in aging. In sum, high levels of DHA, vitamin D, and physical activity were related to cortical sparing, whereas high cholesterol and BMI were related to greater cortical thinning. No effects were found of EPA or blood pressure on cortical atrophy. In the following, implications of the findings are discussed.

Higher level of DHA at follow-up was associated with regional cortical sparing in the left middle and superior temporal cortex, supporting our first hypothesis. Although not surviving proper statistical corrections, similar effects were seen in the right lateral temporal cortex, suggesting that the effect is not strongly lateralized. The finding that DHA is associated with cortical sparing, is in line with recent studies showing effects of DHA on risk of dementia and cognitive decline (Cederholm and Palmblad, 2010; Dangour et al., 2010; Kalmijn et al., 1997; Kalmijn et al., 2004; Samieri et al., 2008), as well as cross-sectionally estimated neuroanatomical characteristics (Brenner, 2012; Tan et al., 2012; Titova et al., 2012). Inconsistent findings (Devore et al., 2009; Engelhart et al., 2002) may be because of different methodologies used and populations studied (Huang, 2010). No effects of EPA were observed in the present study. Both DHA and EPA may have neuroprotective effects through anti-inflammatory, anti-oxidant, and energy-metabolism pathways (Samieri et al., 2012). However, EPA is present in the brain in much lower amounts than DHA, which is the principle omega-3 fatty acid in the brain (McNamara and Carlson, 2006). DHA is an important component of membranes in the CNS accounting for approximately 15% of the fatty acids in the human brain (McNamara and Carlson, 2006). A recent supplementation study (Witte et al., 2013) with increases in both DHA and EPA, also found gray matter increases in the left superior temporal gyrus, like in the present study, as well as left hippocampus and parietal areas and right middle temporal gyrus. Another recent longitudinal study found effects of EPA, but not DHA on medial temporal lobe (MTL) atrophy (Samieri et al., 2012). That study included older adults aged 65 years and above only. It is possible that some effects may be more pronounced in the MTL with increasing age, as accelerated MTL decline in aging is an established finding (Fjell et al., 2009, 2012; Walhovd et al., 2009). Interactions of age were not observed for DHA in the present study, but as the sample is of moderate size, we think it is premature to conclude strongly about this. In the study by Witte et al. (Witte et al., 2013), gray matter increases were related to decreases in fasting insulin and glucose, and there were also reductions in diastolic blood pressure and carotid intima media thickness in women, hinting at anti-atherogenic and anti-inflammatory effects of the long chain polyunsaturated acids. In addition, the mechanisms of the observed cortical sparing may include pathways lowering beta amyloid ( $A\beta$ ), as higher intake of long chain polyunsaturated acids have been associated with lower plasma amyloid beta levels (Gu et al., 2012).

The present study for the first time provided evidence that higher levels of vitamin D relate to regional cortical sparing in normal aging. This effect was seen in the right hemisphere, primarily in lateral prefrontal cortex, extending to inferior frontal areas. Although vitamin D deficiency has previously been related to cognitive decline and MRI-indicators of cerebrovascular disease (Buell et al., 2010), the lack of longitudinal studies has made it uncertain whether deficiency precipitates decline, or, alternatively, if neurodegeneration precipitates vitamin D deficiency (Annweiler et al., 2012). Our present study involved well-screened, primarily high-functioning adults. This indicates that vitamin D level and its associations with longitudinal brain anatomy are not only related to neurodegenerative disorders. Moreover, our results hint that effects may not be restricted to insufficiency or deficiency of vitamin D. The vitamin D concentrations were on average in the insufficient range. Although subsamples categorized according to insufficiency or deficiency versus above were small, yielding relatively low power, there was no evidence suggesting that the relationship was stronger in the part of the sample categorized as having insufficient or deficient vitamin D. A similar finding has been reported in a study of vitamin B supplementation promoting lowering of homocysteine levels, where continuous effects of plasma concentrations of B-vitamins were

found well beyond traditional recommended levels (Smith et al., 2010). Experimental studies have shown that vitamin D has anti-neurodegenerative and anti-ischemic effects by binding to the neuronal vitamin D receptors, and the present finding provides hitherto lacking data (Annweiler et al., 2012) on variation in brain characteristics that may be affected by vitamin D levels.

Higher cholesterol was related to cortical thinning in the lateral superior frontal gyrus, in support of hypothesis 2. Little previous data exist on the relationship of cholesterol to normal brain aging. The one study that we are aware of on normal variation among community-dwelling older adults, used a cross-sectional design, and unexpectedly found that higher cholesterol levels were associated with thicker cortex in broad areas (Leritz et al., 2011). It was suggested that this might be related to a complex relationship between blood serum level and brain cholesterol production. Based on the current finding, we believe that cholesterol may constitute a risk factor for increased cortical thinning too, but it is likely that different relationships may be observed in different groups, according to their cognitive and health status. In keeping with our present result, increased brain atrophy has been documented in individuals with cardiovascular risk factors including higher cholesterol (Kin et al., 2007), and high serum cholesterol in midlife has been identified as a risk factor for dementia (Kivipelto et al., 2002). Although other studies of older populations have found no association (Reitz et al., 2004) or a positive association (Mielke et al., 2005) between low cholesterol and AD risk, these opposing findings may be attributed to differences in plasma-brain cholesterol levels in different groups. Of note, higher cholesterol levels were related to reduced gray matter volumes in memory clinic patients with subjective cognitive impairment, but in AD-patients in the same study, cholesterol levels were positively associated with brain volumes (Solomon et al., 2009). The authors pointed to the possibility of brain neurodegeneration promoting lower serum cholesterol levels because of a disruption of the production of brain cholesterol (Solomon et al., 2009). Based on the finding of a negative relationship between cholesterol and brain volumes in subjective cognitive impairment, one would expect that cholesterol should longitudinally be negatively related to brain volumes also in normal aging. It is noteworthy that the effect was found only in males. It is possible that this sex difference may be partly related to some unknown selection bias. Animal studies have suggested sex differences in CNS cholesterol homeostasis, including reduction of low-density-lipoprotein receptor protein in the brains of female rats with estrogens decline in aging, but this has rather been linked to an increased risk of neurodegenerative disorder (Segatto et al., 2011). Possible sex differences should be investigated further in larger samples.

Unexpectedly, no effects of blood pressure were seen, despite several previous studies having linked blood pressure to gray matter differences in normal aging (den Heijer et al., 2005; Korf et al., 2004; Leritz et al., 2011; Nagai et al., 2008; Raz et al., 2007a, 2007b). Whereas blood pressure, like cholesterol, can decline in older age and in dementia as a result of brain degenerative changes (Skoog et al., 1998), possibly causing a complex relationship, we find it unlikely that the lack of effects in the present sample can be attributed to incipient neurodegenerative disease. Another possibility may be that effects of blood pressure are primarily seen in older age, and in the larger sample where blood pressure was measured ( $n = 202$ , mean age 53 years, range 23–87 years), relatively many are young. Furthermore, a limitation is that blood pressure was measured at Tp2 only, and fluctuations or newly acquired changes may cause noise in the data.

In support of hypothesis 3, widespread effects of BMI were seen in the left hemisphere, with higher BMI being related to more cortical thinning in most of the lateral and anterior temporal cortex, the

temporo-parietal junction and the inferior parietal cortex, the lingual and fusiform gyrus and lateral and medial prefrontal cortex. These effects are interesting because brain correlates of BMI have so far primarily implicated obesity, which is associated with increased risk for developing dementia (Kivipelto et al., 2005; Profenno et al., 2010). In addition to global volume reduction (Gunstad et al., 2008), previous findings have centered on reduced gray matter volumes in limbic circuits, including frontal and prefrontal areas (Brooks et al., 2013; Kurth et al., 2012; Pannacciulli et al., 2006). As these areas are within appetite-regulatory circuits, it has been suggested that the identified differences make individuals prone to overeating (Brooks et al., 2013). However, the present effects also suggest that higher BMI yields more cortical thinning in these areas with age. A common genetic variation in fat mass and obesity associated gene, carried by 46% of Western Europeans, appears by cross-sectional comparisons to be associated with reduced regional brain volumes in older age (Ho et al., 2010). Although this effect has also been shown on total brain volume in adolescence, and the origin has been suggested to be in early development, by an inverse effect on adipose and brain tissue during fetal life (Melka et al., 2013), the present findings imply that effects are found in aging too, with increased cortical thinning with higher BMI. The presently identified cortical areas partly correspond to regions previously reported to be reduced in obesity and high BMI, including frontal and temporal cortices (Ho et al., 2010), and are prone to age-related and neurodegenerative decline (Fjell et al., 2009). It has been suggested that increased inflammatory responses or cardiovascular complications in obesity may make these brain areas vulnerable to atrophy (Profenno et al., 2010). Thus, it was surprising that the quadratic interaction term was not significant, implying that effects were not significantly greater for high BMI. However, analyses on the sample split according to normal weight versus overweight or obesity indicated that the effect was primarily observed in the overweight and obese group. It is noteworthy, though, that most persons in this group are not obese, and effects are still seen. Moreover, effects indeed appeared to be uniquely associated with BMI, and were not mediated by blood physical activity pressure, smoking, or alcohol consumption.

Higher physical activity level was related to less thinning in the right prefrontal cortex, in keeping with hypothesis 4. This finding corresponds well with previous studies reporting associations between higher physical activity levels and greater neuroanatomical volumes, higher cognitive function, and lower incidence of dementia in older cohorts (Chang et al., 2010; Erickson et al., 2012; Laurin et al., 2001; Weinstein et al., 2012). The underlying mechanisms may be multiple, including induction of brain-derived neurotrophic factor, synaptic plasticity, and reduction of amyloid- $\beta$  ( $A\beta$ )-levels (Brown et al., 2012). The link may also be indirect because physical activity may serve to maintain a low, or reduce BMI, limiting risk factors such as cardiovascular health or inflammatory responses related to obesity. However, BMI and physical activity did not correlate significantly in this sample, and the effect was not explained by BMI, suggesting that physical activity is beneficial in and of itself, perhaps through direct neural effects as mentioned previously.

## 5. Conclusions, limitations, and perspectives for future research

The present study showed that higher levels of DHA, vitamin D, and physical activity were related to cortical sparing, while higher levels of cholesterol and BMI related to increased cortical atrophy in cognitively well-functioning adults. Application of longitudinal exclusion criteria for cognitive decline limits the impact of possible incipient neurodegenerative disease, and underscores that effects are found also throughout the normal and high range of functioning. A

limitation is that we do not know whether other or additional effects may be seen in the low-functioning range. The identified effects appeared to be largely independent. For instance, unique effects of physical activity and BMI were found. A limitation of the present study is that blood biomarkers, blood pressure, BMI, and measures of physical activity were obtained at follow-up only. Because these measures may change over time, this may cause noise in the data. Genotyping, for example of apolipoprotein E, was not included here, but interactions with candidate genes may be a venue of further research. Furthermore, subcortical structures, for example MTL structures such as the hippocampus and amygdala were not included in the present study, but may also be associated with the presently investigated markers. This should be a topic of further research. We note, however, that cortical MTL areas did not show significant associations with the studied markers. Cortical MTL areas are known to show a relatively steeper decline in higher age (Fjell et al., 2012, 2013), and associations within these areas may be further investigated in older samples. Effects were generally not observed bilaterally, but we do not at present want to speculate about possible lateralization effects. All observed effects were in the predicted direction, but the consistency of regions identified should be further evaluated in additional longitudinal studies. One of the most novel findings in the present study is that higher levels of vitamin D were related to cortical sparing in aging. This effect appeared not to be specific to vitamin D deficiency or insufficiency, in correspondence with recent findings on effects of vitamin B supplementation having effects beyond established cut-offs. Unfortunately, vitamin B and homocysteine levels were not included in the present study. The observed effects of DHA, vitamin D, and physical activity levels on cortical sparing show promise for possible lifestyle factors to reduce cortical atrophy in normal aging.

#### Disclosure statement

Kristine Beate Walhovd (KBW) has received a honorarium of 500 Euros and refund of travel costs in relation to work on a review on brain imaging markers and related presentations from International Life Sciences Institute (ILSI) Europe. Christian A Drevon (CAD) and his family hold stocks in the public company VITAS AS, which is an analytical service provider. VITAS has analyzed the nutrient biomarkers in this actual paper. The University of Oslo has screened bidders of the actual analyses and VITAS has been found to be the preferred provider of these actual analyses based on quality and price. CAD is also on the board of VITAS and acts as a consultant for this company. CAD is also a stockowner with his family in a public consulting company called DBG AS. He is or has been a paid consultant for many companies in food industry and pharmaceutical industry and a private medical college named Atlantis Medical College, where he also is the chairman of the board.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2013.11.011>.

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