# Genetic and methodological aspects of statin-induced lipid response

Smit, R.A.J.

# PART III

## Statins and visit-to-visit lipid variability

# CHAPTER 8

## Higher visit-to-visit low-density lipoprotein cholesterol variability is associated with lower cognitive performance, lower cerebral blood flow, and greater white matter hyperintensity load in older subjects

**Roelof AJ Smit**, Stella Trompet, Behnam Sabayan, Saskia le Cessie, Jeroen van der Grond, Mark A van Buchem, Anton JM de Craen, J Wouter Jukema

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## ABSTRACT

Background: Recently it was shown that intra-individual variation in low-density lipoprotein cholesterol (LDL-c) predicts both cerebro- and cardiovascular events. We aimed to examine whether this extends to cognitive function, and examined possible pathways by using an MRI substudy.

Methods and results: We investigated the association between LDL-c variability and four cognitive domains at month thirty in 4428 participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). Additionally, we assessed the association of LDL-c variability with neuroimaging outcomes in a subset of 535 participants. LDL-c variability was defined as the intra-individual standard deviation over four post-baseline LDL-c measurements, and all analyses were adjusted for mean LDL-c levels and cardiovascular risk factors. We observed that higher LDL-c variability was associated with lower cognitive function in both the placebo and pravastatin treatment arm. Associations were present for selective attention, processing speed, and memory. Furthermore, higher LDL-c variability was associated with lower cerebral blood flow in both trial arms, and with greater white matter hyperintensity load in the pravastatin arm. No evidence was found for interaction between LDL-c variability and pravastatin treatment for both cognitive and MRI outcomes.

Conclusions: We found that higher visit-to-visit variability in LDL-c, independent of mean LDL-c levels and statin treatment, is associated with lower cognitive performance, lower cerebral blood flow, and greater white matter hyperintensity load.

## INTRODUCTION

Over eighty-five years ago, Cannon hypothesized that loss of physiological homeostasis, for instance through disease or the ageing process, would lead to disturbances in intrinsic variability (1). This intra-individual variability in various physiological measures has become of increasing interest in recent years, as both lowered heart rate variability and increased blood pressure variability have been repeatedly linked to adverse outcomes such as vascular events, impaired cognition, and mortality (2-6). However, little is known about cholesterol variability, which may be considerable even on a day-to-day basis (7, 8). Recent evidence indicates that, in subjects with coronary artery disease, greater visit-to-visit variability in low-density lipoprotein cholesterol (LDL-c) is associated with higher risks of coronary and other cardiovascular events, stroke, and mortality, independent of mean LDL-c levels (9). Whether visit-to-visit variability in LDL-c is associated with cognitive performance is currently unknown.

Here, we assessed whether visit-to-visit variability in LDL-c is associated with cognitive function, independent of mean LDL-c levels, in 4428 participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). Additionally, to assess potential mechanisms behind this association, we examined the link between LDL-c variability and hippocampal volume, cerebral blood flow, and white matter hyperintensity load in an MRI substudy.



## METHODS

### Study population

All subjects were participants of the PROSPER study, of which the study design has been described in detail elsewhere (10). In short, this multicentre, randomized, placebo-controlled trial aimed to determine whether pravastatin reduces the risk of major cardio- and cerebrovascular events in participants aged 70-82 years with pre-existing vascular disease (coronary, cerebral, or peripheral) or at higher risk for developing vascular disease due to a history of hypertension, cigarette smoking or diabetes mellitus. To be eligible for enrolment, plasma total cholesterol was required to be 4.0-9.0 mmol/L, with triglyceride concentrations lower than 6.0 mmol/L. Participants were recruited in Scotland, Ireland, and the Netherlands. The study was approved by the institutional ethics review boards of each center, and all participants gave written informed consent. LDL-c variability and cognitive measures were available for 4428 participants. In addition, MRI measurements at end of study were available for 535 participants.

## Assessment of LDL-c variability

Lipid levels were assessed after an overnight fast, and LDL-c was measured directly. Lipoprotein profiles were quantified at the Centre for Disease Control certified central lipoprotein laboratory in Glasgow. Visit-to-visit variability of LDL-c was calculated by means of the intra-individual standard deviation over each individual's measurements, using post-baseline measurements after 3, 6, 12 and 24 months. The coefficient of variation, another measure for LDL-c variability but standardized to the intra-individual mean LDL-c level over the same measurement period, was highly correlated with the intra-individual standard deviation (Pearson's $r = 0.87$). Baseline measurements were excluded to avoid including artificially induced variability from commencement of statin therapy or as an initial response to dietary and lifestyle advice given to all participants at baseline. Throughout the trial, subjects received nutritional advice and health counselling, and were stimulated to follow the National Cholesterol Education Program Step 1 diet or a local equivalent that provided <30% of total calories from fat (<10% as saturated fat) and a cholesterol intake of <300 mg/day.

## Assessment of cognition

Subjects with poor cognitive function (Mini Mental State Examination (MMSE) score < 24) were excluded from enrolment in the main PROSPER study. Serving as outcome variables, cognitive function was evaluated through four cognitive measures (11). The Stroop-Colour-Word-Test (Stroop) was employed to test selective attention, with total number of seconds needed to complete the third test part used as the outcome parameter. The Letter-Digit Coding Test (LDT) assessed information processing speed, taking the number of correct digits filled in within 60 seconds, with higher scores denoting better performance. The Picture-Word Learning Test (PLT) was used as a verbal memory test, separately assessing immediate (number of recalled pictures over three learning trials) and delayed recall after twenty minutes, with higher scores denoting better performance. All cognitive outcomes were assessed at month thirty to maximize the availability of cognitive outcomes following the measurement of LDL-c variability.

## Magnetic Resonance Imaging Substudy

Of the eligible Dutch participants of the main PROSPER study, 646 consented to participate in a nested MRI substudy, of which the methods and results have been published previously (12). Subjects with intraorbital vascular clips,

collagen disease, cardiac pacemakers, hearing implants, multiple sclerosis, or claustrophobia were excluded from participating. In the current study, we examined results from imaging performed after a mean±SD follow-up of 33±1.4 months. Data on visit-to-visit LDL-c variability and MRI outcomes were available for 535 participants.

A clinical MR-system operating at a field strength of 1.5 Tesla was employed for all imaging (Philips Medical Center, Best, the Netherlands). The Oxford Centre for Functional MRI of the Brain's integrated registration and segmentation tool (FIRST) was utilized to estimate the hippocampal volume (13). Using the phase contrast technique, cerebral blood flow was calculated by adding the flow from the left and right internal carotid arteries to the flow in both vertebral arteries, and was subsequently standardized to whole-brain parenchymal volume (12). Quantification of white matter hyperintensity load was performed using Software for Neuro-Image Processing in Experimental Research (SNIPER), an in-house developed fully automatic segmentation method combining information from proton density, T2-weighted and fluid-attenuated inversion recovery (FLAIR) images (14).



## Demographic and clinical characteristics

Participant characteristics were assessed at baseline. These included age, education (age of leaving school), body-mass index, current smoking status (yes/no), alcohol intake (measured in units per week), and history of various clinical diseases.

## Statistical analyses

All analyses were conducted separately for the placebo and pravastatin arm. Demographic and clinical characteristics are presented as numbers with percentage, means with standard deviations, or medians with interquartile range when appropriate. Participant characteristics were compared over tertiles of LDL-c variability using analysis of variance and Pearson's chi-square test. Using multivariable linear regression models, the association between post-baseline LDL-c variability and cognitive performance at month 30, and MRI measures at end of study, was determined. Subjects with a minimum of two out of four LDL-c measurements were included. While reporting mean (SE) cognitive scores and MRI measures over tertiles of LDL-c variability to gain insight into the underlying distribution of neurocognitive function, intra-individual variability was used as a continuous covariate in the linear regression models. Adjusted unstandardized

regression coefficients, 95% confidence intervals, and p-values were reported. Covariate adjustments were made based on their biological plausibility as potential confounders for the association between LDL-c variability and neurocognitive outcomes. These covariates consisted of diseases and factors that are known to influence LDL-c levels, and have been linked to either cognitive or neurovascular impairment. For the minimally adjusted model we included age, gender, country, education, average LDL-c, and cognitive test version and whole-brain parenchymal volume where appropriate. The fully adjusted model additionally included body-mass index, current smoking status, alcohol intake, and history of diabetes, hypertension, and vascular disease. Data on these baseline covariates was complete for all participants. Possible violations of the assumptions of multiple linear regression were examined by visually inspecting the distribution of residuals through both histograms and normal P-P plots. We further checked for deviations of linearity and homoscedasticity by visually inspecting scatterplots of standardized residuals by standardized predicted values. In addition, we assessed Variance Inflation Factors to examine the possibility of multicollinearity. We considered p-values of 0.05 or smaller statistically significant. All analyses were conducted using IBM SPSS Statistics version 20.0.

## Sensitivity analyses

Several sensitivity analyses were conducted in order to measure how robust the findings were to different subsets of the data, and to elucidate possible mechanisms through which LDL-c variability might associate with cognitive function. First, the association with cognitive performance at end of study was assessed, rather than cognition at month thirty, whilst using the same exposure measurement period. On average, this meant cognitive performance was assessed 9 months later. A further consideration was possible influence of the number of lipid measurements. Therefore, we restricted our analyses to those participants with all four measurements. We additionally performed separate analyses excluding history of, and incident events of, cerebro- and cardiovascular disease. As both cancer and serious infection may influence levels of LDL-c, we also carried out analyses excluding these incident disease states. Furthermore, blood pressure variability has been shown to associate with cognition in recent years (4). As variability in LDL-c and blood pressure could arise from a common cause, we adjusted for systolic blood pressure (SBP) variability to distinguish effects of LDL-c variability from those mediated by blood pressure variability. SBP variability was defined as the intra-individual standard deviation over months 3-24, with blood pressure measured every three months, and these

analyses were additionally adjusted for mean SBP over the same measurement period. Further, it is possible that LDL-c variability reflects consistent trends over time rather than an undulating pattern, e.g. due to progressively reduced dietary intake in the context of overall decline in health status. Therefore, we carried out analyses whilst adjusting for the average slope of LDL-c during the measurement period. Finally, as concomitant medication usage may underlie differences in lipid variability, we performed analyses adjusting for baseline medication usage of diuretics, ACE I- or II-inhibitors, beta-blockers, calcium channel blockers, nitrates, anticoagulants, anti-arrhythmic medication, and glucose-lowering medication (insulin and non-insulin separately). For all sensitivity analyses, we report the results from the fully adjusted model only, which were similar to those seen for the minimally adjusted model.

## RESULTS



### Demographic and clinical characteristics

Participant characteristics are described in **Table 1**. In both the placebo and pravastatin arms, participants in higher tertiles of visit-to-visit LDL-c variability had a higher SBP variability (p=0.003, p=0.006, respectively), higher average LDL-c (both p<0.001), were more often female (p=0.001, p=0.002), and less likely to be Dutch rather than Scottish or Irish when compared to the other tertiles (p=0.047, p=0.014). However, the difference in the proportion of females and males disappeared after standardizing variability to the intra-individual mean LDL-c, by means of the coefficient of variation, in both trial arms (p=0.67, p=0.23, respectively). As shown in **Supplemental Table 1**, the participants of the MRI substudy were largely representative of the Dutch participants.

### Effect of pravastatin on LDL-c

Statin therapy was associated with a reduction of both average LDL-c (-1.18 mmol/L, 95% CI: -1.14 to -1.22) and mean visit-to-visit LDL-c variability (-0.02 mmol/L, 95% CI: -0.01 to -0.04), as measured by the intra-individual standard deviation.

### Association between LDL-c variability and cognitive performance

In both the placebo and pravastatin group, higher LDL-c variability was significantly associated with lower cognitive test scores (**Table 2**). While most consistent for the memory measures

**Table 1.** Baseline characteristics over tertiles of LDL-c variability

| | Placebo (n=2226) | | | |
|---|---|---|---|---|
| | Lowest tertile n=742* | Middle tertile n=742† | Highest tertile n=742‡ | p-value |
| **Continuous variables (mean ± SD)** | | | | |
| Age (years) | 75.3 ± 3.4 | 75.1 ± 3.2 | 74.9 ± 3.3 | 0.10 |
| Education (age left school, years) | 15.3 ± 2.3 | 15.0 ± 1.9 | 15.2 ± 2.0 | 0.15 |
| Alcohol intake (units/month) | 5.4 ± 8.7 | 5.1 ± 8.7 | 5.2 ± 9.6 | 0.79 |
| Body mass index (kg/m$^2$) | 26.9 ± 4.2 | 27.0 ± 4.4 | 27.1 ± 4.1 | 0.79 |
| Mean SBP (mmHg)** | 153.9 ± 16.0 | 153.4 ± 17.2 | 153.8 ± 16.1 | 0.82 |
| SBP variability (mmHg)** | 13.8 ± 5.0 | 14.2 ± 5.4 | 14.8 ± 14.8 | **0.003** |
| Mean LDL cholesterol (mmol/L)** | 3.5 ± 0.7 | 3.7 ± 0.7 | 3.9 ± 0.8 | **<0.001** |
| | | | | |
| **Categorical variables (n, %)** | | | | |
| Female | 350 (47.2) | 368 (49.6) | 420 (56.6) | **0.001** |
| History of hypertension | 462 (62.3) | 458 (61.7) | 464 (62.5) | 0.95 |
| History of diabetes mellitus | 88 (11.9) | 84 (11.3) | 70 (9.4) | 0.29 |
| History of stroke or TIA | 87 (11.7) | 82 (11.1) | 72 (9.7) | 0.44 |
| History of myocardial infarction | 101 (13.6) | 104 (14.0) | 91 (12.3) | 0.58 |
| History of vascular disease | 320 (43.1) | 301 (40.6) | 330 (44.5) | 0.30 |
| Current smoker | 190 (25.6) | 187 (25.2) | 189 (25.5) | 0.98 |
| | | | | |
| **Country of origin (n, %)** | | | | |
| Scotland | 296 (39.9) | 300 (40.4) | 301 (40.6) | **0.047** |
| Ireland | 261 (35.2) | 288 (38.8) | 301 (40.6) | |
| The Netherlands | 185 (24.9) | 154 (20.8) | 140 (18.9) | |

P-values calculated using analysis of variance and Pearson's chi-square test when appropriate. LDL-c denotes low-density lipoprotein cholesterol; SBP, systolic blood pressure; TIA, transient ischemic attack.
LDL-c variability ranges (mmol/L): *0.02-0.22, †0.22-0.35, ‡0.35-1.71, §0.00-0.18, ||0.18-0.30, #0.30-1.56; ** calculated over months 3 to 24, similar to LDL-c variability.

| Pravastatin (n=2202) | | | |
|---|---|---|---|
| Lowest tertile n=734§ | Middle tertile n=735‖ | Highest tertile n=733# | p-value |
| | | | |
| 75.4 ± 3.3 | 75.0 ± 3.4 | 75.1 ± 3.2 | 0.13 |
| 15.3 ± 2.2 | 15.3 ± 2.3 | 15.2 ± 2.1 | 0.76 |
| 4.8 ± 8.4 | 5.5 ± 8.4 | 5.9 ± 11.1 | 0.07 |
| 26.8 ± 1.8 | 26.8 ± 3.9 | 26.9 ± 4.0 | 0.89 |
| 153.0 ± 16.4 | 153.7 ± 17.4 | 153.7 ± 16.4 | 0.62 |
| 13.7 ± 5.3 | 14.0 ± 5.2 | 14.6 ± 5.5 | **0.006** |
| 2.3 ± 0.5 | 2.5 ± 0.6 | 2.8 ± 0.7 | **<0.001** |
| | | | |
| | | | |
| 364 (49.6) | 360 (49.0) | 419 (57.2) | **0.002** |
| 480 (65.4) | 450 (61.2) | 467 (63.7) | 0.25 |
| 83 (11.3) | 76 (10.3) | 58 (7.9) | 0.08 |
| 86 (11.7) | 78 (10.6) | 72 (9.8) | 0.50 |
| 84 (11.4) | 101 (13.7) | 91 (12.4) | 0.41 |
| 310 (42.2) | 342 (46.5) | 323 (44.1) | 0.25 |
| 172 (23.4) | 170 (23.1) | 192 (26.2) | 0.32 |
| | | | |
| | | | |
| 287 (39.1) | 296 (40.3) | 311 (42.4) | **0.014** |
| 259 (35.3) | 278 (37.8) | 290 (39.6) | |
| 188 (25.6) | 161 (21.9) | 132 (18.0) | |

**Table 2.** Cognitive function, at month thirty, over tertiles of LDL-c variability

| | | Lowest tertile | Middle tertile |
|---|---|---|---|
| **Placebo** (n=2226) | | | |
| Stroop card III, seconds needed | Model 1 | 62.25 (0.91) | 65.16 (0.93) |
| | Model 2 | 65.06 (1.21) | 68.00 (1.23) |
| LDT, digits coded correctly | Model 1 | 23.67 (0.24) | 22.93 (0.24) |
| | Model 2 | 23.05 (0.32) | 22.29 (0.32) |
| PLTi, pictures remembered | Model 1 | 9.72 (0.07) | 9.51 (0.07) |
| | Model 2 | 9.60 (0.10) | 9.38 (0.10) |
| PLTd, pictures remembered | Model 1 | 10.56 (0.10) | 10.24 (0.10) |
| | Model 2 | 10.36 (0.13) | 10.03 (0.13) |
| **Pravastatin** (n=2202) | | | |
| Stroop card III, seconds needed | Model 1 | 62.39 (0.90) | 61.77 (0.88) |
| | Model 2 | 65.17 (1.21) | 64.70 (1.20) |
| LDT, digits coded correctly | Model 1 | 23.35 (0.25) | 23.80 (0.25) |
| | Model 2 | 22.41 (0.34) | 22.81 (0.34) |
| PLTi, pictures remembered | Model 1 | 9.66 (0.07) | 9.65 (0.07) |
| | Model 2 | 9.38 (0.10) | 9.36 (0.09) |
| PLTd, pictures remembered | Model 1 | 10.54 (0.10) | 10.42 (0.10) |
| | Model 2 | 10.14 (0.14) | 10.00 (0.14) |

Data are presented as mean cognitive test scores (SE). The adjusted unstandardized regression coefficient and p-value for trend were calculated using LDL-c variability (mmol/L) as a continuous measure.
LDT denotes Letter-Digit Coding test; PLTi, 15-Picture Learning test immediate; PLTd, 15-Picture Learning test delayed.
Model 1: adjusted for age, gender, country, education, mean LDL cholesterol, and test version where appropriate.
Model 2: as model 1, additionally for BMI, smoking status, alcohol intake, history of diabetes mellitus, hypertension, and vascular disease.

| Highest tertile | Beta (95% CI) | p-value |
|---|---|---|
| | | |
| 65.12 (0.94) | 6.24 (0.92, 11.56) | **0.021** |
| 68.00 (1.23) | 6.44 (1.13, 11.75) | **0.017** |
| 22.99 (0.25) | -0.92 (-2.32, 0.48) | 0.196 |
| 22.39 (0.32) | -0.91 (-2.30, 0.49) | 0.204 |
| 9.48 (0.07) | -0.68 (-1.09, -0.27) | **0.001** |
| 9.36 (0.10) | -0.66 (-1.07, -0.25) | **0.002** |
| 10.16 (0.10) | -1.02 (-1.60, -0.44) | **0.001** |
| 9.97 (0.13) | -1.00 (-1.58, -0.42) | **0.001** |
| | | |
| 64.66 (0.94) | 3.94 (-0.88, 8.75) | 0.109 |
| 67.54 (1.23) | 3.89 (-0.92. 8.70) | 0.113 |
| 22.69 (0.27) | -1.51 (-2.86, -0.15) | **0.030** |
| 21.72 (0.34) | -1.51 (-2.86, -0.15) | **0.029** |
| 9.37 (0.08) | -0.56 (-0.95, -0.16) | **0.006** |
| 9.08 (0.10) | -0.55 (-0.94, -0.15) | **0.006** |
| 9.87 (0.11) | -1.22 (-1.78, -0.66) | **<0.001** |
| 9.46 (0.14) | -1.20 (-1.76, -0.64) | **<0.001** |

(immediate recall: p=0.002, p=0.006; delayed recall: p=0.001, p=<0.001), statistically significant associations were also seen for Stroop (p=0.017, p=0.11) and LDT (p=0.20, p=0.029) test scores. These fully adjusted associations were essentially unchanged from those seen for the minimally adjusted model. We found no evidence for interaction between LDL-c variability and pravastatin treatment, for all cognitive outcomes (**Supplemental table 2**).

## Sensitivity analyses for cognitive outcomes

As shown in **Figure 1,** the associations between LDL-c variability and cognitive performance were essentially unchanged by restricting the analyses to different subsets, c.q. adjusting for various possible common causes of LDL-c variability and cognitive performance, in both trial arms.

## Association between LDL-c variability and MRI measures

We found no evidence for an association between LDL-c variability and hippocampal volume (p=0.779, p=0.864, respectively). However, higher LDL-c variability was associated with lower total cerebral blood blow in the fully adjusted model (**Table 3**), in both placebo and pravastatin group **(**p=0.031, p=0.050, respectively). Furthermore, higher LDL-c variability was associated with greater white matter hyperintensity load in the pravastatin group (p=0.046), but this association did not reach statistical significance in the placebo group (p=0.184). Additionally, no interaction was observed between LDL-c variability and pravastatin treatment, for all MRI measures (**Supplemental table 3**). Further adjustments for whole-brain, or grey-matter specific, atrophy did not markedly change any of the results (data not shown).

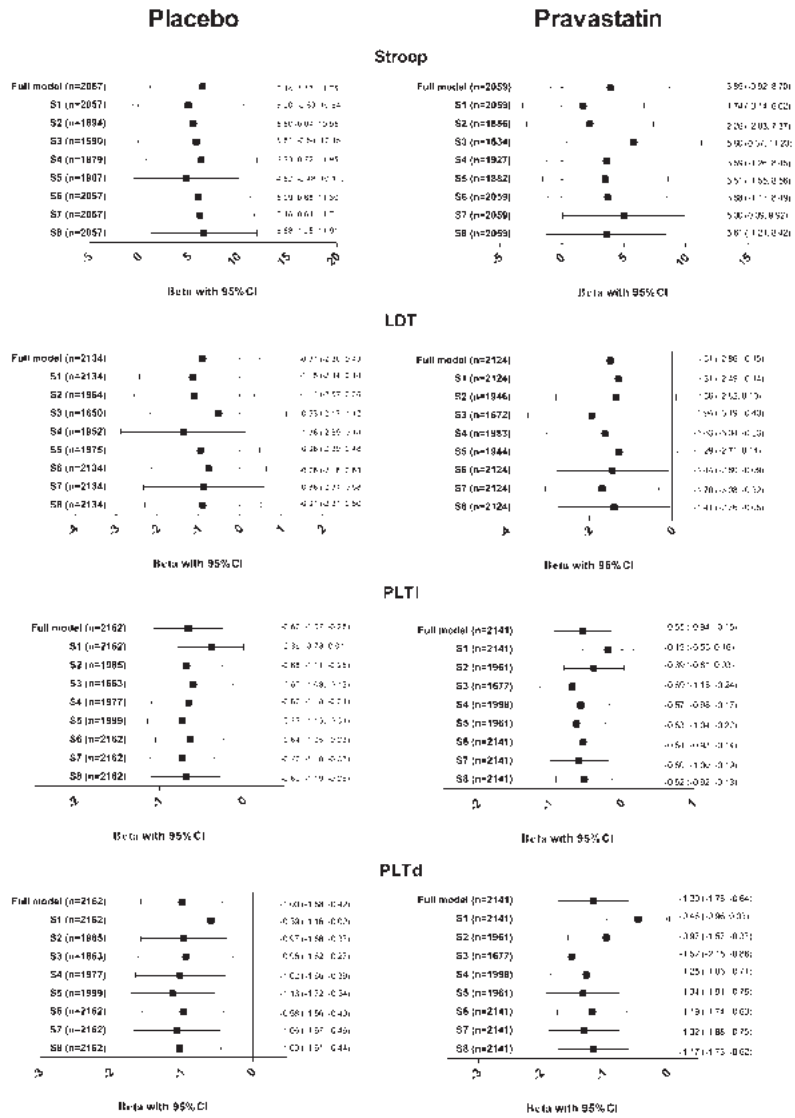


**Figure 1.** Sensitivity analyses of the association between LDL-c variability and cognitive performance. Consecutively listed, these are: (S1) assessing cognition at end of study, (S2) only subjects with four (complete) LDL-c measurements, (S3) excluding history of cerebro- and cardiovascular disease, (S4) excluding incident cerebro- and cardiovascular disease, (S5) excluding incident serious infection and cancer, (S6) adjusting for visit-to-visit systolic BP variability, (S7) adjusting for mean LDL-c slope during measurement period, (S8) adjusting for concomitant baseline medication usage. Results are presented as adjusted unstandardized regression coefficients with 95% confidence intervals. LDT denotes Letter-Digit Coding test; PLTi, 15-Picture Learning test immediate; PLTd, 15-Picture Learning test delayed.

**Table 3.** MRI measures, at end of study, over tertiles of LDL-c variability

| | | Lowest tertile | Middle tertile |
|---|---|---|---|
| **Placebo** (n=269) | | n=89* | n=90† |
| Hippocampal volume (ml) | Model 1 | 9.21 (0.13) | 9.26 (0.13) |
| | Model 2 | 9.14 (0.15) | 9.08 (0.16) |
| Cerebral blood flow (ml/min/100 ml) | Model 1 | 48.09 (1.10) | 48.43 (1.14) |
| | Model 2 | 46.95 (1.33) | 47.21 (1.44) |
| WMHL (ml) | Model 1 | 7.79 (1.35) | 7.06 (1.34) |
| | Model 2 | 7.76 (1.62) | 7.10 (1.71) |
| **Pravastatin** (n=266) | | n=88§ | n=89‖ |
| Hippocampal volume (ml) | Model 1 | 9.31 (0.13) | 9.34 (0.12) |
| | Model 2 | 9.17 (0.17) | 9.18 (0.16) |
| Cerebral blood flow (ml/min/100 ml) | Model 1 | 48.19 (1.05) | 49.17 (1.02) |
| | Model 2 | 48.80 (1.38) | 49.89 (1.46) |
| WMHL (ml) | Model 1 | 5.21 (1.30) | 7.54 (1.22) |
| | Model 2 | 4.50 (1.69) | 6.88 (1.71) |

Data are presented as mean MRI measure (SE). The adjusted unstandardized regression coefficient and p-value for trend were calculated using LDL-c variability (mmol/L) as a continuous measure.
WMHL denotes white matter hyperintensity load.
Model 1: adjusted for age, gender, education, mean LDL cholesterol, and whole-brain parenchymal volume.
Model 2: as model 1, additionally for BMI, smoking status, alcohol intake, history of diabetes mellitus, hypertension, and vascular disease.
LDL-c variability ranges (mmol/L): *0.05-0.20, †0.20-0.32, ‡0.32-1.18, §0.03-0.16, ‖0.16-0.25, #0.25-1.52

| Highest tertile | Beta (95% CI) | p-value |
|---|---|---|
| n=90‡ | | |
| 9.15 (0.13) | 0.19 (-0.57, 0.95) | 0.622 |
| 9.07 (0.16) | 0.11 (-0.66, 0.88) | 0.779 |
| 45.30 (1.14) | -6.39 (-13.13, 0.34) | 0.063 |
| 44.12 (1.40) | -7.66 (-14.61, -0.70) | **0.031** |
| 8.56 (1.33) | 4.74 (-3.33, 12.80) | 0.249 |
| 8.75 (1.66) | 5.69 (-2.72, 14.09) | 0.184 |
| n=89# | | |
| 9.36 (0.11) | -0.19 (-0.83, 0.45) | 0.557 |
| 9.27 (0.15) | -0.06 (-0.72, 0.61) | 0.864 |
| 46.93 (1.09) | -6.17 (-12.78, 0.44) | 0.067 |
| 47.33 (1.40) | -6.82 (-13.63, -0.01) | **0.050** |
| 8.47 (1.26) | 5.62 (-1.50, 12.75) | 0.121 |
| 8.43 (1.62) | 7.42 (0.15, 14.69) | **0.046** |

### Multiple linear regression assumptions

We found no evidence of non-normality, curvilinearity, heteroscedasticity, or multicollinearity in any of our models. This held true for all cognitive tests and magnetic-resonance imaging outcomes.

## DISCUSSION

We found that higher visit-to-visit variability in LDL-c is robustly associated with lower cognitive performance, independent of mean LDL-c levels. While most consistent for both immediate and delayed memory-related outcomes, similar trends were present for selective attention and processing speed. In addition, we observed that higher variability is associated with lower cerebral blood flow and greater white matter hyperintensity load within an MRI substudy. All associations were independent of clinically overt cerebro- and cardiovascular disease and comorbidities. Of particular importance is that these associations were present within both placebo and pravastatin treatment arm, with no evidence for interaction by pravastatin treatment. This advocates against increased LDL-c variability purely reflecting the known beneficial and harmful pleiotropic effects of statins, or behavioral factors which may undermine response to lipid lowering treatment, most notably non-adherence. Nonetheless, our findings that higher LDL-c variability associates with lower neurocognitive function highlight the need for further investigations into the potential influence of lipid-lowering treatment on LDL-c variability and consequent adverse events. While it should be noted that these events are uncommon, and the adverse event reporting not part of a systematic evaluation of neurocognitive function, currently available trial evidence suggests that neurocognitive adverse events may occur more frequently in individuals receiving proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors, independent of on-treatment LDL levels (15). At the same time, high-dose monthly regimens of PCSK9 monoclonal antibodies are known to produce substantial fluctuations of LDL-c between doses (16). Based on our results, this increased variability could possibly contribute to the observed higher rate of neurocognitive events, and should be examined by currently ongoing PCSK9 trials. To our knowledge, this is the first study examining the association between lipid variability and cognitive performance, and provides further evidence that lipid variability could be of clinical significance. The implications of our findings are thus in line with those from the recently published results from the Treating to New Targets trial (9), but extending these to cognitive and neuroimaging outcomes.

Major strengths of the current study are its size, with over 4400 participants providing data on lipid variability and cognitive performance, and the opportunity to perform these analyses both in the presence, and absence, of lipid-lowering therapy. Moreover, due to the exclusion of participants with MMSE scores lower than 24 we were able to examine a fairly homogenous population with regard to cognitive function. A limitation of the current study is the observational nature of the data, due to which we are unable to infer causal relationships. Furthermore, our ability to look at cognitive performance at different time points and perform longitudinal analyses was limited by the number of, and varying time intervals between, post-baseline LDL-c measurements. In addition, we included a limited neurocognitive test battery, which did not provide information on various important cognitive domains such as visual-constructive function or language. A further possible limitation could be that we did not adjust for multiple testing. However, we did not consider our analyses to be hypothesis-free, as we included neurocognitive tests specifically known to be affected by neurovascular impairment, which are additionally known to be correlated. Applying multiple comparison methods like Bonferroni in this case would therefore yield too conservative results. Finally, though lipid levels were measured after an overnight fast, we did not have data on the exact nature and timing of last consumed meal. While this might have influenced our results, it is very likely that any potential dietary effect would be random in nature.



There are several explanations for our findings, which roughly fall within two categories. First, it is possible that LDL-c variability is causally related to cognition function. Histological studies have demonstrated that lipid-lowering treatments such as statins may lower the lipid content of human carotid plaques (17), with recent animal studies suggesting that complete atherosclerotic regression of early lesions is possible through the lowering of lipid levels (18). As such, varying levels of LDL-c could theoretically lead to fluctuations in the composition of atherosclerotic plaques, possibly inducing plaque instability and thereby increasing the risk of (sub)clinical cerebrovascular damage (19). Another pathway might be through endothelial dysfunction, which can be caused by many of the risk factors that predispose to atherosclerosis (20). As individuals with elevated serum markers of endothelial dysfunction are at higher risk for developing cognitive impairment (21), possibly through changes in cerebral blood flow (22, 23), increased LDL-c variability might lead to cognitive impairment. In line with this hypothesis, we observed that higher LDL-c variability associated with lower cerebral blood flow, but also with greater white matter hyperintensity load, which has been linked to endothelial (dys)function (24).

Explanations within the second category dismiss a causal role for LDL-c variability. Here, visit-to-visit variability would rather reflect other processes leading to cognitive dysfunction. For example, despite excluding participants with a diagnosis of cancer or serious infection from the analyses in a sensitivity analysis, undetected subclinical disease might have led both to increased lipid variability and cognitive impairment. This also holds true for liver disease, though participants with clinically significant liver damage were explicitly excluded from enrolling in the trial. Exploratory analyses with inflammatory markers (fibrinogen, IL-6, IL-10, CRP) measured at baseline did not reveal evidence of an association with LDL-c variability (all p-values > 0.1, data not shown). Furthermore, numerous drugs may have unintended effects on lipid levels (25). While adjusting for baseline medication usage did not materially change our findings, exact timing of new drug commencement, although known to be few, was unfortunately not available within our study, and it was therefore not possible to take this into account. The observation that the associations were independent of blood pressure variability might imply that loss of homeostatic function does not underlie our current findings. However, more likely, it may signify that the different regulatory systems involved in homeostasis may be affected through different pathological pathways. Finally, due to the cross-sectional design of our analyses we cannot rule out that subclinical cerebrovascular damage, for which cognitive dysfunction may be a marker, leads to increased LDL-c variability.

In conclusion, we showed for the first time that in older participants at risk for vascular disease, higher visit-to-visit LDL-c variability is associated with lower cognitive performance, lower cerebral blood flow and greater white matter hyperintensity load. Our findings underscore the potential of LDL-c variability being a useful prognostic marker for different clinical outcomes. Future replication studies are needed to corroborate these findings, and should ideally also employ longitudinal assessments of neuroimaging to further elucidate the possible relationship between LDL-c variability, cerebral blood flow, and white matter hyperintensities.

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## REFERENCE LIST


1 Cannon WB. Organization for physiological homeostasis. Physiol Rev. 1929;9:399-431.

2 Tai C, Sun Y, Dai N, et al. Prognostic significance of visit-to-visit systolic blood pressure variability: a meta-analysis of 77,299 patients. J Clin Hypertens (Greenwich). 2015;17:107-15.

3 Rothwell PM, Howard SC, Dolan E, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. Lancet. 2010;375:895-905.

4 Sabayan B, Wijsman LW, Foster-Dingley JC, et al. Association of visit-to-visit variability in blood pressure with cognitive function in old age: prospective cohort study. BMJ. 2013;347:f4600.

5 Kim DH, Lipsitz LA, Ferrucci L, et al. Association between reduced heart rate variability and cognitive impairment in older disabled women in the community: Women's Health and Aging Study I. J Am Geriatr Soc. 2006;54:1751-7.

6 Tsuji H, Larson MG, Venditti FJ Jr, et al. Impact of Reduced Heart Rate Variability on Risk for Cardiac Events: The Framingham Heart Study. Circulation. 1996;94:2850-5.

7 Cooper GR, Myers GL, Smith SJ, et al. Blood lipid measurements. Variations and practical utility. JAMA. 1992;267:1652-60.

8 Smith SJ, Cooper GR, Myers GL, et al. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. Clin Chem. 1993;39:1012-22.

9 Bangalore S, Breazna A, DeMicco DA, et al. Visit-to-visit low-density lipoprotein cholesterol variability and risk of cardiovascular outcomes: insights from the TNT trial. J Am Coll Cardiol. 2015;65:1539-48.

10 Shepherd J, Blauw GJ, Murphy MB, et al. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. Am J Cardiol. 1999;84:1192-7.

11 Houx PJ, Shepherd J, Blauw GJ, et al. Testing cognitive function in elderly populations: the PROSPER study. PROspective Study of Pravastatin in the Elderly at Risk. J Neurol Neurosurg Psychiatry. 2002;73:385-9.

12 ten Dam VH, Box FM, de Craen AJ, et al. Lack of effect of pravastatin on cerebral blood flow or parenchymal volume loss in elderly at risk for vascular disease. Stroke. 2005;36:1633-6.

13 Patenaude B, Smith SM, Kennedy DN, et al. A Bayesian model of shape and appearance for subcortical brain segmentation. Neuroimage. 2011;56:907-22.

14 Admiraal-Behloul F, van den Heuvel DM, Olofsen H, et al. Fully automatic segmentation of white matter hyperintensities in MR images of the elderly. Neuroimage. 2005;28:607-17.

15 Swiger KJ, Martin SS. PCSK9 inhibitors and neurocognitive adverse events: exploring the FDA directive and a proposal for N-of-1 trials. Drug Saf. 2015;38:519-26.

16 Ballantyne CM, Neutel J, Cropp A, et al. Results of bococizumab, a monoclonal antibody against proprotein convertase subtilisin/kexin type 9, from a randomized,

placebo-controlled, dose-ranging study in statin-treated subjects with hypercholesterolemia. Am J Cardiol. 2015;115:1212-21.

17 Crisby M, Nordin-Fredriksson G, Shah PK, et al. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. Circulation. 2001;103:926-33.

18 Björkegren JL, Hägg S, Talukdar HA, et al. Plasma cholesterol-induced lesion networks activated before regression of early, mature, and advanced atherosclerosis. PLoS Genet. 2014;10:e1004201.

19 Chen Z, Ichetovkin M, Kurtz M, et al. Cholesterol in human atherosclerotic plaque is a marker for underlying disease state and plaque vulnerability. Lipids Health Dis. 2010;9:61.

20 Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109:III27-32.

21 Quinn TJ, Gallacher J, Deary IJ, et al. Association between circulating hemostatic measures and dementia or cognitive impairment: systematic review and meta-analyzes. J Thromb Haemost. 2011;9:1475-82.

22 de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol. 2004;3:184-90.

23 Sabayan B, Westendorp RG, van der Grond J, et al. Markers of endothelial dysfunction and cerebral blood flow in older adults. Neurobiol Aging. 2014;35:373-7.

24 Hoth KF, Tate DF, Poppas A, et al. Endothelial function and white matter hyperintensities in older adults with cardiovascular disease. Stroke. 2007;38:308-12.

25 Mantel-Teeuwisse AK, Kloosterman JME, Maitland-van der Zee AH, et al. Drug-Induced lipid changes - a review of the unintended effects of some commonly used drugs on serum lipid levels. Drug Saf. 2001;24:443-56.

**Supplemental table 1**. Characteristics of study participants included in the whole group, Dutch subsample, and magnetic resonance imaging (MRI) substudy.

| | Overall cohort (n=4428) | Dutch subsample (n=960) | MRI substudy (n=535) |
|---|---|---|---|
| **Continuous variables (mean ± SD)** | | | |
| Age (years) | 75.2 (3.3) | 75.1 (3.3) | 75.0 (3.2) |
| Education (age left school, years) | 15.2 (2.1) | 15.4 (2.9) | 15.5 (2.9) |
| Alcohol intake (units/month) | 5.3 (9.2) | 6.9 (8.3) | 6.7 (8.2) |
| Body mass index (kg/m$^2$) | 26.9 (4.1) | 26.8 (3.8) | 26.7 (3.7) |
| Mean SBP (mmHg)* | 153.6 (16.6) | 156.6 (16.8) | 156.6 (17.4) |
| SBP variability (mmHg)* | 14.2 (5.4) | 13.2 (5.2) | 13.2 (5.4) |
| Mean LDL cholesterol (mmol/L)* | 3.1 (0.9) | 3.2 (0.9) | 3.2 (0.9) |
| LDL-c variability (mmol/L)* | 0.31 (0.21) | 0.28 (0.20) | 0.28 (0.20) |
| Stroop card III, seconds needed † | 64.5 (26.1) | 55.2 (20.0) | 54.8 (20.0) |
| LDT, digits coded correctly † | 22.9 (7.8) | 26.7 (7.3) | 27.1 (7.1) |
| PLTi, pictures remembered † | 9.5 (2.0) | 10.2 (2.1) | 10.3 (2.0) |
| PLTd, pictures remembered † | 10.2 (2.9) | 11.3 (2.8) | 11.3 (2.8) |
| | | | |
| **Categorical variables (n, %)** | | | |
| Female | 2281 (51.5) | 461 (48.0) | 233 (43.6) |
| History of hypertension | 2781 (62.8) | 619 (64.5) | 339 (63.4) |
| History of diabetes mellitus | 459 (10.4) | 158 (16.5) | 88 (16.4) |
| History of stroke or TIA | 477 (10.8) | 158 (16.5) | 87 (16.3) |
| History of myocardial infarction | 572 (12.9) | 144 (15.0) | 64 (12.0) |
| History of vascular disease | 1926 (43.5) | 407 (42.4) | 234 (43.7) |
| Current smoker | 1100 (24.8) | 228 (23.8) | 113 (21.1) |

LDL-c denotes low-density lipoprotein cholesterol; SBP, systolic blood pressure; TIA, transient ischemic attack; LDT, Letter-Digit Coding test; PLTi, 15-Picture Learning test immediate; PLTd, 15-Picture Learning test delayed.
* calculated over months 3 to 24, † at month 30.

**Supplemental Table 2**. Cognitive function, at month thirty, over treatment-specific tertiles of LDL-c variability (n=4428)

| | Lowest tertile | Middle tertile | Highest tertile | Beta (95% CI) | $P_{trend}$ | $P_{interaction}$ |
|---|---|---|---|---|---|---|
| Stroop card III, seconds | 65.09 (0.85) | 66.34 (0.86) | 67.77 (0.86) | 5.10 (1.59, 8.62) | **0.004** | 0.504 |
| LDT, digits coded | 22.73 (0.23) | 22.56 (0.23) | 22.06 (0.23) | -1.26 (-2.22, -0.31) | **0.010** | 0.549 |
| PLTi, pictures remembered | 9.39 (0.07) | 9.38 (0.07) | 9.22 (0.07) | -0.63 (-0.91, -0.35) | **<0.001** | 0.730 |
| PLTd, pictures remembered | 10.26 (0.10) | 10.03 (0.10) | 9.72 (0.10) | -1.12 (-1.52, -0.73) | **<0.001** | 0.790 |

Data are presented as mean cognitive test scores (SE). The adjusted unstandardized regression coefficient, p-value for trend, and p-value for interaction between treatment and LDL-c variability were calculated using variability (mmol/L) as a continuous measure. LDT denotes Letter-Digit Coding test; PLTi, 15-Picture Learning test immediate; PLTd, 15-Picture Learning test delayed.

Adjusted for age, gender, country, education, mean LDL cholesterol, pravastatin use, test version where appropriate, BMI, smoking status, alcohol intake, history of diabetes mellitus, hypertension, and vascular disease.

**Supplemental Table 3**. MRI measures, at end of study, over treatment-specific tertiles of LDL-c variability (n=535)

| | Lowest tertile | Middle tertile | Highest tertile | Beta (95% CI) | $P_{trend}$ | $p_{interaction}$ |
|---|---|---|---|---|---|---|
| Hippocampal volume (ml) | 9.16 (0.11) | 9.14 (0.11) | 9.22 (0.11) | 0.11 (-0.38, 0.61) | 0.646 | 0.848 |
| Cerebral blood flow (ml/min/100 ml) | 47.70 (0.96) | 48.37 (1.01) | 45.60 (0.97) | -6.13 (-10.80,-1.47) | **0.010** | 0.746 |
| WMHL (ml) | 6.20 (1.15) | 7.02 (1.18) | 8.71 (1.13) | 6.64 (1.36, 11.93) | **0.014** | 0.840 |

Data are presented as mean MRI measure (SE). The adjusted unstandardized regression coefficient, p-value for trend, and p-value for interaction between treatment and LDL-c variability were calculated using variability (mmol/L) as a continuous measure. WMHL denotes white matter hyperintensity load. Adjusted for age, gender, education, mean LDL cholesterol, whole-brain parenchymal volume, pravastatin use, BMI, smoking status, alcohol intake, history of diabetes mellitus, hypertension, and vascular disease.