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Chapter 7

Homocysteine levels and treatment effect in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER)

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ABSTRACT

Objectives

To assess the effect of preventive pravastatin treatment on coronary heart disease (CHD) morbidity and mortality in older persons at risk for cardiovascular disease, stratified for plasma levels of homocysteine.

Design

A post-hoc subanalysis in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), started 1997, which is a double-blinded randomized placebo-controlled trial with a mean follow-up of 3.2 years.

Setting

Primary care setting in two of the three PROSPER study sites (Netherlands and Scotland).

Participants

Individuals (n=3522, aged 70-82 years, 1765 men) with a history of or risk factors for cardiovascular disease, were ranked in three groups depending on baseline homocysteine, sex and study site.

Intervention

40 mg pravastatin versus placebo.

Measurements

Fatal and nonfatal CHD and mortality.

Results

In the placebo group, participants with high homocysteine (n=588) had a 1.8 higher risk (95% CI 1.2–2.5, $p=0.001$) of fatal and nonfatal CHD compared to low homocysteine (n=597). The absolute risk reduction in fatal and nonfatal CHD with pravastatin treatment was 1.6% (95% CI -1.6–4.7) in the low homocysteine group vs. 6.7% (95% CI 2.7–10.7) in the high homocysteine group (difference 5.2%, 95% CI 0.11–10.3, $p=0.046$). Therefore, the number needed to treat (NNT) with pravastatin for 3.2 years for benefit related to fatal and nonfatal CHD events was 14.8 (95% CI 9.3–36.6) for high homocysteine compared to 64.5 (95% CI 21.4– ∞) for low homocysteine.

Conclusion

In older persons at risk for cardiovascular disease, those with high homocysteine are at highest risk for fatal and nonfatal CHD. With pravastatin treatment, this group has the highest absolute risk reduction and the lowest NNT to prevent fatal and nonfatal CHD.

INTRODUCTION

The aim of cardiovascular risk management is to reduce the incidence of cardiovascular events in a high-risk population. To select those with high cardiovascular risk, clinical cardiovascular risk scores, such as the Framingham Risk Score¹ and the Systematic Coronary Risk Evaluation (SCORE)², are used worldwide. However, their accuracy to predict risk on cardiovascular outcomes declines with advancing age.³⁻⁶ Because the prevalence and incidence of cardiovascular disease increases exponentially with age,⁷⁻⁹ it is suggested to offer preventive treatment to everyone over a specified age without measuring other risk factors.¹⁰ Others, however, emphasize the need for risk stratification in old age.^{11;12} Recently, the Leiden 85-plus Study (and others) showed that homocysteine is predictive of cardiovascular events in old age.¹³⁻¹⁸

Since, according to Wilson and Jungner, risk predictors are only clinically meaningful when effective preventive treatment is available,¹⁹ we need to establish which treatment possibilities exist (and are appropriate) for older persons with high homocysteine to lower their cardiovascular risk. Large randomized controlled trials (RCTs) and meta-analyses show that lowering plasma homocysteine by treatment with folate has no beneficial effect on the incidence of cardiovascular events.^{16;20;21} The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)²² has shown that pravastatin lowers the risk of coronary heart disease (CHD) in older people in general, but not the risk for fatal or non-fatal strokes. We questioned whether older persons with high homocysteine levels would benefit more from this conventional preventive treatment, also compared to those with lower levels. Therefore, we performed a post-hoc subanalysis in PROSPER (a large double-blinded randomized placebo-controlled trial) to assess the effect of pravastatin on CHD risk and mortality in older persons, stratified for plasma levels of homocysteine.

METHODS

Study design

The protocol of PROSPER has been published elsewhere.²³ Briefly, in 1997-1999 a total of 5804 individuals were enrolled in Scotland (n=2520), Ireland (n=2184) and the Netherlands (n=1100). Men and women aged 70-82 years were recruited, with either pre-existing vascular disease (coronary, cerebral, or peripheral) or an increased risk of such disease because of smoking, hypertension or diabetes. Their plasma total cholesterol was required to be 154-347 mg/dl (4.0-9.0 mmol/L) and their triglyceride concentrations ≤531 mg/dl (≤6.0 mmol/L). Individuals with congestive heart failure (New York Heart

Association functional class III and IV) or poor cognitive function (Mini-Mental State Examination (MMSE) score <24 points) were excluded. Participants were randomized either to a group receiving 40 mg pravastatin a day or to a control group receiving a placebo, and were followed (on average) for 3.2 years. Throughout the study, all study personnel was unaware of the allocated study medication status of the patients. The institutional ethics review boards of all centers approved the protocol, and all participants gave written informed consent.

Determination of homocysteine

After blood drawing, blood samples were kept at room temperature until they were processed in the laboratory to be stored in the biobank (-80°C). In 2010 homocysteine concentrations were measured in the biobank EDTA plasma samples, from samples taken at baseline (blood samples n=5757, missing n=47) and again at six months. Measurements were done in batches after reduction to the free form with a fluorescence polarization immunoassay on an IMx analyzer (Abbott, Abbott Park, IL, USA).

The median plasma level of homocysteine in The Netherlands (n=1100) was 14.1 µmol/L (IQR 11.8-17.0), in Scotland (n=2505) 17.9 µmol/L (IQR 15.3-21.8), and in Ireland (n=2152) 18.8 µmol/L (IQR 15.6-23.3). However, there were differences in standard operating procedures per study site, i.e. the Dutch and Scottish blood samples were processed within eight hours, whereas in Ireland this processing frequently exceeded eight hours. Statistical analysis showed that the variance in log homocysteine for Scotland and the Netherlands was comparable ($F=2.4$, $p=0.120$), but both showed a significant difference in variance compared with Ireland (Scotland vs. Ireland $F=5.4$, $p=0.020$ and the Netherlands vs. Ireland $F=11.2$, $p=0.001$). Since plasma homocysteine levels increase by 0.5-1.0 µmol/L per hour in blood at room temperature,²⁴⁻²⁶ differences in lag time could explain the differences in variance between Ireland and the other countries. Therefore, we decided to exclude participants from Ireland from this analysis.

Outcomes

The outcomes, described in the design of PROSPER,²³ were the incidence of fatal and nonfatal CHD (including definite or suspected CHD mortality and non-fatal myocardial infarction), non-fatal myocardial infarction (MI), CHD mortality, non-CHD mortality, and all-cause mortality. All CHD endpoints were assessed by the PROSPER Endpoints Committee, which was blinded for study medication and for plasma levels of homocysteine.

Data analysis

At baseline, participants were ranked in three equal groups (low, medium and high homocysteine) based on plasma homocysteine level, sex and study site. Per homocysteine

group, characteristics between placebo and treatment were compared using independent t-tests for continuous data and Pearson Chi-square tests ($df=1$) for categorical data.

Predictive value of homocysteine in the placebo group

The cumulative incidence rates of fatal and nonfatal CHD and all-cause mortality for the three homocysteine groups are presented in Kaplan-Meier curves and compared with the log rank test ($df=2$). Hazard ratios (HRs) and corresponding 95% confidence intervals (95% CI) for sex- and study site-specific tertiles of homocysteine were calculated for the endpoints using Cox proportional hazard models (reference low homocysteine), with adjustment for age. To further investigate the independent predictive value of homocysteine, we additionally adjusted for baseline history of cardiovascular disease, for baseline Framingham risk factors [smoking, diabetes, left ventricle hypertrophy, systolic blood pressure, total cholesterol, high density lipoprotein (HDL)] and for earlier published predictors in PROSPER [CRP, HDL and creatinine clearance (Cockcroft-Gault)].

Treatment effect comparing placebo and treatment group

The treatment effects of pravastatin vs. placebo per homocysteine group were calculated with two methods. *First*, per homocysteine group, the cumulative incidence rate for fatal and nonfatal CHD and all-cause mortality are presented for those on placebo and those on pravastatin with Kaplan-Meier curves and compared with the log rank test ($df=1$) and with Cox proportional hazard models. No adjustments were made. The presence of multiplicative interaction was formally tested by adding the interaction term (treatment x homocysteine group) to the Cox regression model. All analyses were on an intention-to-treat basis.

Second, the absolute risk reduction by treatment with pravastatin was calculated. Differences in absolute risk reductions between the homocysteine groups were tested with a z-test. Numbers needed to treat (NNT) to benefit were calculated over the mean follow-up of the trial (3.2 years) based on the difference in cumulative proportion of surviving in the placebo and pravastatin group.^{27,28} Because creatinine clearance seems to be associated with the level of homocysteine, we carried out a subgroup analysis for absolute risk reduction for fatal and nonfatal CHD and for all-cause mortality in people with creatinine clearance ≥ 30 ml/min.

Influence of treatment with pravastatin on homocysteine

To investigate whether pravastatin treatment influences the plasma levels of homocysteine, we measured homocysteine concentrations after six months treatment for 1832 participants (183 on placebo and 1649 on pravastatin). The effect of treatment of

pravastatin on plasma levels of homocysteine was tested after six months with linear regression analysis, adjusted for baseline homocysteine.

RESULTS

In total, 3620 PROSPER participants from the study sites in The Netherlands and Scotland were eligible for this study. Since 15 participants had missing biobank samples and 83 had missing homocysteine measurements, 3522 participants (1764 placebo and 1758 pravastatin) were included in the analyses. The cut-off values of the homocysteine tertiles (33% and 67%) in the Netherlands ($n=1049$) were 11.7 and 14.7 $\mu\text{mol/L}$, respectively, for women, and 13.5 and 16.9 $\mu\text{mol/L}$, respectively, for men; for Scotland ($n=2473$) these limits were 15.4 and 19.6 $\mu\text{mol/L}$, respectively, for women, and 16.9 and 21.0 $\mu\text{mol/L}$, respectively, for men.

Baseline characteristics

Table 1 presents baseline characteristics of the total group of participants and per homocysteine group, stratified for placebo or pravastatin. In the total group, mean age of the participants was 75 (SD 3.4) years and 48% had a history of cardiovascular disease. The mean homocysteine level at baseline was 18.3 (SD 7.1) $\mu\text{mol/L}$. Per homocysteine group, there were no differences in baseline characteristics between the pravastatin and placebo groups. Remarkably, the proportion of diabetic patients was lower in the high homocysteine group.

Predictive value of homocysteine in the placebo group

Figure 1 shows the cumulative incidence of fatal and nonfatal CHD and all-cause mortality for the three homocysteine groups within the placebo group. Compared to participants with low homocysteine, those with medium homocysteine had no increased risk of fatal and nonfatal CHD (HR 1.1, 95% CI 0.76–1.6, $p=0.569$), but those with high homocysteine had a 1.8 fold increased risk (95% CI 1.2–2.5, $p=0.001$). For overall mortality, the HRs were 1.0 (95% CI 0.67–1.5, $p=0.992$) and 1.7 (95% CI 1.2–2.5, $p=0.003$), respectively. These estimates did not change by additional adjustments for history of cardiovascular disease, for Framingham Risk Factors, and for CRP, HDL and creatinine clearance (data not shown).

Similarly, participants with high homocysteine also had an increased risk for non-fatal MI, CHD mortality and non-CHD mortality. Furthermore, for all these outcomes no differences in risk were found between the medium and low homocysteine groups (data not shown). t

Table 1. Baseline characteristics of the participants stratified for treatment per homocysteine group (n=3522).

	All n=3522	Homocysteine group					
		Low		Medium		High	
		Placebo n=597	Pravastatin n=575	Placebo n=579	Pravastatin n=598	Placebo n=588	Pravastatin n=585
Demographic and functional characteristics							
Study site Scotland	2473 (70)	424 (71)	400 (70)	400 (69)	425 (71)	416 (71)	408 (70)
Men	1765 (50)	296 (50)	291 (51)	286 (49)	304 (51)	285 (49)	303 (52)
Age (years)	75 (3.4)	75 (3.4)	75 (3.3)	75 (3.2)	75 (3.3)	76 (3.5)	76 (3.4)
Mini-Mental State Examination (points)	28 (1.5)	28 (1.4)	28 (1.4)	28 (1.4)	28 (1.5)	28 (1.5)	28 (1.6)
Barthel index (points)	20 (0.7)	20 (0.7)	20 (0.8)	20 (0.7)	20 (0.5)	20 (0.8)	20 (0.9)
Instrumental activities of daily living (points)	14 (0.9)	14 (0.9)	14 (0.9)	14 (0.9)	14 (0.7)	14 (1.1)	14 (1.0)
Clinical history and cardiovascular risk factors							
History of cardiovascular disease*	1675 (48)	267 (45)	272 (47)	269 (47)	279 (47)	298 (51)	290 (50)
History of diabetes mellitus	386 (11)	88 (15)	85 (15)	68 (12)	57 (9.5)	45 (7.7)	43 (7.4)
Creatinine clearance <30 ml/min†	96 (2.7)	12 (2.0)	15 (2.6)	14 (2.4)	16 (2.7)	17 (2.9)	22 (3.8)
Body mass index (kg/m²)	27.0 (5.5)	27 (5.6)	27 (5.5)	27 (5.7)	27 (5.3)	27 (5.6)	27 (5.4)
Current smoker	943 (27)	157 (26)	137 (24)	156 (27)	166 (28)	167 (28)	160 (27)
Alcohol (units per week) ‡	5.3 (8.4)	4.9 (7.3)	5.0 (7.5)	5.5 (8.7)	5.6 (9.5)	4.9 (7.7)	5.7 (9.4)
Systolic blood pressure (mmHg)	155 (21)	153 (21)	154 (21)	156 (21)	154 (21)	155 (23)	155 (22)
Total cholesterol (mg/dl)	221 (35)	218 (35)	220 (36)	221 (34)	222 (35)	220 (36)	224 (37)
LDL cholesterol (mg/dl)	148 (31)	147 (31)	148 (30)	149 (30)	149 (31)	148 (32)	151 (32)
HDL cholesterol (mg/dl)	49 (13)	50 (13)	49 (13)	49 (13)	49 (13)	49 (15)	49 (14)
Triglycerides (mg/dl)	137 (61)	131 (59)	138 (63)	136 (59)	140 (66)	136 (61)	137 (58)
Homocysteine (µmol/L)	18.3 (7.1)	13.0 (2.1)	13.1 (2.1)	16.8 (2.2)	17.0 (2.2)	25.2 (8.6)	24.6 (8.2)

n (%) or mean (SD)

*Any of stable angina, intermittent claudication, stroke, transient ischaemic attack, myocardial infarction, peripheral arterial disease surgery, or amputation for vascular disease ≥6 months before study entry.

†Calculated with the Cockcroft-Gault formula; ‡1 unit = 60 ml distilled spirits, 170 ml wine or 300 ml beer

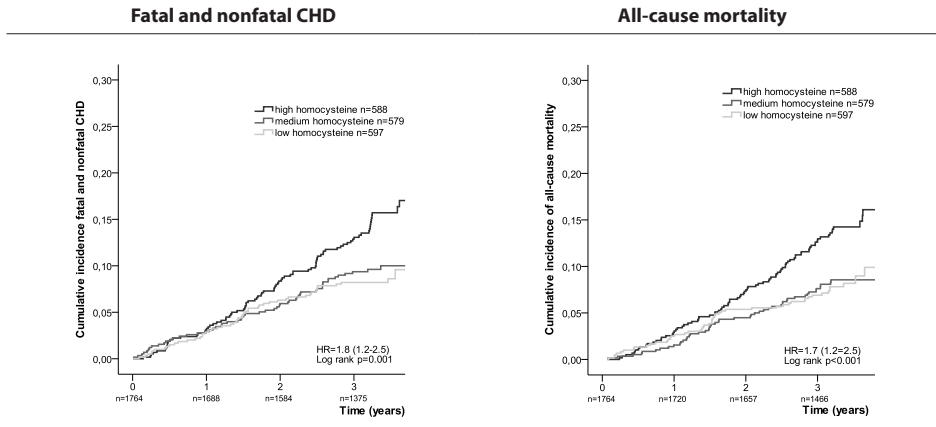


Figure 1. Cumulative incidence of fatal and nonfatal CHD and all-cause mortality depending on baseline plasma levels of homocysteine in the placebo group (n=1764).

HR=Hazard ratio (95% CI) high homocysteine group vs. low homocysteine group, adjusted for age.

Treatment effect depending on homocysteine

Figure 2 presents the cumulative incidence of fatal and nonfatal CHD and all-cause mortality in participants with and without pravastatin per homocysteine group. In participants with high homocysteine, an HR of 0.57 (95% CI 0.41–0.81, $p=0.002$) was found as treatment effect of pravastatin on fatal and nonfatal CHD, and an HR of 0.70 (95% CI 0.50–0.98, $p=0.036$) as treatment effect on all-cause mortality. In medium and low homocysteine, there was no significant difference in cumulative incidence between placebo and pravastatin treatment. Formal testing of multiplicative statistical interaction was not significant (Figure 2). Similar patterns were seen for non-fatal MI and CHD mortality. For non-CHD mortality, we found no effect of treatment with pravastatin in all three homocysteine groups (data not shown).

Table 2 presents absolute treatment effects of pravastatin per homocysteine group. The absolute risk reduction in fatal and nonfatal CHD by pravastatin treatment was 1.6% (95% CI -1.6–4.7) in the low homocysteine group, and 6.7% (95% CI 2.7–10.7) in the high homocysteine group (absolute risk reduction difference 5.2%, 95% CI 0.11–10.3, $p=0.046$). For all-cause mortality the absolute risk reductions were -0.66% (95% CI -4.0–2.7) and 4.6% (95% CI 0.78–8.4), respectively (absolute risk reduction difference 5.2%, 95% CI 0.19–10.3, $p=0.042$).

In persons with creatinine clearance ≥ 30 ml/min ($n=3426$) we found a mean homocysteine value of 18.3 $\mu\text{mol/L}$ and in persons with creatinine clearance <30 ml/min ($n=96$) a mean homocysteine value of 19.0 $\mu\text{mol/L}$ ($p=0.292$). Because creatinine clearance is known to be associated with the level of homocysteine we carried out a subgroup

analysis in persons with creatinine clearance ≥ 30 ml/min. The absolute risk reductions by pravastatin treatment remained similar (fatal and nonfatal CHD 6.0% (95% CI 0.84–11.1, $p=0.023$) and all-cause mortality 5.8% (95% CI 0.70–11.0, $p=0.026$)).

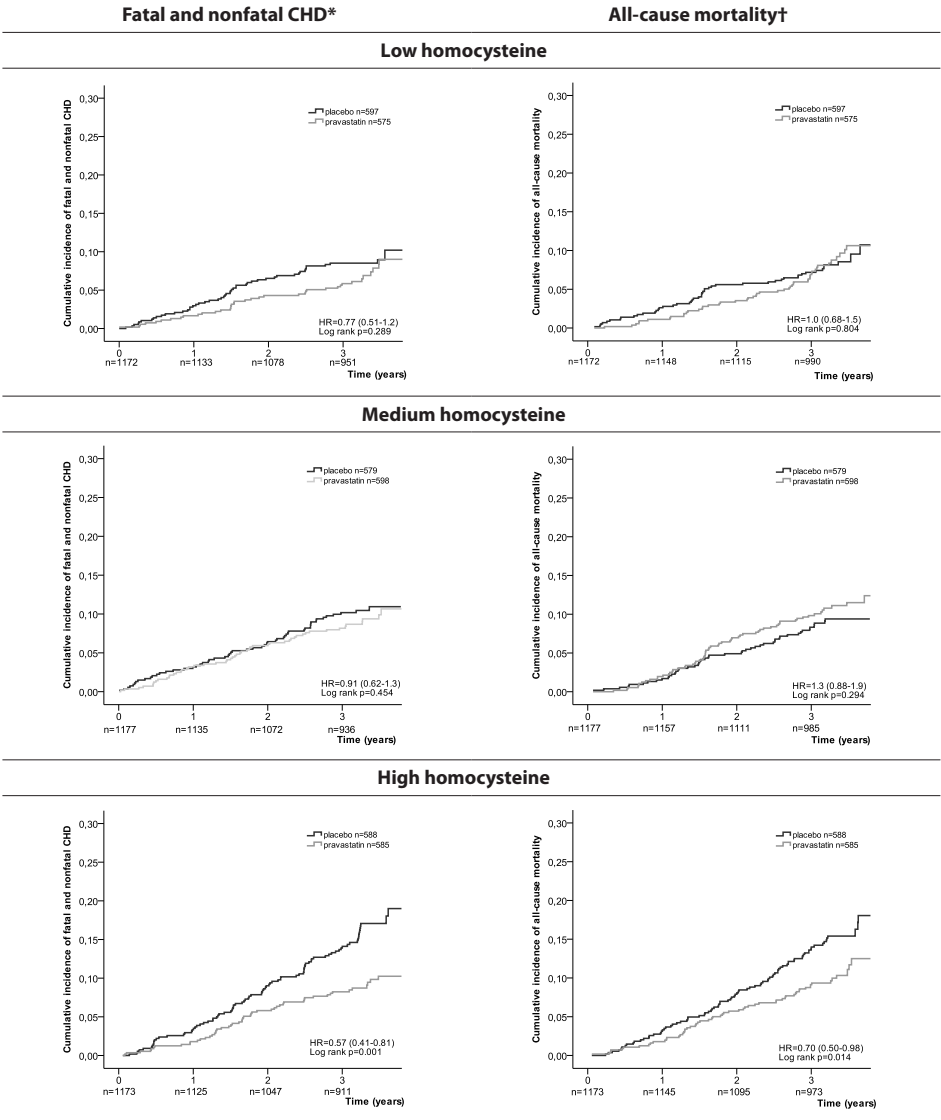


Figure 2. Cumulative incidence fatal and nonfatal CHD and all-cause mortality depending on pravastatin treatment, stratified for plasma levels of homocysteine at baseline.

* p for multiplicative interaction = 0.208

† p for multiplicative interaction = 0.097

Table 2. Absolute effect of treatment with pravastatin on cardiovascular outcomes and mortality after 3.2 years per homocysteine group.

Outcomes	Homocysteine groups*	n	% events (95% CI)	n	% events (95% CI)	Pravastatin	ARR (95% CI)	Diff ARR (95% CI)	p
Fatal and nonfatal CHD	Low	48	8.2 (6.0–10.5)	32	6.7 (4.5–8.9)		1.6 (-1.6–4.7)		
	Medium	54	9.9 (7.4–12.4)	47	8.7 (6.3–11.0)		1.2 (-2.2–4.7)	-0.32 (-5.0–4.3)	0.889
	High	76	15.9 (12.8–19.1)	47	9.2 (6.7–11.7)		6.7 (2.7–10.7)	5.2 (0.11–10.3)	0.046
Non-fatal MI	Low	29	5.1 (3.3–6.9)	26	5.6 (3.6–7.7)		-0.5 (-3.2–2.2)		
	Medium	39	7.3 (5.1–9.5)	26	5.1 (3.2–7.0)		2.2 (-0.73–5.0)	2.7 (-1.3–6.7)	0.184
	High	51	11.3 (8.5–14.1)	32	6.4 (4.3–8.5)		4.9 (1.4–8.4)	5.5 (1.0–9.9)	0.016
CHD mortality	Low	20	3.4 (1.9–4.9)	11	2.0 (0.83–3.2)		1.4 (-0.45–3.3)		
	Medium	18	3.3 (1.8–4.7)	27	4.7 (3.0–6.4)		-1.5 (-3.7–0.83)	-2.9 (-5.8–0.08)	0.057
	High	33	6.5 (4.4–8.6)	18	3.5 (1.9–5.1)		3.0 (0.35–5.6)	1.5 (-1.7–4.8)	0.347
Non-CHD mortality	Low	25	4.8 (3.0–6.6)	31	6.9 (4.6–9.1)		-2.1 (-4.9–0.84)		
	Medium	30	5.4 (3.5–7.3)	32	5.9 (3.9–7.8)		-0.46 (-3.2–2.3)	1.6 (-2.4–5.6)	0.430
	High	46	8.4 (6.1–10.7)	34	6.5 (4.4–8.5)		2.0 (-1.2–5.1)	4.0 (-0.24–8.2)	0.064
All-cause mortality	Low	45	8.0 (5.8–10.3)	42	8.7 (6.3–11.1)		-0.66 (-4.0–2.7)		
	Medium	48	8.5 (6.2–10.8)	59	10.3 (7.8–12.8)		-1.8 (-5.2–1.6)	-1.1 (-5.9–3.6)	0.638
	High	79	14.3 (11.4–17.2)	52	9.8 (7.3–12.2)		4.6 (0.78–8.4)	5.2 (0.19–10.3)	0.042

*Group sizes: low: placebo n=597, pravastatin n=575; medium: placebo n=579, pravastatin n=598; high: placebo n=588, pravastatin n=585

% events (95% CI) = Cumulative incidence of events after 3.2 years with corresponding 95% confidence intervals

ARR (95% CI) = Absolute risk reduction in % with corresponding 95% confidence intervals

Diff ARR (95% CI) = Difference in absolute risk reduction in % with corresponding 95% confidence intervals compared to reference group low homocysteine

p-value of difference in absolute risk reduction compared to reference group low homocysteine estimated by z-test

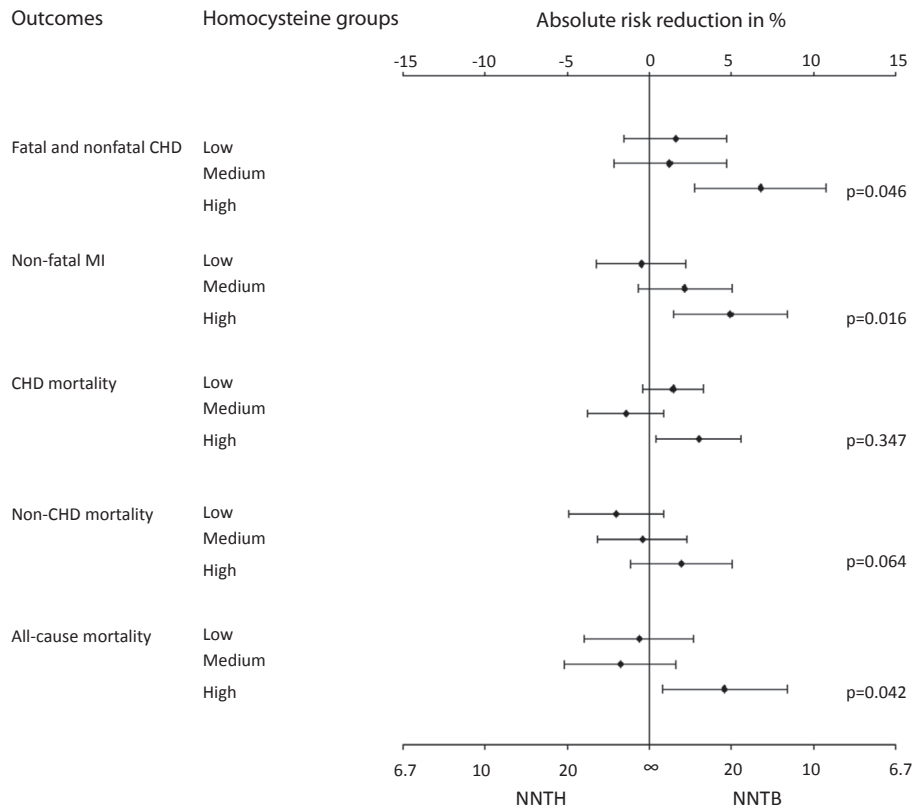


Figure 3. Number needed to treat with pravastatin after 3.2 years dependent on homocysteine level at baseline.

NNTH= Number needed to treat to harm

NNTB= Number needed to treat to benefit

CHD=coronary heart disease; MI=myocardial infarction

p-value of difference between high and low homocysteine group for absolute risk reduction in % and for number needed to treat estimated by z-test

For fatal and nonfatal CHD the NNT with pravastatin to benefit for 3.2 years is 14.8 (95% CI 9.3–36.6) in the high homocysteine group, 81.3 (95% CI 21.5–∞) in the medium group, and 64.5 (95% CI 21.4–∞) in the low homocysteine group (p high vs. low=0.046) (Figure 3). For all-cause mortality we found a beneficial result in the high homocysteine group (NNT 21.8, 95% CI 11.9–129), but no benefit in the medium and low homocysteine groups (p high vs. low=0.042).

Influence of pravastatin on homocysteine

After six months treatment, the difference in homocysteine levels was -0.52 $\mu\text{mol/L}$ (95% CI -1.1–0.07) for those on pravastatin compared to baseline (linear regression, p=0.082).

DISCUSSION

This study suggests that homocysteine may be a promising new CHD risk predictor in older people, since high plasma homocysteine not only selects older persons at high risk for fatal and nonfatal CHD and all-cause mortality, but also identifies those with the highest absolute risk reduction by pravastatin and lowest NNT to prevent fatal and nonfatal CHD.

Earlier studies showed that older persons with high levels of homocysteine are at increased risk for cardiovascular events and homocysteine level may provide additional risk stratification beyond traditional risk factors.¹³⁻¹⁸ For example, Veeranna et al. examined whether adding homocysteine to a model based on traditional cardiovascular disease risk factors improved classification. In two younger population cohorts, they found that addition of homocysteine level to the Framingham Risk Score significantly improved risk prediction.¹⁸ Moreover, for persons aged 85+ years, De Ruijter et al. showed that the classic risk factors as included in the Framingham Risk Score no longer accurately predicted cardiovascular mortality in those with no history of cardiovascular disease, while a single measurement of homocysteine did accurately identify those at high risk of cardiovascular mortality.¹³ Our findings not only confirm studies reporting that homocysteine predicts CHD risk in old age, but also show the independent predictive capacity in a selected population of older persons with increased cardiovascular risk. A recent meta-analysis showed that a moderate homocysteine elevation due to genetic variance does not meaningfully affect CHD risk.²⁹ This finding indicates that circulating homocysteine levels within the normal range are not causally related to CHD risk. Moreover, large RCTs and their meta-analyses show that lowering plasma homocysteine by treatment with folate has no beneficial effect on the incidence of cardiovascular events.^{16;20;21} Therefore, the underlying biological pathway to explain predictive value of high homocysteine for cardiovascular diseases, if there is one, to date is still unknown. Homocysteine may be seen as an epiphenomenon rather than a causal agent, but this does not refute its predictive abilities.

Since homocysteine showed to be not causally related to cardiovascular disease, it was unknown if preventive treatment could be offered to those with high homocysteine to reduce their cardiovascular risk. In the AFCAPS/TexCAPS study,³⁰ with only a small proportion of old individuals, the beneficial effect of statin treatment in people with elevated homocysteine levels was limited to people with an LDL level higher than 149 mg/dL. Our results further extend the findings from the AFCAPS/TexCAPS study by demonstrating that the benefits of statin treatment may differ by homocysteine levels among high-risk patients with an average LDL level of 148 mg/dL. If these findings hold

true in a subsequent study, there could be a role for measurement of homocysteine levels to help guide decisions on statin use in older individuals, which is a widely available treatment, also for older persons. This is an important criterion of Wilson and Jungner underlying screening.¹⁹

The present study revealed other findings that deserve further examination. First, although we only found a clear CHD risk benefit from pravastatin therapy over the trial period of 3.2 years in older persons with high homocysteine and not in older persons with low and medium homocysteine, we did not find multiplicative interaction for the treatment effect. Therefore, there is a possibility that pravastatin has the same treatment effect in the three homocysteine groups. However, even when the relative treatment effect is similar between these groups, those at highest absolute risk will have most benefit expressed in absolute risk reduction. This absolute risk reduction and corresponding NNT is very important in clinical practice and guidelines, since this indicates the number of persons who need to be treated in order to prevent one event.

Second, the effect of pravastatin over the homocysteine groups does not show a linear trend. This finding could be explained by a lack of power due to a small number of events in the low and medium homocysteine groups, therefore a possibility of random variation cannot be excluded. However, it is also possible that pravastatin therapy is only effective in people with homocysteine beyond a certain cut-off value. The possibility of absolute cut-off values requires more in-depth study, investigated in a population with consistent blood sampling and storage procedures.

Third, we found that plasma levels of homocysteine did not change significantly with pravastatin treatment during a six-month period, although a small reduction was seen. Further examination is needed to determine the effect of pravastatin treatment on homocysteine levels. If pravastatin does not affect the homocysteine level, homocysteine measurement might be useful to evaluate the need for continuing cardiovascular preventive therapy in persons under pravastatin treatment.

For new biomarkers, others have investigated whether high cardiovascular risk and corresponding benefit from treatment could be predicted. A large-scale RCT³¹ and an earlier analysis in PROSPER³² showed that baseline C-reactive protein concentration predicts cardiovascular risk, but does not predict the relative CHD risk benefits of pravastatin therapy. Other analyses in PROSPER showed that high-density lipoprotein³³ and creatinine clearance³⁴ can predict benefit for prevention of fatal and nonfatal CHD by pravastatin therapy. However, a high plasma level of homocysteine remained predictive for a beneficial effect of pravastatin even in persons with creatinine clearance ≥ 30 ml/

min. Furthermore, pravastatin was more effective in preventing cardiovascular events in those without diabetes.²² We showed that adjustment for these predictors did not influence the predictive value of homocysteine. A next step is to study the clinical value of homocysteine and other biomarkers by comparing their predictive value in combination with treatment response, to develop the most effective predictors of cardiovascular risk and treatment benefit. This is particularly important since statins are not without side-effects or costs, and targeting those at maximal risk and with most to gain would be both clinically and economically advantageous.

Strength and limitations

This study was embedded in the PROSPER trial, a large double-blinded randomized placebo-controlled trial in older persons. This landmark clinical cardiovascular trial with older participants was performed following guidelines of good clinical practice, including endpoints that were uniformly assessed by the Endpoint Committee. Since homocysteine was assessed after closure of the trial, plasma levels of homocysteine had no influence on the study procedures, clinical treatment during follow-up, or on the decisions of the Endpoint Committee.

A limitation of this study is that the PROSPER study procedures were not originally designed to collect optimal blood samples for the assessment of plasma homocysteine. Therefore, it was appropriate to use data from only two of the three PROSPER study sites. However, in these two sites, it is still possible that some samples were stored at room temperature until maximally eight hours before processing. Since this could lead to misclassification, that was assumed to be non-differential, this could have resulted in underestimation of the differences in treatment effect by homocysteine levels. Because it is also known that homocysteine levels vary between countries,^{13;35;36} more studies are needed to validate the absolute cut-off values of homocysteine to select elderly at highest risk in clinical practice. Moreover, data about the use of B vitamins, that lower the levels of homocysteine, are not available. Another limitation of this study is that the treatment-by-homocysteine group multiplicative interaction was not significant, although the absolute risk reduction by high versus low homocysteine was significant ($p=0.046$ for fatal and nonfatal CHD and $p=0.042$ for all-cause mortality). The possibility of a type 1 error from multiple comparisons cannot be excluded. It might also be seen as a limitation that these analyses focused only on the value of homocysteine to predict the CHD risk and treatment effect, rather than investigating the etiological mechanisms behind our findings. Predictive and etiological studies will contribute to the further development of cardiovascular risk management in old age, both on their own merits.

Implications

A recent analysis on cost-effectiveness of statin treatment in primary care showed that even in high age groups it is useful to stratify for risk of cardiovascular outcomes.¹² Our study shows that homocysteine may usefully predict CHD risk in the PROSPER population of old persons with increased cardiovascular risks. As a consequence, individuals without traditional risk factors and, thus, with the lowest risks were excluded. Before these results can be implemented in current guidelines, further research is needed to find a cutoff value of homocysteine and confirm that high-risk older adults with high homocysteine levels get more benefit from pravastatin treatment. Moreover, whether homocysteine is useful in predicting benefits from pravastatin treatment for low-risk or intermediate-risk older adults remains to be investigated.

In conclusion, homocysteine is a promising CHD risk predictor in old age, not only because high plasma homocysteine identifies older persons at high risk for fatal and nonfatal CHD, but also because those persons have the highest absolute risk reduction by pravastatin treatment and lowest number needed to treat to prevent fatal and non-fatal CHD. This is an important step in the further development of CHD risk stratification and treatment for older people.

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