The risk association of plasma total homocysteine with acute myocardial infarction is modified by serum vitamin A

Thomas Olsen, MSc[1], Kathrine J Vinknes, PhD[1], Gard FT Svingen, MD, PhD[2], Eva R Pedersen, MD, PhD[2,3], Indu Dhar[4] Grethe S Tell, PhD, MPH[5,6], Rune Blomhoff, PhD[1,7], Per M Ueland MD, PhD[8,9], Øivind Midttun PhD[9], Helga Refsum MD, PhD[1] and Ottar K Nygård, MD, PhD[2,3,4]

[1]Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway

[2]Department of Heart Disease, Haukeland University Hospital, Bergen Norway

[3]KG Jebsen Centre for Diabetes Research, University of Bergen, Norway

[4]Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway

[5]Department of Global Public Health and Primary Care, University of Bergen, Norway

[6]Division of Mental and Physical Health, Norwegian Institute of Public Health, Norway

[7]Department of Clinical Service, Division of Cancer Medicine, Oslo University Hospital, Norway

[8]Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway

[9]Bevital AS, Bergen, Norway

Corresponding author: Thomas Olsen, Institute of Basic Medical Sciences, Domus Medica, Sognsvannveien 9, 0372 Oslo, Norway, phone: +47 406 24 230, e-mail: thool@uio.no

Total word count including references: 5348

ABSTRACT

Background: Plasma total homocysteine (tHcy) has been implicated in the development of cardiovascular disease (CVD), but the mechanisms remain unclear. Vitamin A (Vit-A) is involved in homocysteine metabolism and we therefore explored the potential interaction between plasma tHcy and serum Vit-A in relation to incident acute myocardial infarction (AMI).

Methods: Cox proportional hazards models were used to assess the prospective relationships between tHcy and AMI in 2205 patients from Western Norway undergoing elective coronary angiography for suspected stable angina pectoris. Results are reported as hazard ratio per standard deviation (HR per SD) increase in log-transformed tHcy. An interaction term for tHcy × Vit-A was added to multivariate models including age, sex, smoking, apolipoprotein B, and estimated glomerular filtration rate.

Results: Geometric mean (geometric standard deviation) age of the participants (64.3% men) was 62.3 (1.24) y. Plasma tHcy was higher among participants in the upper vs. lower Vit-A tertile. During 7 (2.4) y of follow-up, 15.1% suffered an AMI. A significant association of plasma tHcy with AMI in the total study population was observed. When we stratified the population according to Vit-A tertiles, plasma tHcy was only associated with AMI only in the upper Vit-A tertile (HR per SD: 1.25, 95% CI: 1.04-1.53, $P_{\text{interaction}}$ = 0.03).

Conclusions: The risk relationship between plasma tHcy and AMI was modified by serum concentrations of Vit-A in patients with suspected stable angina pectoris. This finding may clarify the relationship between tHcy and CVD.

Abstract word count: 238

Key words: Homocysteine, vitamin A, cardiovascular disease

INTRODUCTION

The non-protein, sulfur-containing amino acid homocysteine is produced by intracellular transmethylation reactions where a methyl group is transferred from the universal methyl donor S-adenosylmethionine (SAM) to a methyl acceptor[1]. This reaction produces S-adenosylhomocysteine (SAH), which is readily hydrolyzed to adenosine and homocysteine in a reversible reaction. In the circulation, homocysteine undergoes disulphide exchange reactions forming symmetrical and mixed disulphides, which are collectively referred to as total homocysteine (tHcy)[2]. Elevated plasma concentrations of tHcy have been linked to increased risk of cardiovascular disease (CVD) in observational studies[3], findings that were recently suggested to depend on oxidative stress and inflammation[4]. However, tHcy-lowering treatment with B-vitamins did not improve prognosis in secondary prevention trials, and the potential underlying pathological mechanisms for the risk relationship of tHcy with CVD remain unclear[5].

Vitamin A (Vit-A) designates a class of fat-soluble compounds abundant in Western diets[6]. In target cells, retinol is a precursor for the major bioactive retinoid, retinoic acid (RA), which is a ligand for nuclear RA receptors. The role of Vit-A in CVD development is unclear[7], but we have shown that serum Vit-A modified the risk association between lipid parameters and acute myocardial infarction in the same core population as the current study[8]. Moreover, synthesis of *all-trans*-RA is increased in some inflammatory conditions[9] and is promoted by mediators of oxidative stress and inflammation such as oxysterols[10]. This potentially increased synthesis of *all-trans*-RA may have metabolic ramifications to homocysteine. Circulating tHcy has been positively associated with Vit-A in an observational study[11], and *all-trans*-RA is a potent activator of glycine *N*-methyltransferase (GNMT), which produces SAH and

homocysteine under conditions of excess intracellular SAM[12-15]. GNMT is not only involved in the regulation of intracellular homocysteine concentrations[16], but also lipid metabolism[17] and cellular immune activation[18].

Taken together, the association of plasma tHcy with risk of CVD may be modified by Vit-A through several biological mechanisms. However, evidence is limited, predominantly experimental and little is known about this relationship in humans. The aim of this observational study among more than 2000 patients with suspected stable angina pectoris, was to explore whether the relationship of plasma tHcy with risk of AMI was modified by serum concentrations of Vit-A.

SUBJECTS AND METHODS

A total of 4164 patients undergoing elective coronary angiography for suspected stable angina pectoris at Haukeland ($n = 3413$) and Stavanger ($n = 751$) University Hospitals, Norway, in the period 2000-2004 were enrolled in this study. In total, 61.8% ($n = 2573$) participated in the Western Norway B-Vitamin Intervention Trial (WENBIT) (clinicaltrials.gov: NCT00354081) and received either 1) folic acid, vitamin B_{12} and vitamin B_6, 2) folic acid and vitamin B_{12}, 3) vitamin B_6 or 4) placebo [19]. Subjects with missing data on plasma tHcy or serum Vit-A and those who received B-vitamin treatment ($n = 1959$) in the WENBIT were excluded yielding a total of 2205 subjects available for the present study. Written informed consent was obtained from all participants and the study protocol was in accordance with the principles of the Declaration of Helsinki, and approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Norwegian Data Inspectorate.

Baseline data and biochemical analyses

CVD history and prescription of medications at baseline were self-reported and verified by hospital records[19]. Patients with diabetes were classified by previous diagnosis (type 1 or 2), having fasting or non-fasting serum glucose ≥ 7.0 or ≥ 11.1 mmol/L, respectively, or having baseline glycated hemoglobin $> 6.5\%$. Definitions of smoking and severity of coronary artery disease as well as the calculation of glomerular filtration rate (eGFR) have been described previously[20].

Blood samples, mostly non-fasting (80%), were collected at baseline. Plasma and serum samples were stored at -80°C until analysis. Plasma concentrations of tHcy were analyzed using gas chromatography mass spectrometry[21], whereas serum Vit-A

(as *all-trans* retinol)[22] and neopterin[23] by liquid chromatography tandem mass spectrometry by BEVITAL AS (www.bevital.no). Serum concentrations of apolipoprotein B and C-reactive protein (CRP), and dietary Vit-A intake was measured as described previously[8, 20].

Follow-up and clinical endpoints

The participants were followed from baseline to the date of AMI diagnosis, death, or through December 31, 2009 (the end of follow-up), whichever came first. AMI cases were ascertained via record linkage to national hospital discharge diagnoses obtained from the Cardiovascular Disease in Norway project (www.cvdnor.no) through 2009[24-26]. The primary outcome was hospitalization or death attributed to AMI (coded I21, I22, I46.1, R96, and R98 according to the *International Statistical Classification of Diseases 10th version*). If more than one AMI event occurred in a participant during the follow-up period, only the first event was considered. An 11-digit personal identifier, unique to each Norwegian resident, was used to link baseline variables with study endpoints.

Statistical analysis

Due to non-normality, continuous variables are presented as geometric means (gM) (geometric standard deviation (gSD)). Categorical variables are given as percentages (%). Baseline characteristics are presented for the total population and according to serum Vit-A tertiles. Trends across Vit-A tertiles were assessed with unadjusted ordinary least squares or logistic regression. Skewed variables were log-transformed before analyses. Survival analyses were carried out using Cox regression and reported as hazard ratios (HRs) (95% confidence intervals (CIs)) per 1 SD log-transformed

plasma tHcy in one model containing log-transformed tHcy (continuous), age (continuous) and sex (binary), and an additional model including additional adjustment for smoking (binary), statin prescription (binary), aspirin prescription (binary), fasting status (binary), eGFR (continuous) and apolipoprotein B (continuous). To assess the effect-modification by Vit-A, Vit-A (continuous), log-transformed tHcy (continuous) and their interaction term (Vit-A × tHcy) was added to the models. To assess the interaction in the presence of competing risks, we computed subdistribution HRs from identical models based on the approach proposed by Fine and Gray[27]. To explore whether the results differed in patients with and without significant coronary stenosis, we evaluated the Vit-A × tHcy interaction in patients with 1-3 stenotic epicardial vessels as well as patients with no significant stenosis in models adjusted for age and sex. The assumption of proportionality was evaluated visually by log-log plots and quantitatively by calculating Schoenfeld residuals. To assess any non-linear relationships between tHcy and risk of AMI according to Vit-A tertiles, we constructed generalized additive model (GAM) dose-response curves based on the age and sex-adjusted models in each Vit-A tertile. To visualize the interaction of log-transformed tHcy with Vit-A, we constructed an interaction plot. In brief, we plotted the HR of log-transformed tHcy against a given range of Vit-A (0-7 μmol/L), and thus illustrated how the association of log-transformed tHcy with AMI changes with increasing concentrations of Vit-A. We considered p-values <0.05 significant. The statistical basis and R-codes for this procedure has been published[28]. Statistical analyses were carried out using the "base", "survival" and "cmprsk" packages, whereas the GAM plot was created using the "mgcv" package for R 3.0.2 (the R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Baseline characteristics of the total study population and according to Vit-A tertiles are presented in **Table 1.** The total population included a total of 2205 participants of which 64.3% were male with a gM (gSD) age of 62.3 (1.24) years. The gM (gSD) serum concentration of Vit-A was 2.93 (1.28) μmol/L. Cutoffs for serum Vit-A in the tertiles were <2.65, 2.65-3.20 and >3.20 μmol/L in the 1st, 2nd and 3rd tertile, respectively. Participants in the upper Vit-A tertile tended to be men, have hypertension and higher concentrations of LDL-cholesterol, apolipoprotein B, triglycerides, apolipoprotein A1, neopterin and tHcy, whereas CRP and eGFR tended to be lower. No trend for Vit-A intake was observed across serum Vit-A tertiles.

Association between plasma tHcy and AMI

During gM (gSD) follow-up time of 7.0 (2.4) y, 333 (15.1%) patients were diagnosed with AMI. As expected, there was a positive risk relationship between plasma tHcy and incident AMI; however the association was somewhat attenuated in the multivariate model (**Table 2**).

Interaction with Vit-A

As presented in **Table 3,** we found a significant interaction between tHcy and Vit-A in relation to AMI in both models. We constructed GAM plots to assess non-linearity between tHcy and AMI in each Vit-A (**Figure 1**). As can be seen from **Figure 1**, we found no associations between tHcy and AMI in the 1st and 2nd Vit-A tertiles; however a positive association was seen in the 3rd Vit-A tertile. This is further illustrated in

Figure 2, which shows that the risk relationship of AMI associated with tHcy increase in a fully adjusted model when Vit-A concentrations exceed ~3 μmol/L.

Competing risks analysis

In total, 505 patients died of which 274 (54.3 %) due to non-coronary causes. Based on this finding, we calculated subdistribution HRs for getting an AMI in the presence of non-cardiovascular mortality. The association of tHcy with AMI was generally unchanged in all multivariate models including the upper tertile of Vit-A (Subdistribution HR: 1.24, 95% CI: 1.04-1.49, p for interaction = 0.03).

Interaction with Vit-A in patients with and without significant stenosis

We stratified the population according to the prevalence of significant coronary artery disease to evaluate whether the interaction between tHcy and Vit-A differed in the two groups. In patients with at least one significant stenosis (n = 1380), 286 (21%) events occurred, whereas 47 events (5.6%) occurred in patients without significant stenosis (n = 825). Risk estimates and confidence intervals are presented in **Table 4**. There was a significant interaction between tHcy and Vit-A in relation to AMI in patients with, but not without, significant coronary stenosis.

DISCUSSION

Principal findings

In this prospective study among more than 2000 patients with stable angina pectoris, we showed that plasma tHcy was higher in patients with elevated systemic Vit-A concentrations and that the association between tHcy and risk of AMI was primarily confined to patients in the highest tertile of serum Vit-A.

Homocysteine and Vit-A

Our findings are of interest considering our previously published results indicating effect modification by serum Vit-A on the association between traditional lipid parameters and incident CVD[8]. We also demonstrated a positive association between baseline plasma tHcy and serum Vit-A, which is in line with findings among overweight but otherwise healthy individuals[11]. Positive associations for Vit-A[29], its transport protein retinol-binding protein 4[30], and cardiovascular endpoints has been reported from some prospective cohorts, but no interaction with other common risk factors have been reported. To our knowledge, epidemiological evidence on the interaction between tHcy and Vit-A with respect to incident AMI or CVD in general has not been assessed previously that we know of.

Potential mechanisms

Experimental data on the interaction between homocysteine and Vit-A are limited, but possible mechanisms may include inflammation and lipid metabolism. Inflammatory conditions such as non-alcoholic fatty liver disease increase hepatic expression of genes involved in *all-trans*-RA synthesis[9]. Inflammatory markers such as oxysterols

stimulate synthesis of *all-trans*-RA in the liver[10], which in turn may affect homocysteine through induction of GNMT[13, 15, 31]. Notably, GNMT plays a role in the production of interferon-γ, which drives synthesis of the macrophage-specific inflammatory marker neopterin[18, 32]. This pteridine has been related to risk of major cardiovascular events in humans[32], and potentiated tHcy-mediated risk of CVD in the same core population as the present study[4]. Neopterin was higher among patients with elevated Vit-A in the present study, and the tHcy-associated risk we observed in our study may reflect pro-inflammatory conditions that contribute to atherosclerosis. In contrast, CRP, which unlike neopterin is produced in the liver as part of the acute phase response[33] and is associated with aspects of CVD risk[34] and complications[35], tended to be lower among patients with elevated Vit-A. Hence, the interaction between Vit-A and tHcy and the potential link with inflammation seems to relate to specific components of immune activation. This hypothesis needs further exploration in future studies.

Accumulation of cholesterol is crucial for atherosclerotic plaque progression and leads to foam cell formation from endothelial-derived smooth muscle like cells[36], and may relate to impaired reverse cholesterol transport to high-density lipoprotein (HDL)[37]. We recently showed that low concentrations of apolipoprotein A1 (apoA1), the major protein constituent of HDL, was associated with excess risk of cardiovascular events in patients with elevated Vit-A concentrations[8]. Homocysteine, partially produced by GNMT in the liver, accelerates hepatic clearance of HDL[38] and inhibits synthesis of apoA1 in the liver of mice and men with coronary heart disease[39]. Thus, homocysteine may exert detrimental effects on reverse cholesterol transport from atherosclerotic plaques and the dynamic exchange of cholesterol between HDL and low-density lipoprotein particles in the circulation by limiting HDL availability.

However, this proposed mechanism is complicated by the demonstration that GNMT ablation in macrophages impaired reverse cholesterol transport[37], indicating that the outlined mechanism involving Vit-A and GNMT may be tissue-dependent.

Taken together, we speculate that elevated Vit-A activity modifies the tHcy-associated risk of CVD. Notably, tHcy-associated risk among these patients may reflect immune activation and disrupted lipid metabolism. However, well-defined mechanistic studies are needed to explore this potential biological interaction in greater detail, and possibly with regards to different tissues.

Relevance

Recent dietary intake of Vit-A is not likely to affect the observed relationship as it does not affect serum Vit-A concentrations except in individuals with extreme excess or deficient intake[40]. Thus, the high circulating Vit-A levels may reflect metabolic disturbances as has been shown in animal models[41]. Adjustment for important confounders such as kidney function, which is a determinant of both serum Vit-A[42] and plasma tHcy[43], did not attenuate the interaction significantly. Although the interaction between tHcy and Vit-A appeared to be independent of other known risk factors, it is essential to replicate the findings in other studies.

In an attempt to explore whether the interaction differed according to the prevalence of coronary artery disease we stratified our patients based on the presence of significant coronary stenosis. We observed a significant interaction between tHcy and Vit-A in patients with 1-3 stenotic epicardial vessels, but not in patients without significant stenosis. These observations indicate that the interaction was confined to patients with more severe disease; however, the statistical power to detect the interaction in patients without coronary stenosis was weak (47 AMI events). These

subgroup observations are exploratory but highlight the necessity of investigating this interaction in large and initially healthy cohorts.

Strengths and limitations

This study included a large and well-characterized population with established CVD. Given the high levels of circulating Vit-A compared with other populations[29, 44], this cohort may have been particularly suitable for the current study objective. In contrast, the results reported in this study may not be generalizable to the general population because the patients were at increased CVD risk upon recruitment.

The majority of samples were drawn from non-fasting subjects, and tHcy has been found to be modestly increased following food intake[45-47], but adjustment for fasting state in the multivariate models did not alter the risk estimates significantly. The findings and proposed mechanisms are further complicated by the possibility that Vit-A concentrations do not necessarily reflect biological activity in tissues. Finally, as this study is of observational nature, it does not allow for causal inference due to the possibility of residual confounding such as long-term use of high-dose supplements[40, 48].

Conclusions

The results from the present exploratory study suggest that the association of circulating concentrations of tHcy with incident AMI is confined to patients with elevated serum concentrations of Vit-A. These findings may shed light on the hitherto unclear relationship between tHcy and CVD. We speculate that tHcy-associated risk may reflect disturbed lipid metabolism and thus experimental and mechanistic studies are needed to elucidate this interaction in a biological system.

Acknowledgements

Conflicts of interest

The authors have nothing to declare.

Author contributions

OKN conceived and designed the study; OKN, GFTS, ERP and PMU conducted the research; TO and KJV conducted the statistical analyses; ØM and PMU were responsible for measuring vitamin A in serum; TO, KJV, GFTS, ERP, GST, RB, ID, PMU, HR and OKN interpreted the results; TO, KJV, GFTS, ERP, GST, RB, ID, PMU, ØM, HR and OKN wrote, critically revised and approved the final version of the manuscript.

References

1.   Brosnan JT and Brosnan ME. The sulfur-containing amino acids: an overview. *The Journal of nutrition*. 2006; 136: 1636S-40S.

2.   Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem*. 2004; 50: 3-32.

3.   Clarke R and Lewington S. Homocysteine and coronary heart disease. *Seminars in vascular medicine*. 2002; 2: 391-9.

4.   Bjornestad EO, Borsholm RA, Svingen GFT, et al. Neopterin as an Effect Modifier of the Cardiovascular Risk Predicted by Total Homocysteine: A Prospective 2-Cohort Study. *J Am Heart Assoc*. 2017; 6.

5.   Clarke R, Halsey J, Lewington S, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. *Archives of internal medicine*. 2010; 170: 1622-31.

6.   Blomhoff R and Blomhoff HK. Overview of retinoid metabolism and function. *J Neurobiol*. 2006; 66: 606-30.

7.   Rhee EJ, Nallamshetty S and Plutzky J. Retinoid metabolism and its effects on the vasculature. *Biochim Biophys Acta*. 2012; 1821: 230-40.

8.   Olsen T, Vinknes KJ, Svingen GFT, et al. Cardiovascular disease risk associated with serum apolipoprotein B is modified by serum vitamin A. *Atherosclerosis*. 2017.

9.   Ashla AA, Hoshikawa Y, Tsuchiya H, et al. Genetic analysis of expression profile involved in retinoid metabolism in non-alcoholic fatty liver disease. *Hepatol Res*. 2010; 40: 594-604.

10.   Huq MD, Tsai NP, Gupta P and Wei LN. Regulation of retinal dehydrogenases and retinoic acid synthesis by cholesterol metabolites. *EMBO J*. 2006; 25: 3203-13.

11.   Brazionis L, Walker KZ, Itsiopoulos C and O'Dea K. Plasma retinol: a novel marker for cardiovascular disease mortality in Australian adults. *Nutr Metab Cardiovasc Dis*. 2012; 22: 914-20.

12.   Ogawa H, Gomi T, Takusagawa F and Fujioka M. Structure, function and physiological role of glycine N-methyltransferase. *Int J Biochem Cell Biol*. 1998; 30: 13-26.

13.   Rowling MJ and Schalinske KL. Retinoid compounds activate and induce hepatic glycine N-methyltransferase in rats. *J Nutr*. 2001; 131: 1914-7.

14.   McMullen MH, Rowling MJ, Ozias MK and Schalinske KL. Activation and induction of glycine N-methyltransferase by retinoids are tissue- and gender-specific. *Arch Biochem Biophys*. 2002; 401: 73-80.

15.   Rowling MJ, McMullen MH and Schalinske KL. Vitamin A and its derivatives induce hepatic glycine N-methyltransferase and hypomethylation of DNA in rats. *J Nutr*. 2002; 132: 365-9.

16.   Luka Z, Mudd SH and Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. *J Biol Chem*. 2009; 284: 22507-11.

17.   Liao YJ, Chen TL, Lee TS, et al. Glycine N-methyltransferase deficiency affects Niemann-Pick type C2 protein stability and regulates hepatic cholesterol homeostasis. *Mol Med*. 2012; 18: 412-22.

18.   Li CH, Lin MH, Chu SH, et al. Role of glycine N-methyltransferase in the regulation of T-cell responses in experimental autoimmune encephalomyelitis. *Mol Med*. 2015; 20: 684-96.

19.   Ebbing M, Bleie O, Ueland PM, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA*. 2008; 300: 795-804.

20.   Svingen GF, Ueland PM, Pedersen EK, et al. Plasma dimethylglycine and risk of incident acute myocardial infarction in patients with stable angina pectoris. *Arterioscler Thromb Vasc Biol*. 2013; 33: 2041-8.

21.   Windelberg A, Arseth O, Kvalheim G and Ueland PM. Automated assay for the determination of methylmalonic acid, total homocysteine, and related amino acids in human serum or plasma by means of methylchloroformate derivatization and gas chromatography-mass spectrometry. *Clin Chem*. 2005; 51: 2103-9.

22.   Midttun O and Ueland PM. Determination of vitamins A, D and E in a small volume of human plasma by a high-throughput method based on liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2011; 25: 1942-8.

23.   Midttun O, Hustad S and Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM*. 2009; 23: 1371-9.

24.   Sulo G, Igland J, Vollset SE, Nygard O, Oyen N and Tell GS. Cardiovascular disease and diabetes mellitus in Norway during 1994-2009 CVDNOR - a nationwide research project. *Nor Epidemiol*. 2013; 23: 101-7.

25.   Sulo G, Igland J, Nygard O, Vollset SE, Ebbing M and Tell GS. Favourable trends in incidence of AMI in Norway during 2001-2009 do not include younger adults: a CVDNOR project. *Eur J Prev Cardiol*. 2014; 21: 1358-64.

26.   Igland J, Tell GS, Ebbing M, Nygard O, Vollset SE and Dimoski T. Cardiovascular Disease in Norway 1994-2009 Description of Data and Data Quality: (2013).

27.   Fine JP and Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association*. 1999; 94: 496-509.

28.   Lamina C, Sturm G, Kollerits B and Kronenberg F. Visualizing interaction effects: a proposal for presentation and interpretation. *J Clin Epidemiol*. 2012; 65: 855-62.

29.   Min KB and Min JY. Relation of serum vitamin A levels to all-cause and cause-specific mortality among older adults in the NHANES III population. *Nutr Metab Cardiovasc Dis*. 2014; 24: 1197-203.

30.   Liu Y, Zhong Y, Chen H, et al. Retinol-Binding Protein-Dependent Cholesterol Uptake Regulates Macrophage Foam Cell Formation and Promotes Atherosclerosis. *Circulation*. 2017; 135: 1339-54.

31.   Rowling MJ and Schalinske KL. Retinoic acid and glucocorticoid treatment induce hepatic glycine N-methyltransferase and lower plasma homocysteine concentrations in rats and rat hepatoma cells. *J Nutr*. 2003; 133: 3392-8.

32.   Sugioka K, Naruko T, Matsumura Y, et al. Neopterin and atherosclerotic plaque instability in coronary and carotid arteries. *J Atheroscler Thromb*. 2010; 17: 1115-21.

33.   Ansar W and Ghosh S. C-reactive protein and the biology of disease. *Immunol Res*. 2013; 56: 131-42.

34.   Gubelmann C, Vollenweider P and Marques-Vidal P. Association of grip strength with cardiovascular risk markers. *Eur J Prev Cardiol*. 2017; 24: 514-21.

35.   Bielas H, Meister-Langraf RE, Schmid JP, et al. Acute stress disorder and C-reactive protein in patients with acute myocardial infarction. *Eur J Prev Cardiol*. 2018; 25: 298-305.

36.   Lao KH, Zeng L and Xu Q. Endothelial and smooth muscle cell transformation in atherosclerosis. *Curr Opin Lipidol*. 2015; 26: 449-56.

37.   Chen CY, Ching LC, Liao YJ, et al. Deficiency of glycine N-methyltransferase aggravates atherosclerosis in apolipoprotein E-null mice. *Mol Med*. 2012; 18: 744-52.

38.   Liao D, Tan H, Hui R, et al. Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein A-I Protein synthesis and enhancing HDL cholesterol clearance. *Circ Res*. 2006; 99: 598-606.

39.   Mikael LG, Genest J, Jr. and Rozen R. Elevated homocysteine reduces apolipoprotein A-I expression in hyperhomocysteinemic mice and in males with coronary artery disease. *Circ Res*. 2006; 98: 564-71.

40.   Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *Journal of the National Cancer Institute*. 1984; 73: 1439-44.

41.   Trasino SE, Tang XH, Jessurun J and Gudas LJ. Obesity Leads to Tissue, but not Serum Vitamin A Deficiency. *Sci Rep*. 2015; 5: 15893.

42.   Chen J, He J, Ogden LG, Batuman V and Whelton PK. Relationship of serum antioxidant vitamins to serum creatinine in the US population. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 2002; 39: 460-8.

43.   Long Y and Nie J. Homocysteine in Renal Injury. *Kidney Dis (Basel)*. 2016; 2: 80-7.

44.   Holvik K, Ahmed LA, Forsmo S, et al. No increase in risk of hip fracture at high serum retinol concentrations in community-dwelling older Norwegians: the Norwegian Epidemiologic Osteoporosis Studies. *Am J Clin Nutr*. 2015.

45.   Verhoef P, van Vliet T, Olthof MR and Katan MB. A high-protein diet increases postprandial but not fasting plasma total homocysteine concentrations: a dietary controlled, crossover trial in healthy volunteers. *Am J Clin Nutr*. 2005; 82: 553-8.

46.   Guttormsen AB, Schneede J, Fiskerstrand T, Ueland PM and Refsum HM. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *J Nutr*. 1994; 124: 1934-41.

47.   Nurk E, Tell GS, Nygard O, Refsum H, Ueland PM and Vollset SE. Plasma total homocysteine is influenced by prandial status in humans: the Hordaland Hhomocysteine Sstudy. *J Nutr*. 2001; 131: 1214-6.

48.   Midttun O, Theofylaktopoulou D, McCann A, et al. Circulating concentrations of biomarkers and metabolites related to vitamin status, one-carbon and the kynurenine pathways in US, Nordic, Asian, and Australian populations. *Am J Clin Nutr*. 2017; 105: 1314-26.

Table 1: Baseline characteristics for the total study population (n = 2205) with suspected stable angina pectoris

	Total study population	1st Vit-A tertile	2nd	3rd	p for trend[1]
	-	< 2.65 μmol/L	2.65-3.20 μmol/L	> 3.20 μmol/L	
	(*n* = 2205)	(*n* = 728)	(*n* = 727)	(*n* = 750)	
Age, y	62.3 (1.24)	61.4 (1.20)	60.3 (1.21)	60.4 (1.20)	0.229
Male sex, n (%)	1418 (64.3)	438 (60.2)	469 (64.5)	511 (68.1)	0.001
Fasting, n (%)	418 (19.8)	150 (21.6)	137 (19.6)	131 (18.2)	0.155
Smoking, n (%)	689 (31.2)	233 (32.0)	235 (32.3)	221 (29.5)	0.323
1-3 stenotic vessels, n (%)	1380 (62.6)	490 (67.3)	454 (62.4)	436 (58.1)	0.005
Diabetes mellitus, n (%)	274 (12.4)	106 (14.6)	75 (10.3)	93 (12.4)	0.157
Hypertension, n (%)	1030 (46.7)	330 (45.3)	317 (43.6)	383 (51.1)	0.025
LVEF < 60, n (%)	1784 (80.9)	593 (81.5)	592 (81.4)	599 (79.9)	0.327
Previous AMI, n (%)	817 (37.1)	273 (37.5)	264 (36.3)	280 (37.3)	0.958
ApoB, g/L	0.89 (1.31)	0.86 (1.30)	0.89 (1.30)	0.93 (1.31)	<0.001
ApoA1, g/L	1.33 (1.22)	1.29 (1.23)	1.33 (1.21)	1.37 (1.22)	<0.001

LDL-C, mmol/L	2.97 (1.39)	2.89 (1.40)	2.98 (1.38)	3.03 (1.39)	0.001
HDL-C, mmol/L	1.26 (1.34)	1.25 (1.35)	1.27 (1.33)	1.27 (1.32)	0.703
Triglycerides, mmol/L	1.51 (1.67)	1.3 (1.61)	1.46 (1.62)	1.79 (1.7)	<0.001
CRP, mg/mL	1.97 (2.99)	2.43 (3.27)	1.85 (2.93)	1.71 (2.7)	<0.001
eGFR, ml/min per 1.73 m^2	83.0 (1.34)	88.5 (1.20)	85.7 (1.21)	75.6 (1.51)	<0.001
Total homocysteine, µmol/L	11.0 (1.4)	10.4 (1.36)	10.7 (1.36)	11.8 (1.44)	<0.001
Neopterin, nmol/L	8.94 (1.51)	8.45 (1.44)	8.65 (1.42)	9.76 (1.64)	<0.001
Retinol intake, RAE/d	1576 (1.83)	1548 (1.73)	1584 (1.91)	1618 (1.91)	0.526
Medications at discharge, n (%)					
Statins	1605 (72.8)	526 (72.3)	525 (72.2)	554 (73.9)	0.286
Aspirin	1642 (74.5)	542 (74.5)	540 (74.3)	560 (74.7)	0.621
Beta-blockers	1479 (67.1)	490 (67.3)	478 (65.7)	511 (68.1)	0.601

[1]P-values for trends across tertiles of vitamin A were calculated by linear regression for continuous variables and logistic regression for categorical variables. Abbreviations: LVEF, left ventricular ejection fraction; AMI, acute myocardial infarction; ApoB, apolipoprotein B;

ApoA1, apolipoprotein A1; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate. Values are geometric mean (geometric standard deviation) for continuous variables and n (%) for categorical variables.

TABLE 2

tHcy-related risk of AMI in the total population[1]

Total population (No of events, 333/2205)	HR per SD (95% CI)	*P*-value
Model 1[2]	1.22 (1.10-1.35)	< 0.01
Model 2[3]	1.12 (0.99-1.26)	0.07

[1] Risk as hazard ratios (95% confidence intervals (CIs) per 1 SD log-transformed plasma tHcy computed by Cox proportional hazards model

[2] Adjusted for age and gender

[3] Adjusted for age, gender, smoking, estimated glomerular filtration rate, statin prescription, aspirin prescription, fasting and apolipoprotein B

TABLE 3

tHcy-related risk of AMI across tertiles of vitamin A[1]

	Tertiles of serum vitamin A[2]			P for interaction
	1st	2nd	3rd	
No. of events/n	117/728	108/727	108/750	
Model 1[3]	1.10 (0.90-1.34)	1.11 (0.90-1.38)	1.41 (1.21-1.64)	<0.001
Model 2[4]	1.08 (0.88-1.33)	1.09 (0.86-1.38)	1.25 (1.04-1.52)	0.03

[1] Risk as hazard ratios (95% confidence intervals (CIs) per 1 SD log-transformed plasma tHcy, computed by Cox proportional hazards model

[2] Cut-offs for serum Vit-A tertiles were <2.65, 2.65-3.20 and >3.20 μmol/L

[3] Adjusted for age and gender

[4] Adjusted for gender, smoking, estimated glomerular filtration rate, statin prescription, aspirin prescription, fasting and apolipoprotein B

TABLE 4

tHcy-related risk of AMI across tertiles of vitamin A in patients with and without significant coronary stenosis at baseline angiography[1]

	Tertiles of serum vitamin A[2,3]			P for interaction
	1st	2nd	3rd	
Stenosis				
No. of events/n	92/461	101/459	93/460	
	1.08 (0.88-1.34)	1.15 (0.93-1.42)	1.47 (1.26-1.71)	0.02
No stenosis				
No. of events/n	19/276	13/274	15/275	
	1.04 (0.63-1.71)	0.73 (0.36-1.48)	1.04 (0.58-1.56)	0.56

[1] Risk as hazard ratios (95% confidence intervals per 1 SD log-transformed plasma tHcy, computed by Cox proportional hazards model adjusted for age and sex

[2] Cut-offs for serum Vit-A tertiles were <2.62, 2.62-3.15 and >3.15 μmol/L for patients with coronary stenosis

[3] Cut-offs for serum Vit-A tertiles were <2.73, 2.73-3.30 and >3.30 μmol/L for patients without coronary stenosis

Figure 1: The dose-response relationship between log-transformed total homocysteine and acute myocardial infarctions in the 1st (A), 2nd (B) and 3rd (C) tertile of vitamin A computed by generalized additive models. Data are adjusted for age and sex. The blue shaded areas around the curve represent the 95% confidence intervals. Kernel distribution plots show the distribution of total homocysteine.

Figure 2: Illustration of the interaction between serum vitamin A and log-transformed total homocysteine on the continuous scale. The blue shaded areas around the curve represent the 95% confidence intervals. HR per SD, hazard ratio per standard deviation; tHcy, total homocysteine
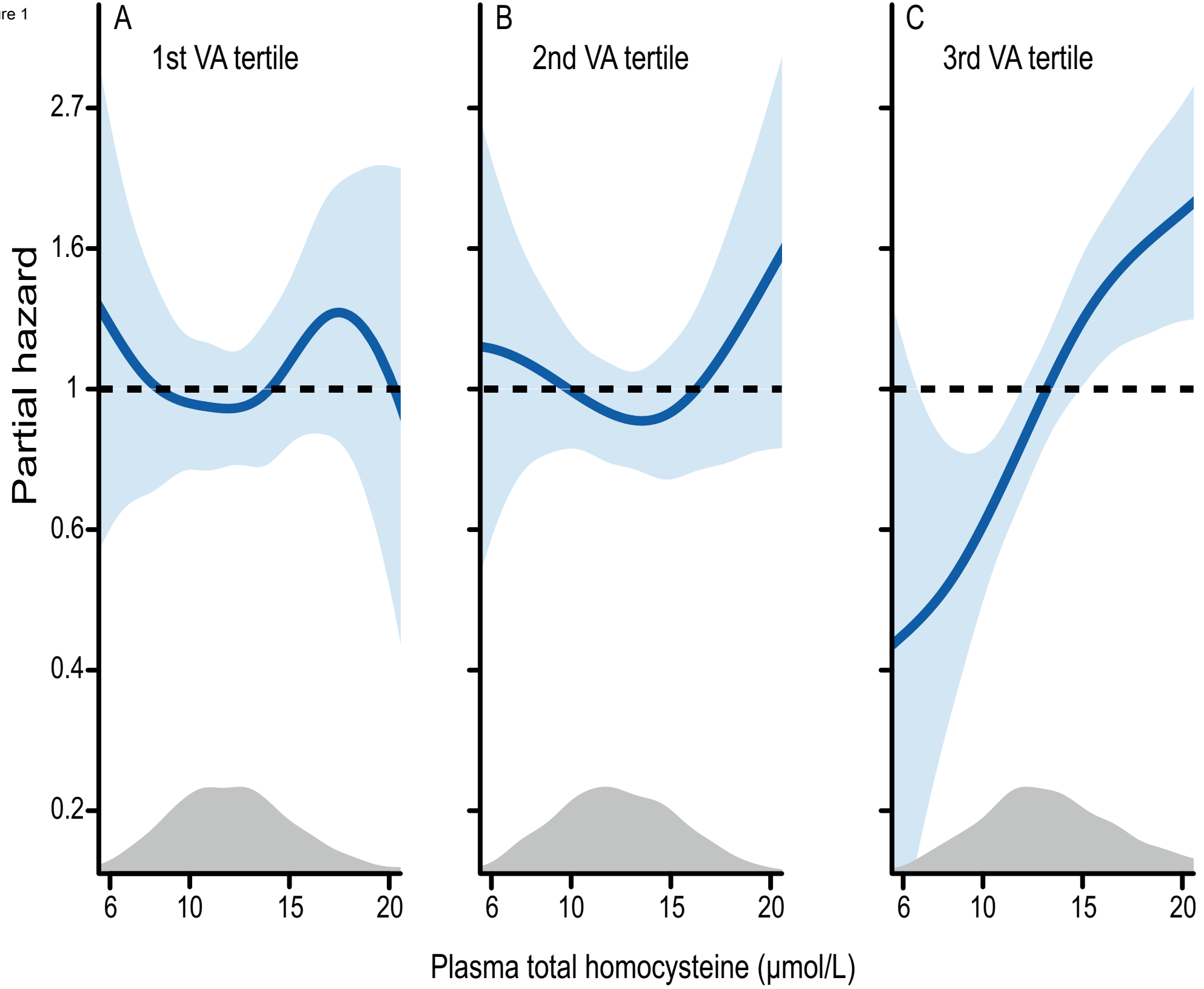
Figure 1

Figure 2