Improving cardiovascular risk assessment in primary care

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CHAPTER 6

Markers of hepatic steatosis do not improve current cardiovascular risk prediction systems

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Abstract

Background Hepatic steatosis is increasingly prevalent worldwide and is associated with a 64% increased cardiovascular disease (CVD) risk.

Objective To examine whether cardiovascular risk prediction can be improved by adding non-invasive markers of hepatic steatosis to the Framingham risk score.

Methods Data was used from the European Prospective Investigation into Cancer and Nutrition-Netherlands study, which comprises 40,011 men and women aged 20-70 years at recruitment in 1993-1997. We analysed participants aged 30-70 years without prevalent CVD and not using preventive treatment. Serological markers of liver function (alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyltransferase (GGT)) and scores developed for the prediction of hepatic steatosis (fatty liver index, hepatic steatosis index, ALT/AST ratio) were added to the calibrated sex-specific Framingham risk score. The outcome was defined as a CVD event in 10 years of follow-up. Model performance was evaluated by measures of discrimination, calibration and reclassification.

Results During 10 years of follow-up, a CVD event occurred in 7% of the men and 5% of the women. None of the markers of hepatic steatosis was a predictor for CVD in men when added to the Framingham risk score. In women, GGT, ALT, the fatty liver index, and the AST/ALT ratio were predictors. Adding these markers did not lead to a relevant improvement in discrimination, calibration or reclassification.

Discussion Easily accessible markers of hepatic steatosis did not improve cardiovascular risk prediction in addition to the established risk factors. Future research is needed to examine the added predictive value of other markers of hepatic steatosis.

Introduction

In clinical practice, patients at increased cardiovascular risk are currently identified by estimating an individual's 10-year risk of cardiovascular disease (CVD) using a risk estimation system. Examples of those risk estimation systems are the Framingham risk score, Pooled Cohort Equations, SCORE, and QRISK.(28) Based on the estimated risk, physicians decide whether preventive treatment is indicated. This requires an optimal estimation of an individual's cardiovascular risk. External validations of the most commonly used risk estimation systems have demonstrated an area under the receiver operating curve (AUC) between 0.75 and 0.80.(28) This means that the prediction of an individual's 10-year cardiovascular risk is not optimal, which may lead to under- and overtreatment of patients.

Possibilities to improve cardiovascular risk prediction are explored in various studies and adding information about the presence of hepatic steatosis, the early stage of non-alcoholic fatty liver disease (NAFLD) is one of the approaches. NAFLD covers a broad clinical spectrum ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis, with varying degrees of inflammation and fibrosis, in the absence of excessive alcohol consumption.(112) Currently, the global prevalence of NAFLD in the general population is 25%, with a prevalence of up to 90% in persons with obesity.(46) NAFLD is strongly related to several cardiometabolic diseases(47) and associated with a 64% increased risk of CVD(48), and is therefore a likely candidate to improve cardiovascular risk prediction.

Proton magnetic resonance spectroscopy and liver biopsy are reference measurements for the assessment of hepatic steatosis and fibrosis.(113) However, these measurements cannot be used in a cardiovascular risk estimation system in clinical practice, due to the invasiveness, availability and costs of the measurements. Serological markers of liver function and combination scores developed for the prediction of hepatic steatosis may be non-invasive alternatives that probably can be used in daily practice. In a previous study in men, cardiovascular risk prediction improved when gamma glutamyltransferase (GGT) was included in the Framingham risk score.(49) However, other studies in men and women showed no improvement when GGT or the ratio of aspartate transaminase (AST) and alanine aminotransferase (ALT) was included in current risk estimation systems.(50, 51) It is suggested that the improvement may differ between men and women.(51) In addition, it is unknown whether combination scores developed for the prediction of hepatic steatosis have any added value in a risk estimation system. Therefore, the aim of this study was to examine whether cardiovascular risk prediction can be improved when non-invasive markers of hepatic steatosis are added to the Framingham risk score and if improvement differed between men and women.

Methods

Study design and study population

The EPIC-NL cohort includes two Dutch contributions to the European Prospective Investigation Into Cancer and Nutrition (EPIC), the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) cohort and Prospect cohort. The study design has been described elsewhere.(53) In both cohorts, the participants were recruited simultaneously between 1993 and 1997. The MORGEN cohort consists of a general population sample of 22,654 men and women aged 20-59 years. Prospect is a prospective cohort study in 17,357 women aged 49-70 years, who participated in the national breast cancer screening programme between 1993 and 1997.

At baseline, the participants completed a questionnaire, a brief physical examination was performed, and a non-fasting blood sample was drawn, fractionated into aliquots, and stored in liquid nitrogen for future analyses. Biochemical measurements were performed in a random sample of 6.5% of the baseline cohort (sub-cohort of 2,604 participants) and in all incident cases of cardiovascular disease that occurred before 2006 for the purpose of a previous case-cohort study.(114, 115)

In the present study, we included all participants from the sub-cohort and all incident cases of CVD during 10 years of follow-up. A total of 2,332 CVD events occurred, of which 2,177 events occurred in participants outside the sub-cohort. The Framingham risk score is developed for persons aged 30-70 years without a history of CVD. So, we consecutively excluded participants outside this age range (n=3,805) and participants with a history of CVD (defined as a medical history of coronary heart disease, cerebrovascular disease, pulmonary embolism, peripheral artery disease, abdominal aortic aneurysm or heart failure, n=1,255). Furthermore, we excluded participants who did not consent to linkage with disease registries as well as participants who were lost to follow-up (n=1,467). The local medical ethics committees approved the cohort studies and all participants gave informed consent.

Cardiovascular risk estimation system

We used the sex-specific Framingham risk score to estimate 10-year CVD risk.(18) This model includes the risk factors age, treated and nontreated systolic blood pressure (SBP), total cholesterol and high-density lipoprotein (HDL) cholesterol concentrations, diabetes mellitus and smoking status. According to the guideline, the risk estimates were categorized into a low, intermediate and high risk, corresponding to a 10-year CVD risk of <10%, 10-20%, and ≥20%, respectively.

The blood pressure was measured twice in supine position, in Prospect on the right arm using a Boso Oscillomat (Bosch & Son, Jungingen, Germany) and in MORGEN on the left arm using a random zero sphygmomanometer. The average of the measurements was used in the analyses. Total cholesterol concentrations were measured using an enzymatic method and HDL cholesterol concentrations were measured using a homogeneous assay with an enzymatic endpoint, both on an autoanalyser (LX20, Beckman Coulter, Mijdrecht, the Netherlands).

Information on the medical history of diabetes mellitus, use of antihypertensive medication and smoking status were reported in the baseline questionnaire. The information on the medical history of diabetes mellitus was clinically validated.(56)

Markers of hepatic steatosis

We examined three serological markers of liver function and three combination scores developed for the prediction of hepatic steatosis. The serological markers ALT, AST and GGT were measured using enzymatic methods on an autoanalyser (LX20, Beckman Coulter, Mijdrecht, the Netherlands). We examined the added value of the markers both as continuous variables and dichotomized. The markers were dichotomized using the upper limit of the normal range: 45 U/L for ALT, 35 U/L for AST and 50 U/L for GGT.

In addition, we examined the combination scores: the fatty liver index, the hepatic steatosis index, and the AST/ALT ratio. The fatty liver index predicts steatosis by using body mass index, waist circumference, triglycerides and GGT.(116) This combination score is the most promising score for the detection of steatosis, with an AUC of 0.84 in the general population.(117) We examined the added value of the continuous score, a threshold of <30 to rule out steatosis and a threshold of ≥60 to rule in steatosis. The hepatic steatosis index is an algorithm based on ALT, AST and body mass index. We examined the added value of the continuous score, a threshold of <30 to rule out steatosis and a threshold of ≥36 to rule in steatosis. The AST/ALT ratio is the ratio between AST and ALT. In the absence of excessive alcohol use, a ratio >1 suggests a more advanced stage of NAFLD.(118) We examined the added value of the continuous ratio and the threshold of 1.

Conform the predictors in the Framingham risk score, all markers were mean standardized and all continuous markers were naturally logarithmically transformed.

Outcome assessment

Data on cardiovascular mortality was obtained through linkage with the municipal population registries. Causes of death were collected from 'Statistics Netherlands'. Morbidity data was obtained from the National Medical Registry (NMR), which keeps a standardized

computerized register of discharge diagnoses coded according to the Ninth Revision of the International Classification of Diseases (ICD-9). The NMR collected and checked these data in the Hospital Discharge Diagnosis Database. This database was linked to the EPIC-NL cohort based on information on the date of birth, sex, postal code, and general practitioner with a validated probabilistic method.(119) CVD was defined according to the definition used to develop the Framingham risk score and included coronary heart disease, heart failure, cerebrovascular disease and peripheral artery disease (ICD-9 402, 410, 411.89, 414.8, 413, 428, 430, 431, 432, 433, 434, 435, 436, 440-449). Follow-up was complete until January 1, 2011. The first fatal or non-fatal CVD event in the first 10 years of follow-up after baseline was used as the endpoint in the analysis.

Statistical analysis

Baseline characteristics were expressed as mean (SD), or percentages. Mean imputation stratified by case status was used for missing data in the original predictors. Participants with missing smoking status were considered as non-smokers. The Framingham risk scores for men and women were calibrated by adjustment for the mean levels of the risk factors and the event rates.(120)

To account for the overrepresentation of cases in our study population, we used inverse probability weighting to weight the participants according to their case-cohort sampling probability.(121) For cases, the weights were set to 1, since all cases were sampled. The non-cases in the sub-cohort were weighted to represent the non-cases in het full cohort. Hereto, weights were calculated by the sampling fraction of the non-cases in the sub-cohort.

Blood samples were missing in cases outside the sub-cohort with an event after 31 December 2005 or an event other than coronary heart disease, stroke or death. In addition, in some participants blood samples were missing due to an unsuccessful blood draw or failed laboratory analysis. The participants with complete blood samples were considered comparable with participants with missing blood samples. Therefore, participants with complete blood samples were weighted to represent the full cohort. Due to the weighted analysis, only proportions could be reported, not counts.

First, cox proportional hazards regression was used to build a prediction model for each marker of hepatic steatosis by adding the marker to the Framingham risk score. Second, we fitted a model by adding all markers of hepatic steatosis as continuous variables to the Framingham risk score, and applied stepwise backward selection of statistically significant variables ($p< 0.05$) on the markers forcing the predictors of the Framingham risk score to be retained in the model.

Third, the models were compared with the Framingham risk score in terms of discrimination and calibration. Discrimination of the models was expressed by Harrell's c-statistics. Calibration was expressed by the calibration slope and visualised by calibration curves. The predicted cardiovascular risk was compared with the observed cardiovascular risk, in deciles of the predicted risk. The observed risks were determined using Kaplan Meier survival estimates.

Furthermore, the benefit for clinical practice was evaluated by measures of reclassification. Participants without a CVD event who were censored before reaching the endpoint of 10 year were considered as having no event. The category-based net reclassification index (NRI) indicates the percentage of correct shifting across risk categories for those with and without an event.(122) We calculated a three-category NRI where the risk categories represented a 10-year CVD risk of <10%, 10-19% and ≥ 20%. Correct shifting is an upward shift after adding new markers in those with an event and a downward shift in those without an event. We have visualised the reclassification in a reclassification graph. The improvement in net benefit (ΔNB) represents the net improvement of true positives calculated *by (Δtrue positives – weight * Δ false positves) / number of subjects*, where the weight is the odds of the decision threshold. The ΔNB was calculated for the treatment thresholds of a 10-year CVD risk of 20%. In this analysis we excluded the cases outside the sub-cohort to be able to calculate with absolute numbers instead of percentages.
Persons with an excessive alcohol consumption are usually excluded in the definition of NAFLD. Therefore, we repeated the analyses after exclusion of participants with an alcohol intake of more than 20 grams per day. We also repeated all analyses after exclusion of participants without a CVD event who were censored before reaching 10 years of follow-up in the analysis on reclassification.

We investigated the robustness of our results by examining the added value of the markers of hepatic steatosis in a second cardiovascular risk prediction system, the Pooled Cohort Equations.(123) The Pooled Cohort Equations is used to estimate the 10-year CVD risk for men and women separately. This model includes the same risk factors as the Framingham risk score. In this model, CVD was defined as a nonfatal myocardial infarction, coronary heart disease death or a fatal or nonfatal stroke (ICD 410, 430-434). According to the guideline, the risk estimates were categorized into a low, intermediate and high risk, corresponding to a 10-year risk of <5%, 5-7.5%, and ≥7,5%, respectively.(123)

For all analyses, STATA statistical software (Statacorp, College Station, Texas, USA), version 12 was used.

Results

The weighted baseline characteristics of the participants included in the analysis, stratified by sex, are shown in Table 1.

Table 1 Baseline characteristics of the participants of the EPIC-NL cohort, stratified by sex[a]

	Men	Women
Risk factors included in the Framingham risk score		
Age (years)	46 (9)	53 (9)
Treated SBP (mmHg)	140 (14)	140 (16)
Untreated SBP (mmHg)	126 (16)	127 (19)
Blood pressure lowering therapy (%)	6	2
Total cholesterol (mmol/L)	4.8 (0.9)	4.9 (0.9)
HDL cholesterol (mmol/L)	1.1 (0.7)	1.3 (0.5)
Smoking (% current)	38	27
Diabetes mellitus (%)	2	1
Markers of hepatic steatosis		
ALT (U/L)	17 (8)	15 (8)
>45 U/L (%)	1	1
AST (U/L)	21 (7)	19 (5)
>35 U/L (%)	3	1
GGT (U/L)	29 (22)	22 (19)
>50 U/L (%)	8	4
Fatty liver index	47 (28)	29 (24)
<30 - rule out hepatic steatosis (%)	33	64
30-60 (%)	32	21
≥60 - rule in hepatic steatosis (%)	35	15
Hepatic steatosis index	33 (7)	34 (5)
<30 - rule out hepatic steatosis (%)	28	20
30-36 (%)	51	52
>36 - rule in hepatic steatosis (%)	21	29
AST/ALT ratio	1.4 (0.4)	1.4 (0.4)
>1 - rule in advanced disease	86	89

Data are expressed as mean (SD) or %

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; SBP, systolic blood pressure

[a] Results are based on weighted analyses and therefore represent the total cohort

Cardiovascular risk prediction in men

Our study population comprised of 23% men, with a mean age of 46 (9) years at baseline. After calibration of the original Framingham risk score, 74% of the men had a low estimated CVD risk, 21% an intermediate risk and 6% a high risk of CVD. The C-statistic of the calibrated Framingham risk score for men was 0.72 (95% CI 0.69-0.75) and the calibration slope was 0.86 (95% CI 0.71-1.01). During 10 years of follow-up, a CVD event occurred in 7% of the male participants.

None of the markers of hepatic steatosis was a predictor for cardiovascular disease when they were added univariably to the calibrated Framingham risk score for men. Also after adding all markers of hepatic steatosis simultaneously to the Framingham risk score and applying backwards selection, none of the markers were retained in the model. (Table 2)

Cardiovascular risk prediction in women

Our study population comprised of 77% women, with a mean age of 53 (9) years at baseline. After calibration of the original Framingham risk score, 87% of the women had a low estimated CVD risk, 11% an intermediate risk and 2% a high risk of CVD. The C-statistic of the calibrated Framingham risk score for women was 0.71 (95% CI 0.69-0.73) and the calibration slope was 0.95 (95% CI 0.84-1.05). During 10 years of follow-up, a CVD event occurred in 5% of the female participants.

The following markers were a predictor when they were added to the calibrated Framingham risk score for women: GGT, ALT, fatty liver index, fatty liver index to rule out hepatic steatosis, AST/ALT ratio or AST/ALT ratio >1. (Table 2) Measures of discrimination, calibration and clinical benefit are shown in Table 3. The C-statistic of these models ranged between 0.71 and 0.72 and the calibration slope ranged between 0.91 and 1.05. Reclassification graphs are visualised in Figure 1. The addition of GGT to the Framingham risk score had the largest improvement in net benefit of 0.08 at the threshold of 20%. After adding all markers of hepatic steatosis simultaneously to the Framingham risk score and applying backwards selection, GGT and ALT were retained in the model. The C-statistic of this model was 0.72 and the calibration slope was 0.98. The improvement in net benefit of the addition of GGT and ALT to the Framingham risk score at the threshold of 20% was 0.12.(Table 3, Figure 1) Calibration curves of all models are shown in Supplemental figure 1 and density plots are shown in Supplemental figure 2.

Table 2 The cox proportional hazards model coefficients of the markers of hepatic steatosis when added to the Framingham risk score in men participating in the EPIC-NL cohort

Markers added to the Framingham risk score[a]	Coefficient (95% CI)
Single marker	
ALT	-0.14 (-0.55;0.28)
ALT >45 U/L	0.54 (-0.77;1.85)
AST	-0.20 (-0.64;0.25)
AST >35 U/L	-0.03 (-0.88;0.82)
GGT	0.00 (-0.31;0.32)
GGT >50 U/L	0.19 (-0.30;0.68)
Fatty liver index	0.02 (-0.20;0.23)
Fatty liver index to rule in hepatic steatosis	-0.01 (-0.31;0.29)
Fatty liver index to rule out hepatic steatosis	0.03 (-0.31;0.37)
Hepatic steatosis index	0.03 (-0.97;1.03)
Hepatic steatosis index to rule in hepatic steatosis	0.18 (-0.16;0.51)
Hepatic steatosis index to rule out hepatic steatosis	0.17 (-0.19;0.52)
AST/ALT ratio	0.00 (-0.50;0.51)
AST/ALT ratio >1	0.11 (-0.30;0.53)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyl transferase

[a] All markers were standardized and all the continuous markers were naturally logarithmically transformed

Sensitivity analyses

Of the study population, 34% of the men and 18% of the women reported alcohol intake of more than 20 grams per day. Exclusion of these participants from the analysis did not markedly change our results (data not shown).

Of the study population, 6% of the men and 4% of the women did not have a CVD event and were censored before completing 10 years of follow-up. When these participants were excluded from the analyses, there was no marked effect on our results (data not shown).

Pooled Cohort Equations

In men, similar to adding the markers to the Framingham risk score, none of the markers of hepatic steatosis improved the risk prediction when they were added to the calibrated Pooled Cohort Equations, both one by one and all at once using backwards selection.

The C-statistic of the calibrated Pooled Cohort Equations for women was 0.73 and the calibration slope was 0.96. The following markers were a predictor when added to the calibrated Pooled Cohort Equations for women: ALT, AST, GGT, AST/ALT ratio or AST/ALT ratio >1. Risk prediction did not improve when the fatty liver index was added to the model. The C-statistic of the models ranged between 0.73 and 0.74 and the calibration slope ranged between 0.96 and 1.02. After adding all markers of hepatic steatosis to the Pooled Cohort Equations and applying backwards selection, ALT and GGT were retained in the model. The C-statistic of this model was 0.72 and the calibration slope was 0.98. Detailed information about the performance of the models is shown in Supplemental table 1.

Table 3 Cox proportional hazard model coefficients, discrimination, calibration and reclassification of the addition of markers of hepatic steatosis to the Framingham risk score in women participating in the EPIC-NL cohort

Markers added to the Framingham risk score[a]	Coefficient (95% CI)	Area under the curve (95% CI)	Calibration slope (95% CI)
Single marker			
ALT	-0.51 (-0.77; -0.25)	0.71 (0.69;0.73)	1.05 (0.88;1.21)
ALT >45 U/L	-0.18 (-1.07;0.71)		
AST	-0.23 (-0.65;0.20)		
AST >35 U/L	0.58 (-0.26;1.41)		
GGT	0.25 (0.06;0.44)	0.71 (0.69;0.73)	0.94 (0.82;1.05)
GGT >50 U/L	0.18 (-0.25;0.60)		
Fatty liver index	0.10 (0.00;0.20)	0.71 (0.69;0.73)	0.92 (0.83;1.01)
Fatty liver index to rule in hepatic steatosis	0.06 (-0.15;0.28)		
Fatty liver index to rule out hepatic steatosis	-0.19 (-0.37; -0.02)	0.71 (0.69;0.73)	0.93 (0.87;0.98)
Hepatic steatosis index	-0.26 (-0.92;0.40)		
Hepatic steatosis index to rule in hepatic steatosis	0.08 (-0.10;0.26)		
Hepatic steatosis index to rule out hepatic steatosis	0.30 (0.05;0.55) [b]		
AST/ALT ratio	0.82 (0.46;1.18)	0.72 (0.70;0.74)	1.04 (0.97;1.10)
AST/ALT ratio >1	0.64 (0.40;0.88)	0.72 (0.70;0.74)	1.04 (0.96;1.11)
Backward selection			
ALT	-0.86 (-1.17;-0.56)	0.72 (0.70;0.74)	0.98 (0.91;1.06)
GGT	0.57 (0.35;0.80)		

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyl transferase; NRI, net reclassification index

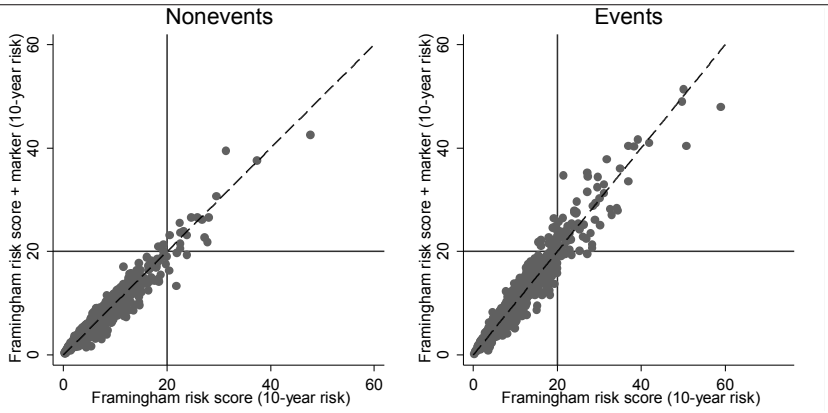
	Threshold 20%			Threshold 10% and 20%	
	NRI non-event (%)	NRI event (%)	Δ Net benefit (%)	NRI non-event (%)	NRI event (%)
	0	0	0.02	1	0
	0	1	-0.03	-1	2
	0	1	-0.05	-1	2
	0	1	-0.05	-1	2
	0	0	0	0	1
	0	1	0.08	0	1
	0	3	0.12	-1	7

[a] All markers were standardized and all the continuous markers were naturally logarithmically transformed

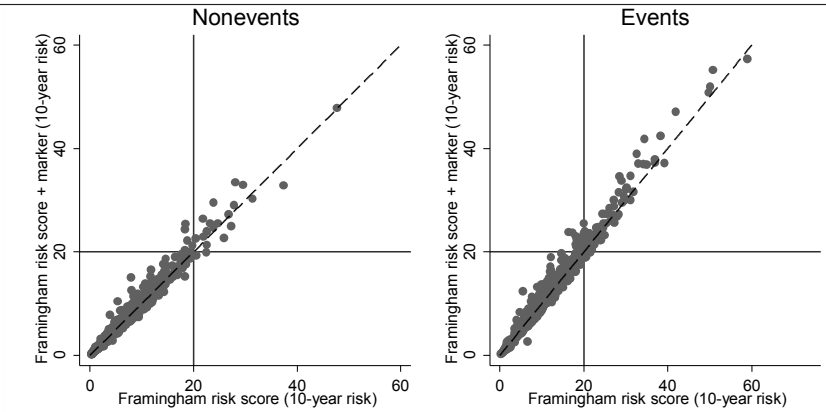
[b] Hepatic steatosis index to rule out hepatic steatosis predicted cardiovascular disease in the opposite direction than expected. Therefore, we did not evaluate the quality of this model

Figure 1 Reclassification graphs for the addition of markers of hepatic steatosis to the Framingham risk score, for participants with and without a cardiovascular event. The threshold of a 10-year risk of 20% is indicated with solid lines, the dashed line indicates no improvement. Correct reclassification of participants without an event is indicated by smaller predicted probabilities, the dots will lie below the diagonal line. Correct reclassification of participants with an event is indicated by larger predicted probabilities, the dots will lie above the diagonal line. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase

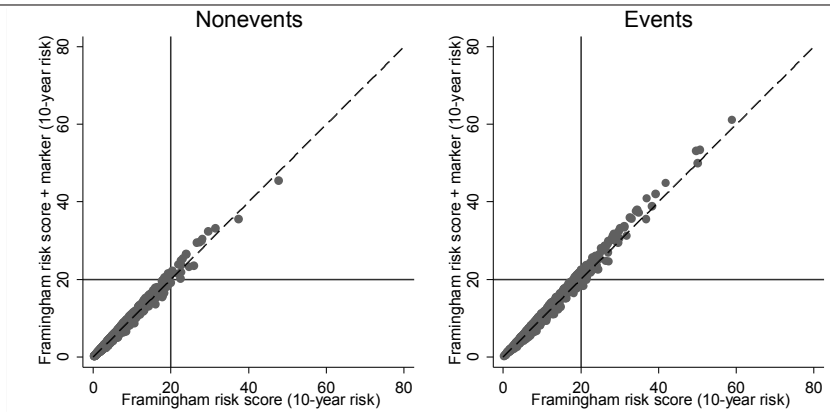
A: ALT



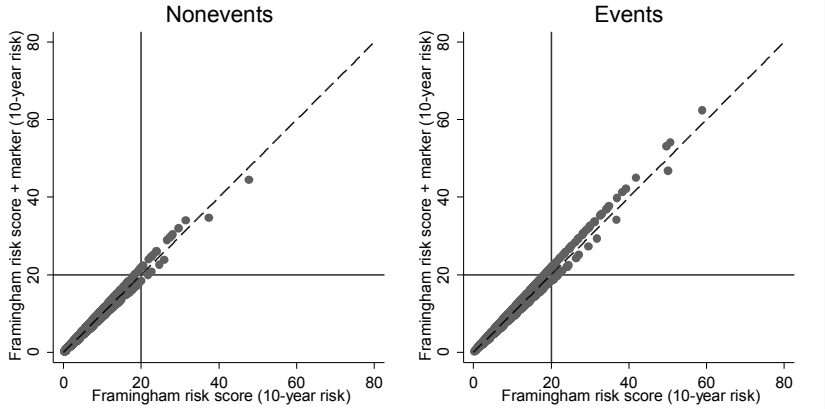
B: GGT



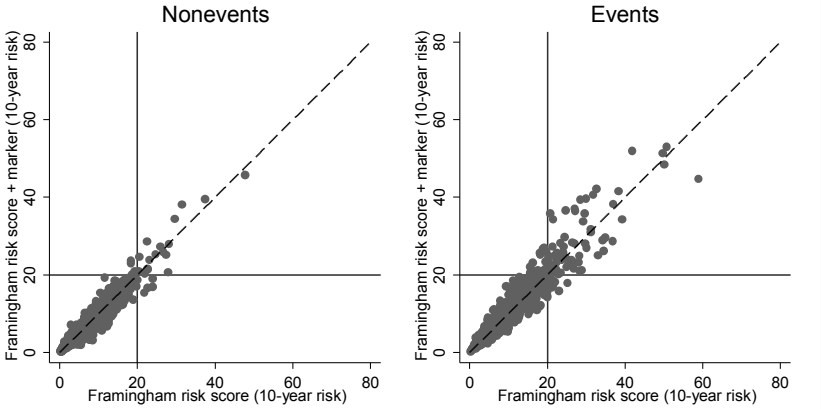
C: Fatty liver index



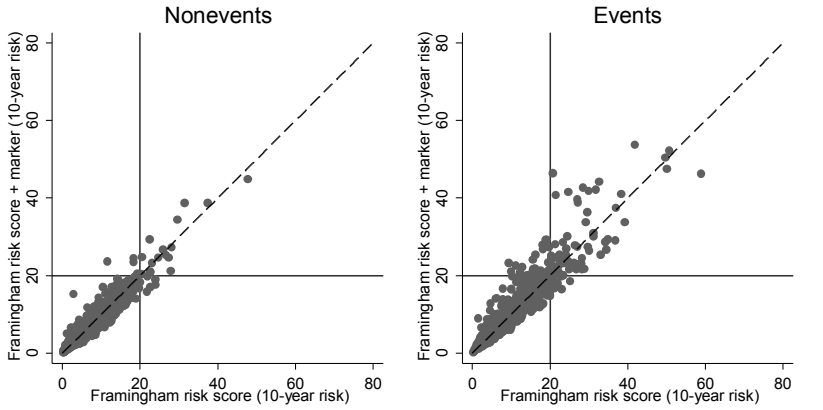
D: Fatty liver index to rule out hepatic steatosis



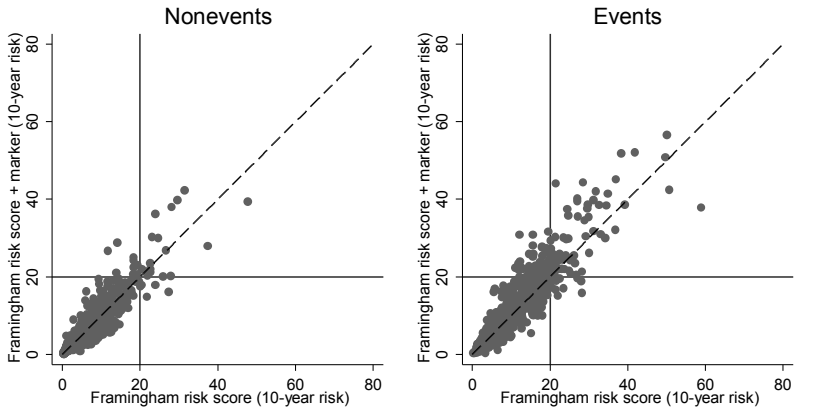
E: AST/ALT ratio



F: AST/ALT ratio >1



G: GGT, ALT



Discussion

In this large prospective cohort study, the addition of markers of hepatic steatosis to the established risk estimation systems Framingham risk score and Pooled Cohort Equations slightly improved the prediction of 10-year CVD risk in women, but not in men. However, this resulted in only a small improvement in discrimination, calibration and reclassification.

In a previous study, the AST/ALT ratio was a predictor of cardiovascular risk in men when the ratio was added to the risk prediction systems Framingham risk score or QRISK, but not in women.(51) However, the addition of the AST/ALT ratio did not improve discrimination. In contrast, in our study, the AST/ALT ratio was a predictor of cardiovascular risk in women only, also without improvement in discrimination. These discrepant results might be due to chance.

In contrast to our findings, a previous prospective study in 6,997 men stated that the addition of GGT to the Framingham risk score may be useful to improve cardiovascular risk prediction.(49) The C-statistic was significantly increased when GGT was added to the model, though the improvement was very small (C-statistic increased from 0.725 to 0.729). No information about calibration or benefit for clinical practice was provided. Another study in 6,969 men and women showed no improvement in discrimination and reclassification when GGT was added to the risk factors in the Framingham risk score.(50) In our study, the addition of GGT did not improve cardiovascular risk prediction in men and we judged the improvement in women not clinically relevant.

A strength of this study is the large study population with a large number of CVD events and the availability of information on all established risk factors and the markers of hepatic steatosis. Another strength is that we investigated the added value of the markers in two different established risk estimation systems, which allowed us to examine the robustness of our findings.

A limitation of this study is the missing data, specifically missing blood samples. These blood samples were missing in cases outside the sub-cohort with an event after 31 December 2005 or an event other than coronary heart disease or stroke or due to an unsuccessful blood draw or failed laboratory analysis. The participants with complete blood samples were representative of all participants, which allowed us to weight these participants in the analysis to represent the full cohort.

Another limitation of this study is the absence of information about the reference standards of hepatic steatosis and fibrosis, proton magnetic resonance spectroscopy and liver

biopsy. The markers of hepatic steatosis used in this study may be not accurate enough to improve risk prediction. The serological markers of hepatic steatosis used in this study are often not very sensitive and specific.(124) Moreover, the combination scores have an AUC between 0.81 and 0.84.(117, 125) Other, more accurate, (bio)markers of hepatic steatosis may have an added value in addition to the established risk factors. However, these markers are currently not measured on a large scale.

Compared with the 1990s, when the participants of the EPIC-NL cohort were recruited, the prevalence of NAFLD has doubled in the last years, in parallel with the increasing prevalence of obesity.(46) The predictive value of markers of hepatic steatosis may be different in a population with a higher prevalence of NAFLD. Furthermore, our study population was predominantly white. Therefore, our results need to be confirmed in other ethnicities.

Different pathophysiological pathways link NAFLD with CVD, including atherogenic dyslipidaemia and insulin resistance.(47) In most established risk estimation systems, cholesterol concentrations are included as marker of dyslipidaemia and diabetes as marker for insulin resistance.(28) As a result, markers of NAFLD may not have added value in addition to the cholesterol concentrations and diabetes.

Interestingly, in our study, some of the markers were predictors of cardiovascular disease in women, but not in men. It is often reported that the strength of cardiovascular risk factors is different between men and women.(126) This emphasizes the importance of making different models for men and women. However, due to source population of the Prospect cohort, more women were included in this study. As a result, predictors with a small effect size may be significant in women and not in men. Nevertheless, this will not change our conclusion that none of the markers of hepatic steatosis showed a clinically meaningful improvement in the prediction of CVD, since predictors with a small effect will probably not affect clinical care.

In conclusion, our findings suggest that indirect and easily accessible markers of hepatic steatosis do not improve cardiovascular risk prediction in addition to the established cardiovascular risk factors. Future research is needed to examine the added predictive value of other markers of hepatic steatosis and also of markers of NASH and advanced fibrosis or cirrhosis.

Supplemental table 1 The cox proportional hazards model coefficients of the markers of hepatic steatosis when added to the Pooled Cohort Equations in men participating in the EPIC-NL cohort

Markers added to the Pooled Cohort Equations[a]	Coefficient (95% CI)
Single marker	
ALT	-0.19 (-0.70;0.32)
ALT >45 U/L	-1.09 (-3.48;1.29)
AST	-0.19 (-0.77;0.40)
AST >35 U/L	0.02 (-1.10;1.13)
GGT	-0.27 (-0.70;0.16)
GGT >50 U/L	-0.19 (-0.83;0.45)
Fatty liver index	-0.16 (-0.44;0.11)
Fatty liver index to rule in hepatic steatosis	-0.26 (-0.64;0.13)
Fatty liver index to rule out hepatic steatosis	0.07 (-0.40;0.53)
Hepatic steatosis index	-0.89 (-2.31;0.54)
Hepatic steatosis index to rule in hepatic steatosis	-0.01 (-0.44;0.43)
Hepatic steatosis index to rule out hepatic steatosis	0.24 (-0.21;0.70)
AST/ALT ratio	0.11 (-0.56;0.78)
AST/ALT ratio >1	0.17 (-0.34;0.68)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyl transferase

[a] All markers were standardized and all the continuous markers were naturally logarithmically transformed

Supplemental table 2 Cox proportional hazard coefficients, discrimination, calibration and reclassification of the addition of markers of hepatic steatosis to the Pooled Cohort Equations in women participating in the EPIC-NL cohort

Markers added to the Pooled Cohort Equations[a]	Coefficient (95% CI)	Area under the curve (95% CI)	Calibration slope (95% CI)
Single marker			
ALT	-0.59 (-0.93;-0.25)	0.73 (0.70;0.75)	1.02 (0.83;1.21)
ALT >45 U/L	-0.87 (-2.38;0.64)		
AST	-0.61 (-1.15;-0.06)	0.73 (0.70;0.75)	0.98 (0.81;1.15)
AST >35 U/L	-0.31 (-1.48;0.86)		
GGT	0.30 (0.05;0.54)	0.73 (0.70;0.75)	0.96 (0.76;1.16)
GGT >50 U/L	0.29 (-0.22;0.79)		
Fatty liver index	0.08 (-0.05;0.21)		
Fatty liver index to rule in hepatic steatosis	0.15 (-0.12;0.43)		
Fatty liver index to rule out hepatic steatosis	-0.20 (-0.42;0.03)		
Hepatic steatosis index	-0.40 (-1.27;0.47)		
Hepatic steatosis index to rule in hepatic steatosis	0.10 (-0.13;0.34)		
Hepatic steatosis index to rule out hepatic steatosis	0.38 (0.07;0.69)[b]		
AST/ALT ratio	0.69 (0.21;1.16)	0.73 (0.70;0.75)	0.98 (0.82;1.14)
AST/ALT ratio >1	0.54 (0.23;0.86)	0.73 (0.70;0.75)	0.98 (0.83;1.14)
Backward selection			
ALT	-1.00 (-1.38;-0.61)	0.74 (0.71;0.76)	1.00 (0.84;1.15)
GGT	0.68 (0.38;0.97)		

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyl transferase; NRI, net reclassification index

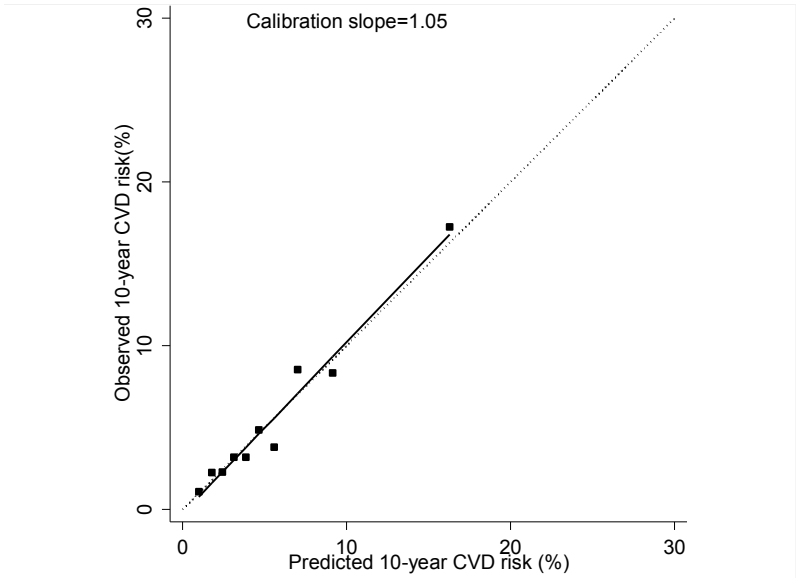
	Threshold 7.5%			Threshold 5% and 7.5%	
	NRI non-event (%)	NRI event (%)	Δ Net benefit (%)	NRI non-event (%)	NRI event (%)
	0	0	0	0	-4
	0	-4	0	0	-7
	0	7	0.11	-1	14
	-1	0	0	0	-7
	-1	0	0	0	-7
	-1	4	0.02	-1	0

[a] All markers were standardized and all the continuous markers were naturally logarithmically transformed

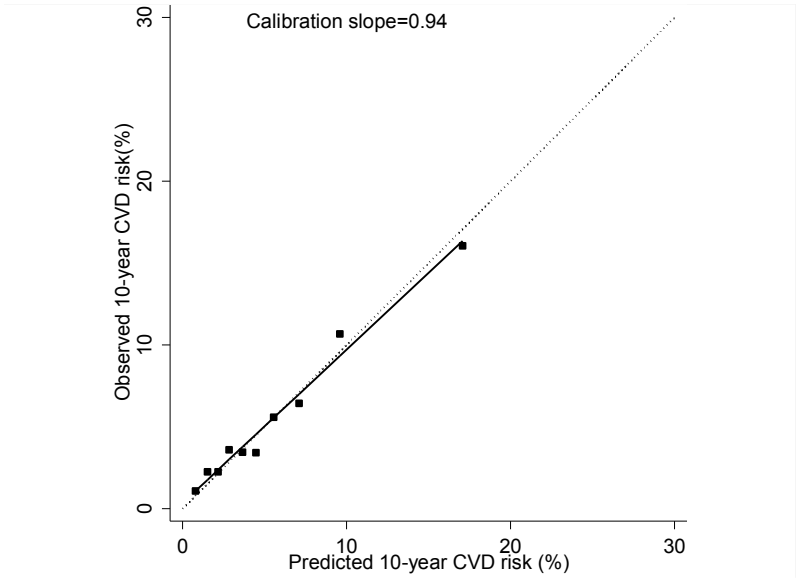
[b] Hepatic steatosis index to rule out hepatic steatosis predicted cardiovascular disease in the opposite direction than expected. Therefore, we did not evaluate the quality of this model

Supplemental figure 1 Calibration curves of the Framingham risk score + markers of hepatic steatosis based on the predicted and observed cardiovascular risk, in deciles of the predicted risk. The solid line represents the calibration slope. The dashed line represents the ideal prediction.
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CVD, cardiovascular disease; GGT, gamma-glutamyl transferase

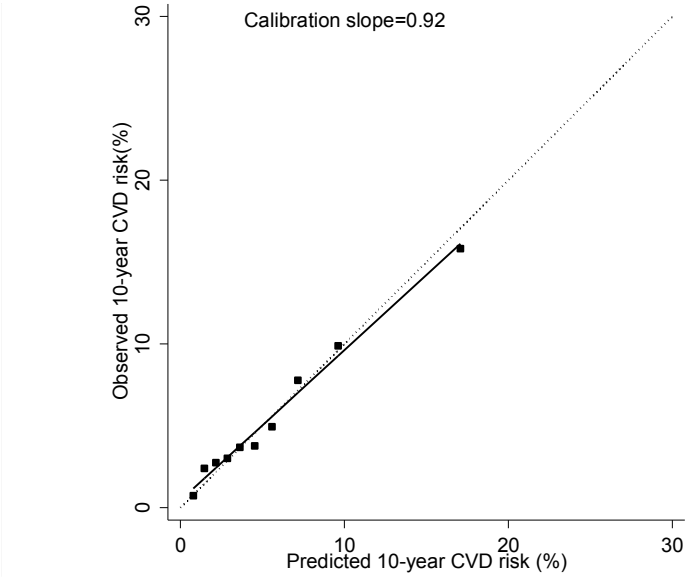
A: ALT



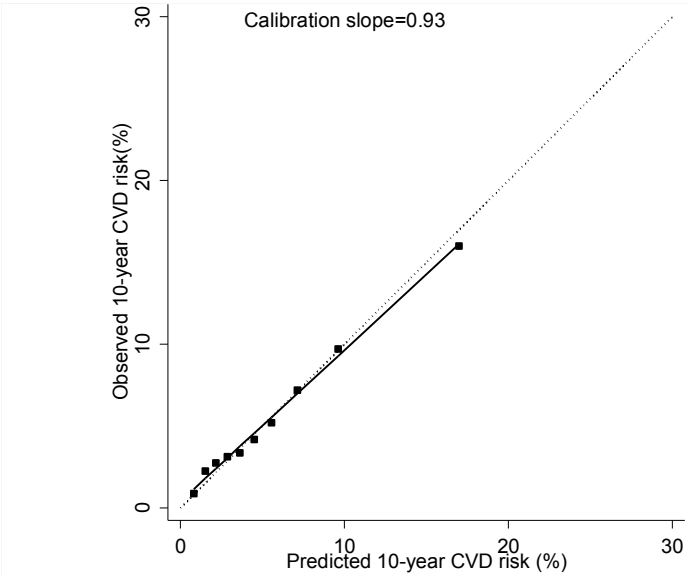
B: GGT



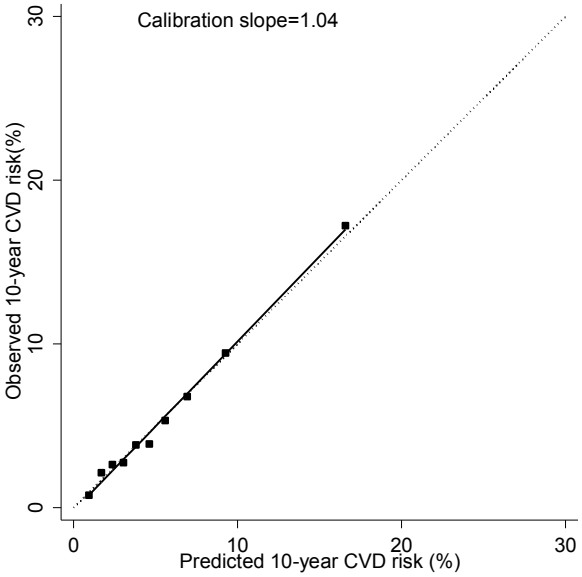
C: Fatty liver index



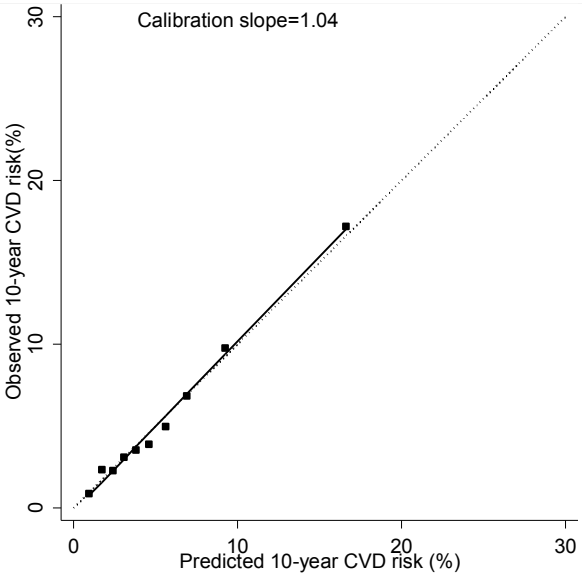
D: Fatty liver index to rule out hepatic steatosis



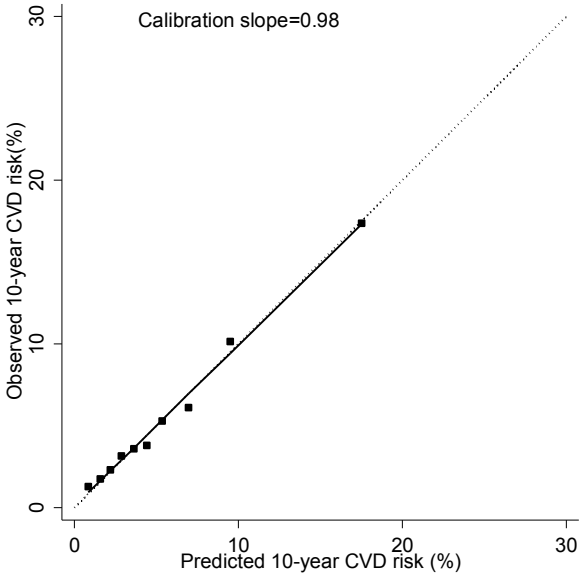
E: AST/ALT ratio



F: AST/ALT ratio >1

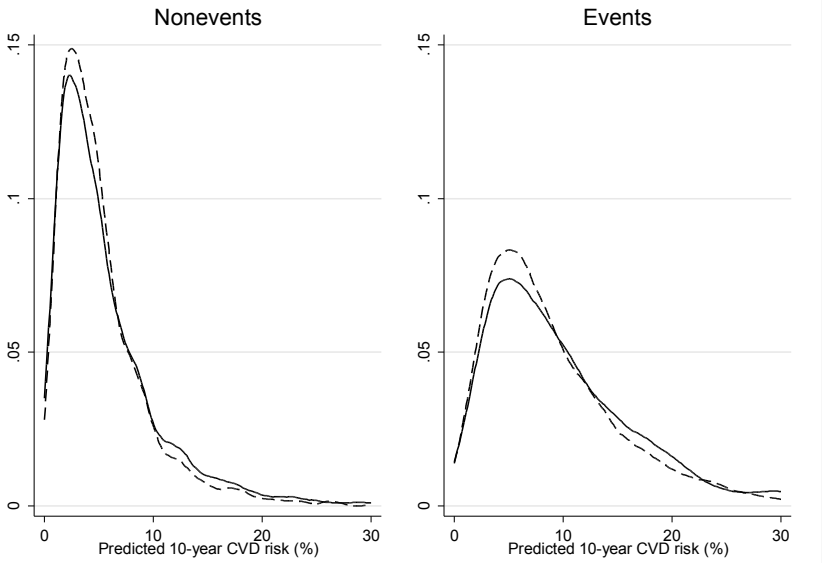


G: GGT, ALT

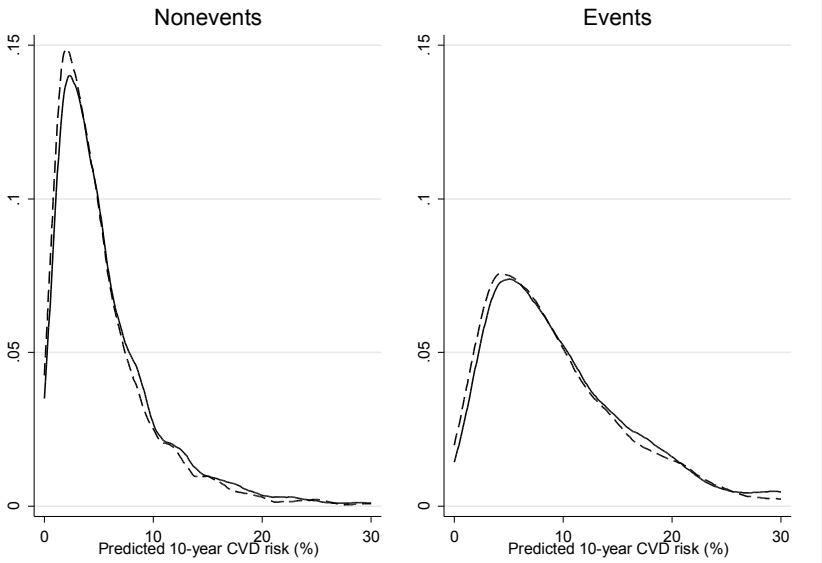

Calibration slope=0.98
Observed 10-year CVD risk(%)
Predicted 10-year CVD risk (%)
0
10
20
30

Supplemental figure 2 Density plots of the predicted 10-year cardiovascular risk, for participants with and without a cardiovascular event. The solid line represents the calibrated Framingham risk score. The dashed line represents the Framingham risk score + markers of hepatic steatosis.
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CVD, cardiovascular disease; GGT, gamma-glutamyl transferase

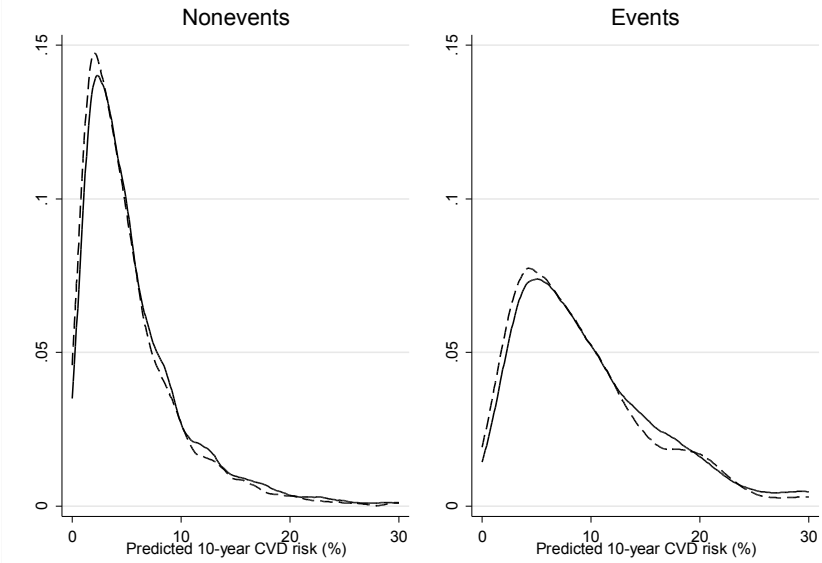
A: ALT



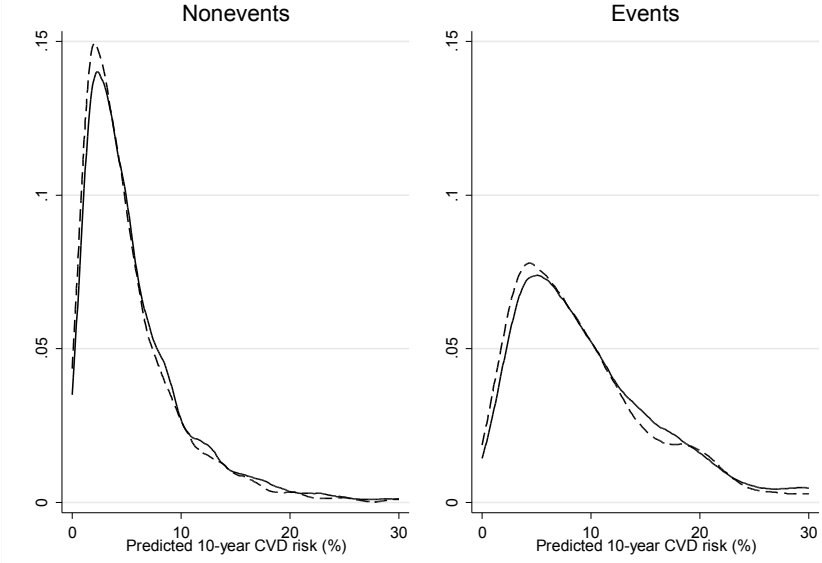
B: GGT



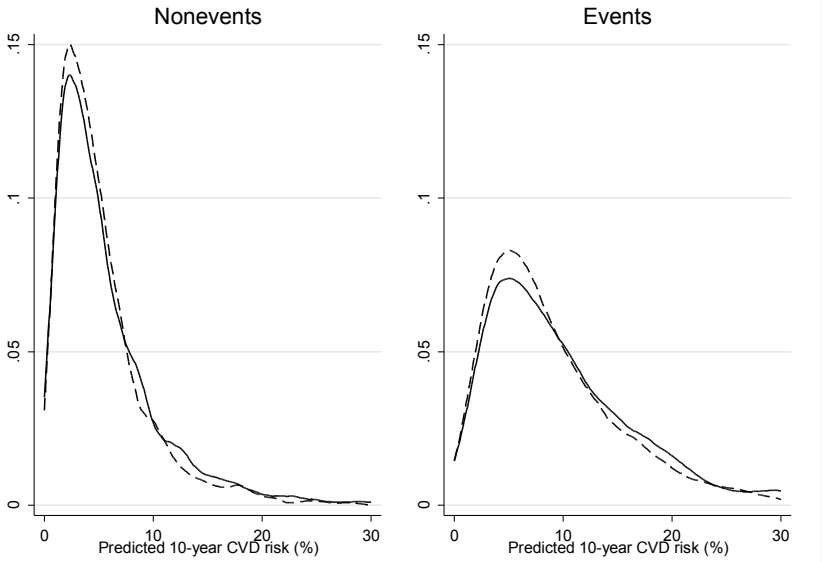
C: Fatty liver index



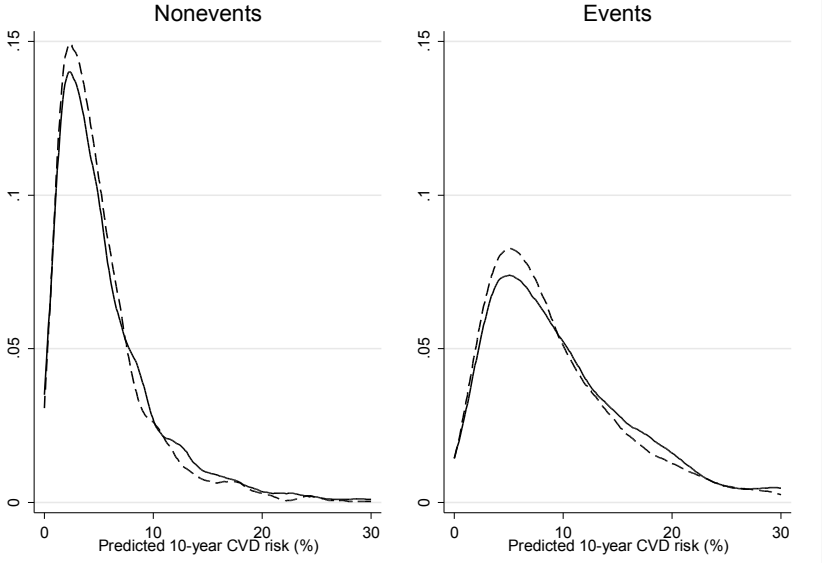
D: Fatty liver index to rule out hepatic steatosis



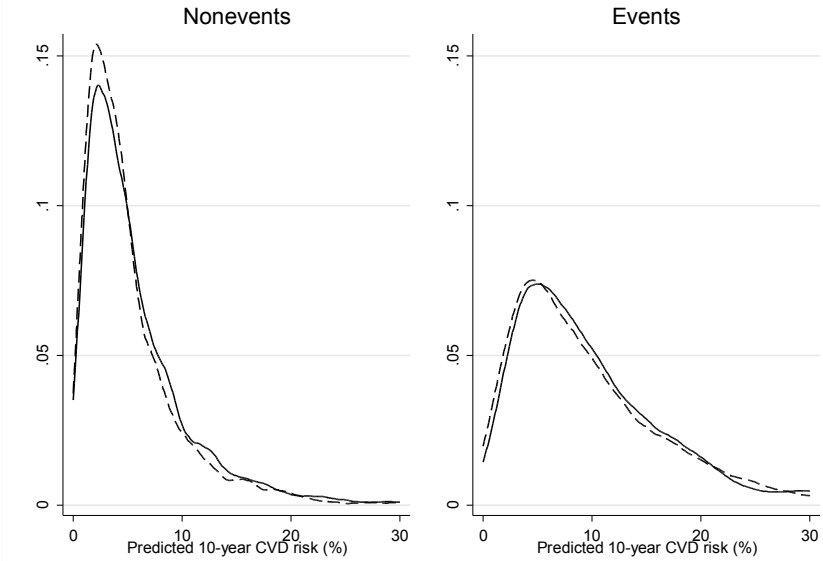
E: AST/ALT ratio



F: AST/ALT ratio >1



G: GGT, ALT


Nonevents
Events
.15
.1
.05
0
0
10
20
30
Predicted 10-year CVD risk (%)