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Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study

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ABSTRACT

Objectives To investigate the associations of the levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) at baseline and their changes over time with mortality.

Design Cohort study.

Setting, participants, and outcome measures We analyzed the data of 484,472 individuals from the National Health Insurance Service-National Health Screening cohort (2002–2013). We used two exposure indices: 1) deciles of baseline ALT, AST, and GGT levels measured in 2002 or 2003 and 2) deciles of changes in ALT, AST, and GGT levels over a 4-year period (2002–2006 or 2003–2007). We constructed Cox and cause-specific proportional hazard models adjusted for potential confounders to evaluate the associations of these exposure indices with all-cause and cause-specific mortality (2008–2013), respectively.

Results We found non-monotonic dose-response associations between the baseline levels of ALT and AST and all-cause mortality. Meanwhile, we found a monotonic non-linear association between the baseline levels of GGT and all-cause mortality. Compared with the 9th, 6th, and 4th deciles of changes in ALT (8–13 U/L), AST (1 U/L), and GGT (-3 to -2 U/L) over time, respectively, the risks of all-cause mortality increased in both the higher and lower deciles of changes in the corresponding liver enzyme levels. These non-monotonic dose-response associations between changes in the liver enzyme levels and mortality remained when analyses were stratified by the medians or quartiles of the baseline liver enzyme levels.

Conclusions The levels of liver enzymes at baseline and over time showed non-linear associations with mortality. These results suggest that both the baseline liver enzyme levels and changes in the levels over time can be used to predict mortality.

Key words

Alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, mortality, non-linear associations

Article Summary

Strengths and limitations of this study

● First study to report non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality.

● Conducted longitudinal analyses using a large-scale cohort constructed from national administration data, which has a negligible follow-up loss.

● Identifed the dates and causes of death using a national database maintained by Statistics Korea, which covers all individuals residing in the Republic of Korea and has high accuracy (>90%).

● Information on albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels was not available.

● Used claims data in which information on sociodemographic factors, lifestyles, past medical histories, and family histories is insufficient.

INTRODUCTION

Serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) are highly sensitive to liver dysfunction and damage.[1] Because assays for these liver enzymes are cost-effective, they are widely used during general health check-ups worldwide.[2,3]

Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality (reviewed by Kunstor et al.[3]). To explain this inconsistency, recent studies suggested non-monotonic dose-response associations between liver enzyme levels and mortality, although studies investigating these associations remain scarce.[3,7,8]

Liver enzyme levels can increase due to factors such as liver damage and injury and can decrease due to factors such as hepatic aging, frailty, and reduced hepatic blood circulation.[9,10] Therefore, changes (increases or decreases) in the levels of these enzymes over time may be associated with higher mortality independent of baseline liver enzyme levels. However, to our knowledge, no study has explored the possibility of non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality.

Therefore, in the present study, we hypothesized that not only higher baseline liver enzyme levels but also lower baseline levels are associated with higher mortality. In addition, we hypothesized that the increase or decrease in liver enzyme levels over time is also associated

with higher mortality independent of baseline liver enzyme levels. We evaluated these hypotheses using a longitudinal study design with a large-scale (n > 500,000) national health screening cohort in which serum liver enzyme levels were repeatedly measured.

METHODS

Study population

The present study used data from the National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS) of the Republic of Korea. Detailed information on the NHIS-HEALS has been presented elsewhere.[11] Briefly, the NHIS-HEALS is a de-identified cohort released to researchers by the NHIS for the purpose of public research. Several epidemiological studies have been published using this data.[12–14] The NHIS-HEALS contains the data of 514,866 individuals, representing a 10% random sample of all participants in the National Health Screening Program between 2002 and 2003 (data collected during this period comprise the baseline) who were followed up to December 31, 2013. In the Republic of Korea, all individuals aged ≥40 years are invited to participate at least every 2 year in this general, free-of-charge health screening program conducted at designated healthcare institutions that meet the quality standards set by the Framework Act on Health Examinations. The rate of participation in the National Health Screening Program was 43.2% in 2003, and increased gradually to 74.8% in 2014.[14] The prevalence rates of common diseases such as hypertension and type 2 diabetes mellitus in the NHIS-HEALS are generally similar to those reported in a nationally representative sample.[11]

The NHIS-HEALS includes various types of information, such as sociodemographic factors, lifestyles, past medical histories, and family histories (collected from self-reported questionnaires during the national health screening), health screening results (from physical examinations and clinical laboratory tests during the national health screening), healthcare usage (from claims data), and dates and causes of death (from Statistics Korea).

In the present study, from a total of 514,866 individuals included in the NHIS-HEALS, we sequentially excluded those who died between 2002 and 2007 (n = 13,278, 2.6%), those without any liver enzyme data between 2002 and 2003 (n = 494, 0.1%), and those with hepatitis or chronic liver disease up to 2007 (n = 16,622, 3.2%). Therefore, 484,472 individuals (94.1%) were included in the final analysis. The Institutional Review Board (IRB) of Seoul National University Hospital reviewed and approved the study protocol (IRB no. E-1804-045-936). Because the present study used claims data released to researchers after de-identification process, which is performed by the NHIS, the IRB of Seoul National University Hospital waived the requirement of informed consent for study participation. All experiments were conducted in accordance with the relevant guidelines and regulations.

Patient and public involvement

Patients were not recruited or involved in this study.

Exposures

We used two exposure indices: 1) deciles of the baseline levels of ALT, AST, and GGT measured in 2002 or 2003 and 2) deciles of changes in the ALT, AST, and GGT levels over a 4-year period (2002–2006 or 2003–2007) (figure 1). The first exposure index was used in an analysis of the association between baseline liver enzyme levels and mortality, while the second was used in an analysis of the association between changes in liver enzyme levels and mortality. If both the earlier and later exposure indices were available, the latter (baseline liver enzyme levels in 2003 or changes in liver enzyme levels between 2003 and 2007) was assigned as the exposure index for each individual.

Outcomes

The outcomes of the present study were all-cause and cause-specific mortality occurring between January 1, 2008 and December 31, 2013 (figure 1). Information on the dates and causes of death was obtained from Statistics Korea and merged with other data by the NHIS via personal identification numbers. The various causes of death were coded according to the International Classification of Disease, 10th Revision (ICD-10). For this analysis, we considered deaths from cardiovascular disease (defined as ICD-10 codes I20–I25, I50, and I60–I70), cancer (C00–C97), diabetes mellitus (E10–E14), and liver disease (B15–B19 and K70–K77), according to previous studies.[7,8,15]

Covariates

Based on previous reports and existing biomedical knowledge,[3,7–9,15–17] we identified the following potential confounders and included them as covariates for further analyses: age (years), sex, household income (decile), smoking status (non-smoker, ex-smoker, or smoker), alcohol consumption (none, ≤1–2, or ≥3–4 times/week), physical activity (did not exercise, ≤1–2, or ≥3–4 times/week), body mass index (<18.5, 18.5–22.9, 23–24.9, 25–29.9, or ≥30 kg/m^2), systolic blood pressure (<120, 120–139.9, or ≥140 mmHg), diastolic blood pressure (<80, 80–89.9, or ≥90 mmHg), fasting glucose levels (<70, 70–99.9, 100–125.9, or ≥126 mmHg), history of heart disease (yes or no), history of stroke (yes or no), and history of cancer (yes or no). Information about these variables was collected from the National Health Screening Program at baseline (2002–2003). We created a missing indicator category for the missing values (0.03–3.4%) of the categorical variables (table 1).

Statistical analysis

We constructed Cox proportional hazard models adjusted for the above-mentioned covariates to investigate the associations of exposure indices (deciles of baseline liver enzyme levels and deciles of changes in liver enzyme levels) with all-cause mortality. Since the associations between changes in liver enzyme levels and mortality may differ according to the baseline liver enzyme levels, we also divided the study population into those with values above and below the medians or quartiles of the baseline levels of ALT, AST, and GGT. We then assessed the associations between deciles of changes in the liver enzyme levels and all-cause mortality in each stratum.

We evaluated the non-linearity of the associations (non-monotonic dose-response or monotonic non-linear associations) by 1) visually inspecting the shapes of the associations in the analysis using categorized exposure indices and 2) testing the squared-terms of the log2-transformed continuous baseline or changes in liver enzyme levels that were added to Cox models including the log2-transformed baseline or changes in liver enzyme levels and the same covariates.

To evaluate the associations of deciles of the baseline and changes in liver enzyme levels with cause-specific mortality (i.e., deaths from cardiovascular disease, cancer, diabetes mellitus, and liver disease), we constructed cause-specific proportional hazard models adjusted for the same covariates. In these competing risk analyses, we estimated cause-specific hazards rather than sub-distribution hazards, because the hazard ratios (HRs) from cause-specific proportional hazard models would be easily interpretable and could be used to draw public health implications.[18,19]

In a sensitivity analysis, we repeated all analyses using sex-specific cut-points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-points, to confirm the robustness of the results.[7]

In both the Cox and cause-specific proportional hazard models, the follow-up duration was calculated in months from January 1, 2008 to the date of death or December 31, 2013 (if death did not occur). For analyses using categorized exposure indices, we designated categories with the lowest betas for each outcome of interest in the association analysis as the referent categories. We used SAS version 9.4 (SAS Institute Inc.) for all analyses.

RESULTS

Of the 484,472 study participants, the mean age was 53.0 years (range: 40–80 years), and 53.3% were men. A larger proportion of participants had higher income levels (household income deciles 9–10, 33.3% vs. deciles 0–2, 15.9%). A majority of the study participants were non-smokers (65.5%), did not drink alcohol (56.1%), and did not exercise regularly (55.8%). Most participants had no history of heart disease, stroke, or cancer (table 1).

Compared with the 4th decile of baseline ALT levels (17–18 U/L), the risk of all-cause mortality was higher in both the higher and lower baseline ALT deciles (10th decile: HR = 1.53, 95% confidence interval [CI]: 1.44, 1.62; 1st decile: HR = 1.16, 95% CI: 1.10, 1.23). Similarly, compared with the 4th decile of baseline AST levels (21 U/L), the risk of all-cause mortality was also higher in both the higher and lower baseline AST deciles (10th decile: HR = 1.70, 95% CI: 1.59, 1.81; 1st decile: HR = 1.15, 95% CI: 1.07, 1.24). When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). We confirmed the non-linearity of these associations by testing the squared-terms of the log2-transformed ALT, AST, and GGT levels added to the Cox models (all *p*-values for the squared-terms < 0.0001).

We also assessed the associations of deciles of changes in the liver enzyme levels over a 4-year period with all-cause mortality. Compared with the 9th decile of ALT changes (8–13 U/L), the 6th decile of AST changes (1 U/L), and the 4th decile of GGT changes (-3 to -2 U/L), respectively, the risk of all-cause mortality was higher in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10th decile: HR = 1.36, 95% CI: 1.24, 1.48, 1st decile: HR =

1.46, 95% CI: 1.34, 1.59 for ALT; 10th decile: HR = 1.55, 95% CI: 1.40, 1.71, 1st decile: HR = 1.53, 95% CI: 1.38, 1.69 for AST; 10th decile: HR = 1.71, 95% CI: 1.56, 1.88, 1st decile: HR = 1.67, 95% CI: 1.52, 1.84 for GGT) (table 3). We then tested the squared-terms of the log2-transformed changes in ALT, AST, and GGT and confirmed the non-linearity of the associations between the changes in liver enzyme levels and mortality (all *p*-values for the squared-terms < 0.0001).

When we performed stratified analyses by the medians or quartiles of the baseline liver enzyme levels, the same non-monotonic dose-response associations between the deciles of changes in liver enzyme levels and all-cause mortality remained in each stratum (figure 2; online supplementary figure 1), similar to the results of the non-stratified analysis (table 3).

In analyses of the associations between the deciles of baseline liver enzyme levels and cause-specific mortality (mortality due to cardiovascular disease, cancer, diabetes mellitus, and liver disease), we found non-monotonic dose-response associations of baseline ALT and AST levels with mortality due to cardiovascular disease, cancer, and diabetes mellitus, whereas we found monotonic non-linear associations in other cases (online supplementary figure 2). When we analyzed the associations between the deciles of changes in the liver enzyme levels and cause-specific mortality, we found non-monotonic dose-response associations in all cases (online supplementary figure 3).

In sensitivity analyses based on sex-specific deciles of the baseline and changes in liver enzyme levels, the results remained robust and did not change appreciably (data not shown).

DISCUSSION

In the present study using the large-scale national health screening cohort, we found non-linear (non-monotonic dose-response or monotonic non-linear) associations of baseline liver enzyme levels and changes in these levels over time with both all-cause and cause-specific mortality. The non-monotonic dose-response associations between changes in the liver enzyme levels and mortality remained after stratifying the study population into subgroups according to the baseline liver enzyme levels.

Although some previous studies have reported inverse associations between ALT levels and all-cause mortality,[9,17,20] other studies have demonstrated positive associations.[21,22] Similarly, some studies have described positive associations between AST levels and all-cause mortality,[21,23,24] whereas other studies found no such associations.[25,26] This heterogeneity may be explained by the increased risk of mortality associated with both higher and lower baseline levels of ALT and AST (non-monotonic dose-response associations), as demonstrated in the present study.[3,7,8] Meanwhile, most previous studies have reported positive associations between baseline GGT levels and all-cause mortality.[15,17] The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.

In the present study, larger changes (both increases and decreases) in the ALT, AST, and GGT levels over a 4-year period were associated with a higher risk of all-cause mortality when compared with smaller changes. These non-linear associations remained even after the participants were stratified according to the baseline liver enzyme levels. These results suggest

that changes in liver enzyme levels over time as well as baseline levels can be used to predict mortality and assess risk by clinicians and public health practitioners. To our knowledge, this is the first study to demonstrate non-monotonic dose-response associations between changes in liver enzyme levels and mortality; further studies are warranted to confirm the findings.

In a study based on a representative U.S. population data, lower baseline ALT levels were found to be associated with higher risks of cardiovascular disease, cancer, and liver disease mortality, whereas higher ALT levels were only associated with a higher risk of liver disease mortality.[7] In another study based on a representative sample of the U.S. population, higher ALT levels were associated with a higher risk of liver disease mortality, whereas higher GGT levels were associated with higher risks of cancer, diabetes mellitus, and liver disease mortality.[15] A study using data from a prospective elderly cohort reported an association of higher AST levels with an increased risk of cancer mortality, as well as an association of higher GGT levels with an increased risk of cardiovascular disease mortality.[8] Although the results of the present study were generally consistent with those of previous studies,[7,8,15] some inconsistencies were also found (e.g., positive associations of baseline ALT levels with cardiovascular, cancer, and diabetes mellitus mortality in the present study but not in previous studies). These discrepancies might be attributable to factors such as differences in the accuracy of cause of death data, study populations, adjusted covariates, and analytical methods. As the associations of baseline and changes in liver enzyme levels with cause-specific mortality have not been thoroughly investigated, additional studies are needed, particularly those using changes in liver enzyme levels as explanatory variables.

Serum liver enzyme levels may increase in response to various factors, including liver damage and cell destruction (e.g., hepatocytes, biliary epithelium, and other cells of the organs such as the heart, skeletal muscle, and kidney),[2,27] and may decrease in response to factors such as age and frailty-related reductions in liver size and blood circulation.[9,10,28] Therefore, changes (both increases and decreases) in liver enzyme levels over time may reflect these conditions related to the deterioration of liver function and could thus be associated with a higher risk of mortality.

The present study has some limitations. First, although the NHIS-HEALS provides information on serum ALT, AST, and GGT levels, data from other standard liver panel tests, such as albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels, are not available. This limits our ability to assess liver function comprehensively. Second, the present study used data that were not primarily collected for the purpose of research. Therefore, information on the sociodemographic factors, lifestyles, past medical histories, and family histories are insufficient, leading to the possibility of residual confounding.

However, the present study also has notable strengths. First, to our knowledge, this is the first study to report non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality. Second, we used a large-scale cohort constructed from national administration data, which has a negligible follow-up loss. The large sample size (n = 484,472) of the present study allowed us to evaluate the potential associations with sufficient power. Third, we identified the dates and causes of death using a national database maintained by Statistics Korea, which covers all individuals residing in the Republic of Korea. The accuracy of the

recorded causes of death in this database is considered high (>90%).[29]

CONCLUSIONS

We found non-linear associations of the baseline liver enzyme levels and their changes over time with risks of mortality. The results of the present study suggest that changes in liver enzyme levels, as well as the baseline levels, can be used to predict health outcomes such as mortality and to assess risk in clinical and public health settings.

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Author Contributions

KK, JJ, HKS, CHK, HK, and YJK contributed to conception and design of the study. KNK conducted statistical analyses and wrote the initial manuscript. JJ, HKS, CHK, HK, and YJK reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Disclaimer

The views in this study are those of the authors and not necessarily those of the Seoul National University Hospital or the National Health Insurance Service of the Republic of Korea.

Competing interests

The authors declare that they have no competing interests.

Patient consent

Not required.

Ethics approval

The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (IRB no. E-1804-045-936).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

The National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS), which is used in the present study, is a de-identified cohort released to researchers by the NHIS for the purpose of public research. The data sets are provided to researchers after the study protocols are approved by the IRB of the researcher's institute and by the NHIS. Researchers can request the data sets through the website https://nhiss.nhis.or.kr/bd/ay/bdaya001iv.do.

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Table 1. Baseline sociodemographic characteristics and liver enzyme levels of the study participants (2002–2003) (n = 484,472)

Characteristics	Total	ALT (U/L)	AST (U/L)	GGT (U/L)
	n (%)	GM ± GSD		
Age (years)				
40–49	212,004 (43.8)	21.8 ± 1.7	23.5 ± 1.4	26.1 ± 2.2
50–59	140,742 (29.1)	22.8 ± 1.6	24.9 ± 1.4	26.8 ± 2.1
60–69	98,759 (20.4)	21.6 ± 1.6	25.4 ± 1.4	24.6 ± 2.0
70–80	32,967 (6.8)	19.5 ± 1.6	25.2 ± 1.4	21.6 ± 1.9
Sex				
Men	258,010 (53.3)	25.3 ± 1.7	26.1 ± 1.5	35.8 ± 2.1
Women	226,462 (46.7)	18.5 ± 1.6	22.6 ± 1.4	17.6 ± 1.8
Household income (deciles)				
0–2	76,994 (15.9)	20.8 ± 1.7	24.2 ± 1.4	23.7 ± 2.1
3–5	106,260 (21.9)	21.6 ± 1.7	24.7 ± 1.5	25.1 ± 2.1
6–8	139,763 (28.9)	22.4 ± 1.7	24.7 ± 1.4	26.6 ± 2.1
9–10	161,455 (33.3)	22.2 ± 1.7	24.0 ± 1.4	26.2 ± 2.1
Smoking status				
Non-smoker	317,199 (65.5)	20.5 ± 1.6	23.6 ± 1.4	21.4 ± 1.9
Ex-smoker	40,797 (8.4)	25.4 ± 1.7	25.8 ± 1.4	34.1 ± 2.0

Smoker	109,868 (22.7)	25.0 ± 1.7	26.0 ± 1.5	39.0 ± 2.1
Missing	16,608 (3.4)	22.0 ± 1.7	24.6 ± 1.4	25.4 ± 2.1
Alcohol consumption				
None	271,551 (56.1)	20.4 ± 1.6	23.2 ± 1.4	20.1 ± 1.8
≤1–2 times/week	151,559 (31.3)	23.4 ± 1.7	24.9 ± 1.4	31.4 ± 2.1
≥3–4 times/week	53,350 (11.0)	25.9 ± 1.7	28.9 ± 1.5	51.1 ± 2.3
Missing	8,012 (1.7)	20.9 ± 1.6	23.5 ± 1.4	21.0 ± 1.9
Physical activity				
Did not exercise	270,329 (55.8)	21.4 ± 1.7	24.3 ± 1.4	24.5 ± 2.1
≤1–2 times/week	112,413 (23.2)	23.0 ± 1.7	24.5 ± 1.4	28.5 ± 2.1
≥3–4 times/week	89,843 (18.5)	21.9 ± 1.6	24.4 ± 1.4	25.9 ± 2.1
Missing	11,887 (2.5)	21.7 ± 1.7	25.4 ± 1.4	25.2 ± 2.1
Body mass index (kg/m^2)				
<18.5	10,599 (2.2)	17.7 ± 1.6	24.6 ± 1.5	21.1 ± 2.2
18.5–22.9	171,108 (35.3)	19.1 ± 1.6	23.4 ± 1.4	21.9 ± 2.1
23–24.9	132,394 (27.3)	21.9 ± 1.6	24.0 ± 1.4	25.9 ± 2.1
25–29.9	156,051 (32.2)	25.1 ± 1.7	25.5 ± 1.4	30.1 ± 2.1
≥30	13,926 (2.9)	29.0 ± 1.8	27.6 ± 1.5	32.1 ± 2.1
Missing	394 (0.1)	21.4 ± 1.6	24.6 ± 1.4	22.2 ± 2.0
Systolic blood pressure				

(mmHg)				
<120	140,993 (29.1)	19.7 ± 1.6	22.8 ± 1.4	21.1 ± 2.0
120–139.9	214,824 (44.3)	22.3 ± 1.7	24.5 ± 1.4	26.4 ± 2.1
≥140	128,464 (26.5)	23.7 ± 1.7	26.0 ± 1.5	30.4 ± 2.2
Missing	191 (0.04)	21.1 ± 1.7	23.3 ± 1.5	24.4 ± 2.0
Diastolic blood pressure (mmHg)				
<80	191,221 (39.5)	20.1 ± 1.6	23.2 ± 1.4	21.7 ± 2.0
80–89.9	167,612 (34.6)	22.4 ± 1.7	24.6 ± 1.4	26.6 ± 2.1
≥90	125,372 (25.9)	24.2 ± 1.7	26.0 ± 1.5	31.5 ± 2.2
Missing	267 (0.1)	21.5 ± 1.7	23.4 ± 1.4	23.8 ± 2.0
Fasting glucose level (mg/dL)				
<70	8,510 (1.8)	20.3 ± 1.7	23.9 ± 1.4	23.2 ± 2.0
70–99.9	324,064 (66.9)	20.9 ± 1.6	23.9 ± 1.4	23.7 ± 2.0
100–125.9	114,597 (23.7)	23.6 ± 1.7	25.3 ± 1.5	29.1 ± 2.2
≥126	37,167 (7.7)	26.5 ± 1.7	26.1 ± 1.6	35.9 ± 2.3
Missing	134 (0.03)	19.7 ± 1.7	22.8 ± 1.7	20.7 ± 2.0
Past history of heart disease				
No	494,401 (98.6)	21.9 ± 1.7	24.4 ± 1.4	25.6 ± 2.1

Yes	7,187 (1.4)	22.4 ± 1.6	25.0 ± 1.4	26.3 ± 2.0
Past history of stroke				
No	499,352 (99.6)	21.9 ± 1.7	24.4 ± 1.4	25.7 ± 2.1
Yes	2,236 (0.5)	22.0 ± 1.6	24.6 ± 1.4	27.4 ± 2.0
Past history of cancer				
No	498,856 (99.5)	21.9 ± 1.7	24.4 ± 1.4	25.7 ± 2.1
Yes	2,732 (0.5)	20.7 ± 1.6	25.0 ± 1.4	21.2 ± 1.9

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; GM, geometric mean; GSD, geometric standard deviation

Table 2. Associations[a] between baseline liver enzyme levels (2002–2003) and all-cause mortality (2008–2013)

Decile[b]	Levels (U/L)	*n* (%)	HR	95% CI	*p*-value
ALT					
1	≤12	56,870 (11.7)	1.16	1.10, 1.23	<0.0001
2	13–14	41,904 (8.7)	1.11	1.04, 1.17	0.0014
3	15–16	46,115 (9.5)	1.07	1.01, 1.13	0.0292
4	17–18	46,272 (9.6)	Ref.	Ref.	Ref.
5	19–21	62,738 (13.0)	1.06	1.01, 1.12	0.0320
6	22–23	33,898 (7.0)	1.04	0.97, 1.11	0.2722
7	24–27	54,157 (11.2)	1.05	0.99, 1.11	0.1046
8	28–32	46,873 (9.7)	1.11	1.05, 1.18	0.0005
9	33–42	49,009 (10.1)	1.17	1.10, 1.24	<0.0001
10	≥43	46,601 (9.6)	1.53	1.44, 1.62	<0.0001
AST					
1	≤16	48,178 (10.0)	1.15	1.07, 1.24	0.0001
2	17–18	45,357 (9.4)	1.09	1.01, 1.18	0.0217
3	19–20	58,461 (12.1)	1.09	1.02, 1.17	0.0175
4	21	30,304 (6.3)	Ref.	Ref.	Ref.
5	22–23	58,761 (12.1)	1.03	0.96, 1.10	0.4151
6	24–25	52,190 (10.8)	1.06	0.99, 1.14	0.0896

7	26–27	41,805 (8.6)	1.09	1.01, 1.17	0.0185
8	28–31	58,055 (12.0)	1.08	1.01, 1.15	0.0257
9	32–37	44,515 (9.2)	1.20	1.12, 1.28	<0.0001
10	≥38	46,802 (9.7)	1.70	1.59, 1.81	<0.0001
GGT					
1	≤11	54,316 (11.2)	Ref.	Ref.	Ref.
2	12–13	36,355 (7.5)	1.01	0.94, 1.08	0.7713
3	14–16	55,121 (11.4)	1.07	1.00, 1.13	0.0412
4	17–19	49,756 (10.3)	1.10	1.04, 1.17	0.0019
5	20–23	53,476 (11.0)	1.12	1.06, 1.19	0.0002
6	24–27	41,003 (8.5)	1.16	1.09, 1.24	<0.0001
7	28–34	50,696 (10.5)	1.24	1.17, 1.32	<0.0001
8	35–45	48,116 (9.9)	1.31	1.23, 1.39	<0.0001
9	46–71	49,144 (10.1)	1.46	1.37, 1.55	<0.0001
10	≥72	46,450 (9.6)	2.05	1.93, 2.18	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

[b]Deciles for baseline levels of alanine aminotransferase, aspartate aminotransferase, and gamma

glutamyltransferase

Table 3. Associations[a] between changes in liver enzyme levels since the baseline survey (2002–2003) and all-cause mortality (2008–2013)

Decile[b]	Levels (U/L)	*n* (%)	HR	95% CI	*p*-value
ALT					
1	≤-16	28,441 (9.6)	1.46	1.34, 1.59	<0.0001
2	-15 to -9	29,670 (10.0)	1.23	1.13, 1.34	<0.0001
3	-8 to -5	34,065 (11.4)	1.11	1.02, 1.21	0.0137
4	-4 to -3	23,295 (7.8)	1.08	0.98, 1.18	0.1334
5	-2 to -1	27,637 (9.3)	1.12	1.03, 1.23	0.0127
6	0–1	28,738 (9.7)	1.11	1.02, 1.22	0.0226
7	2–4	38,771 (13.0)	1.02	0.94, 1.12	0.5916
8	5–7	27,754 (9.3)	1.01	0.92, 1.11	0.8602
9	8–13	29,713 (10.0)	Ref.	Ref.	Ref.
10	≥14	29,842 (10.0)	1.36	1.24, 1.48	<0.0001
AST					
1	≤-12	27,631 (9.3)	1.53	1.38, 1.69	<0.0001
2	-11 to -7	29,839 (10.0)	1.23	1.11, 1.36	0.0001
3	-6 to -4	33,587 (11.3)	1.09	0.98, 1.21	0.1081
4	-3 to -2	30,433 (10.2)	1.15	1.03, 1.28	0.0113
5	-1 to 0	35,094 (11.8)	1.10	0.99, 1.22	0.0773
6	1	17,637 (5.9)	Ref.	Ref.	Ref.

7	2–3	32,282 (10.8)	1.02	0.92, 1.14	0.7010
8	4–6	36,128 (12.1)	1.05	0.95, 1.17	0.3479
9	7–10	26,425 (8.9)	1.04	0.93, 1.16	0.4996
10	≥11	28,874 (9.7)	1.55	1.40, 1.71	<0.0001
GGT					
1	≤-19	28,402 (9.5)	1.67	1.52, 1.84	<0.0001
2	-18 to -9	28,240 (9.5)	1.15	1.05, 1.27	0.0043
3	-8 to -4	35,442 (11.9)	1.09	0.99, 1.20	0.0931
4	-3 to -2	22,232 (7.5)	Ref.	Ref.	Ref.
5	-1 to 1	41,200 (13.8)	1.01	0.92, 1.12	0.7703
6	2–3	26,555 (8.9)	1.00	0.90, 1.12	0.9599
7	4–6	31,623 (10.6)	1.10	1.00, 1.21	0.0602
8	7–10	26,769 (9.0)	1.07	0.96, 1.18	0.2187
9	11–20	28,416 (9.5)	1.22	1.11, 1.35	<0.0001
10	≥21	29,297 (9.8)	1.71	1.56, 1.88	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

[b]Deciles for changes in levels of alanine aminotransferase, aspartate aminotransferase, and

gamma glutamyltransferase during the 4 years since the baseline survey

Figure Legends

Figure 1. Schematic representation of the study design and period.

Figure 2. Associations[a] between the deciles of changes in liver enzyme levels over a 4-year period and all-cause mortality, stratified by the median value of each baseline liver enzyme level.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using Cox proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

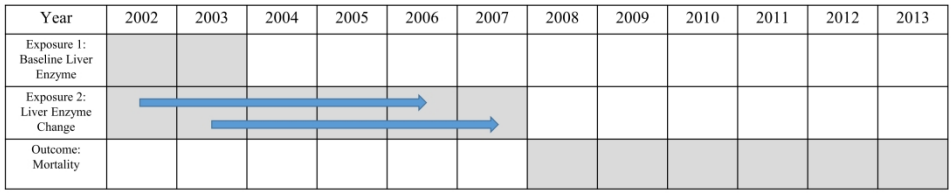


Figure 1

338x190mm (300 x 300 DPI)

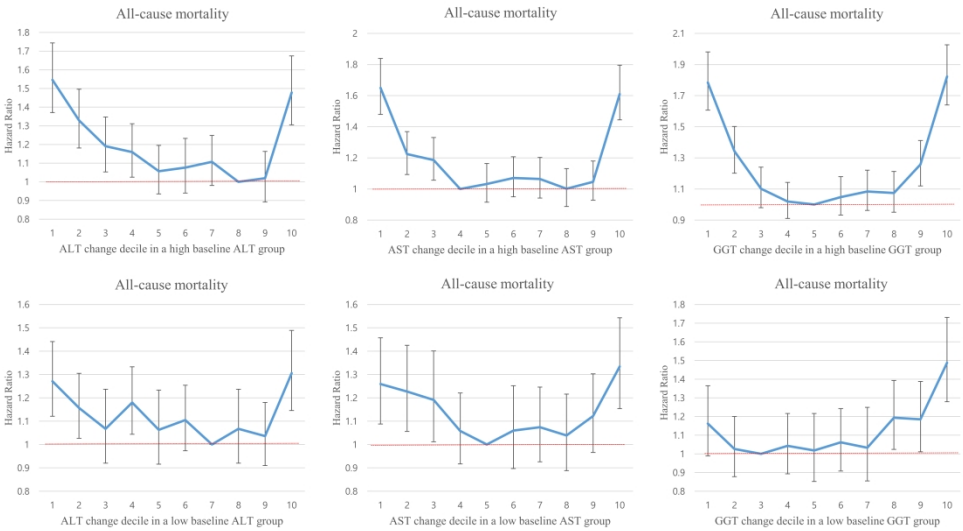


Figure 2

338x190mm (300 x 300 DPI)

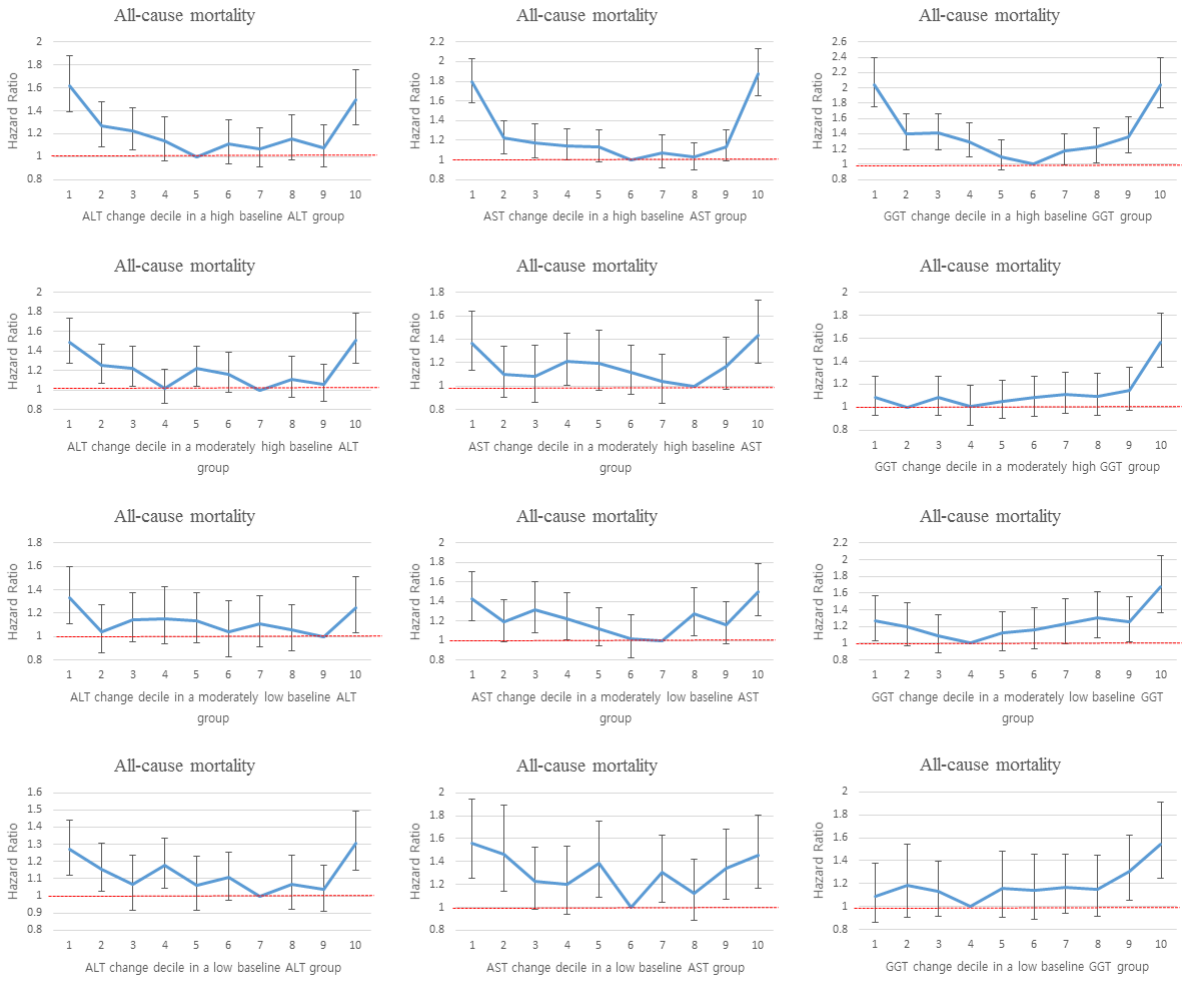
Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study

Kyoung-Nam Kim, Jungmin Joo, Ho Kyung Sung, Chee Hae Kim, Haebin Kim, and Yong-Jin Kwon

Table of Contents:

Figure Legends

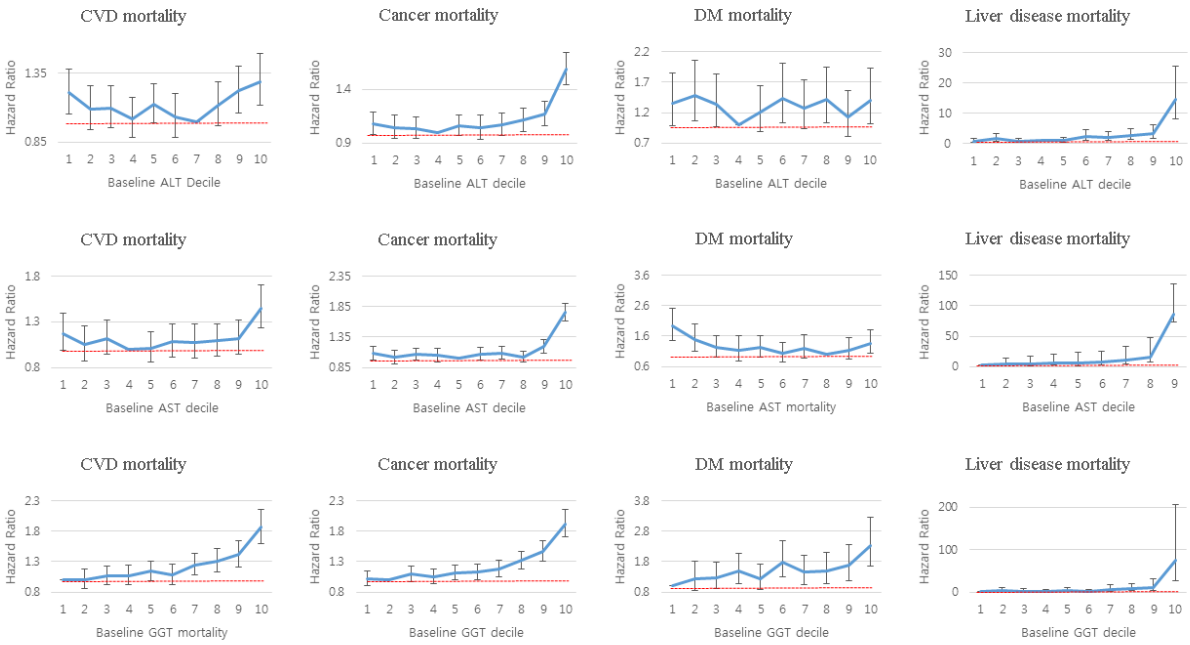
Supplementary Figure 1. Associations[a] between deciles of changes in liver enzyme levels over a 4-year period and all-cause mortality, stratified by the quartiles of each baseline liver enzyme levels.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using Cox proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

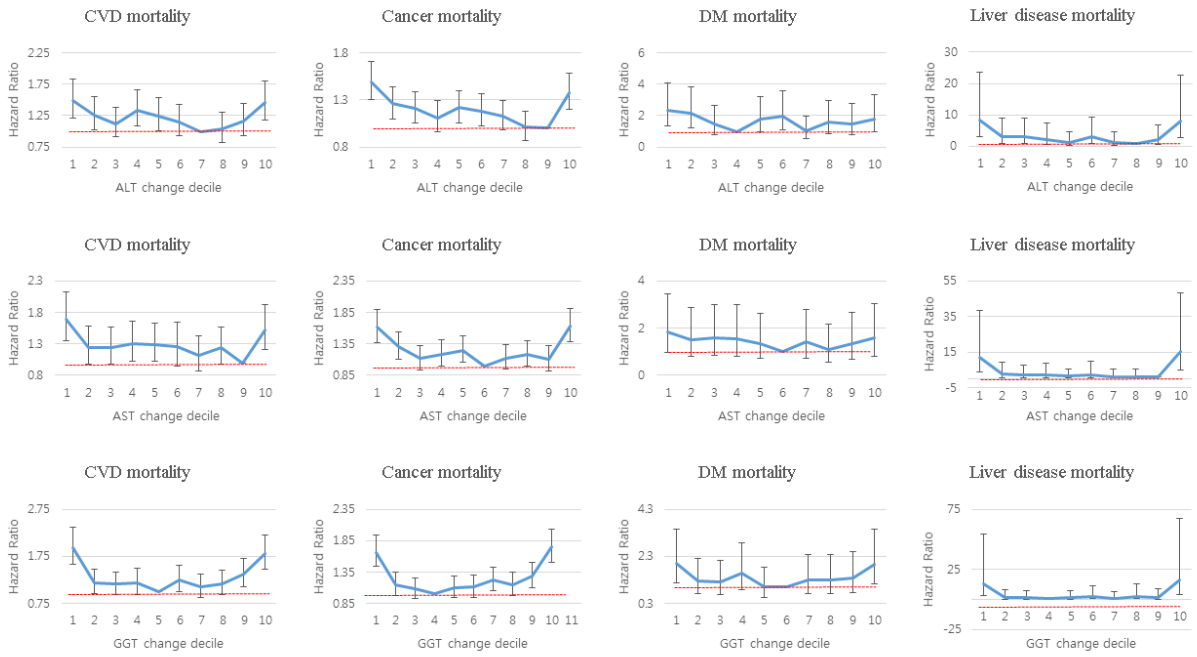
Supplementary Figure 2. Associations[a] between deciles of the baseline liver enzyme levels and cause-specific mortality.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using cause-specific proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

Supplementary Figure 3. Associations[a] between deciles of changes in liver enzyme levels over a 4-year period and cause-specific mortality.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using cause-specific proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5, 6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	9, 10
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	10

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	23, 24
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11, 12
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 12
		(*b*) Report category boundaries when continuous variables were categorized	26, 27, 29, 30
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study

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Keywords:	Alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, mortality, non-linear associations

Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study

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ABSTRACT

Objectives To investigate the associations of the levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) at baseline and their changes over time with mortality.

Design Cohort study.

Setting, participants, and outcome measures We analyzed the data of 484,472 individuals from the National Health Insurance Service-National Health Screening cohort (2002–2013). We used two exposure indices: 1) deciles of baseline ALT, AST, and GGT levels measured in 2002 or 2003 and 2) deciles of changes in ALT, AST, and GGT levels over a 4-year period (2002–2006 or 2003–2007). We constructed Cox models to evaluate the associations of these exposure indices with mortality (2008–2013).

Results We found non-monotonic dose-response associations between the baseline levels of ALT and AST and all-cause mortality. We also found a monotonic non-linear association between the baseline levels of GGT and all-cause mortality (10^{th} decile: hazard ratio [HR] = 2.05, 95% confidence interval [CI]: 1.93, 2.18). Compared with the 9^{th}, 6^{th}, and 4^{th} deciles of changes in ALT (8–13 U/L), AST (1 U/L), and GGT (-3 to -2 U/L) over time, respectively, the risks of all-cause mortality increased in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10^{th} decile: HR = 1.36, 95% CI: 1.24, 1.48, 1^{st} decile: HR = 1.46, 95% CI: 1.34, 1.59 for ALT; 10^{th} decile: 1.55, 95% CI: 1.40, 1.71, 1^{st} decile: HR =1.53, 95% CI: 1.38, 1.69 for AST; 10^{th} decile: HR = 1.71, 95% CI: 1.56, 1.88, 1^{st} decile: HR = 1.67, 95% CI: 1.52, 1.84 for GGT). These non-monotonic dose-response associations remained when

analyses were stratified by the medians or quartiles of the baseline liver enzyme levels.

Conclusions The levels of liver enzymes at baseline and over time showed non-linear associations with mortality.

Key words

Alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, mortality, non-linear associations

Article Summary

Strengths and limitations of this study

● First study to report non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality.

● Conducted longitudinal analyses using a large-scale cohort constructed from national administration data, which has a negligible follow-up loss.

● Identified the dates and causes of death using a national database maintained by Statistics Korea, which covers all individuals residing in the Republic of Korea and has high accuracy (>90%).

● Information on albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels was not available, and information on sociodemographic factors, lifestyles, past medical histories, and family histories was insufficient.

● Could not identify the mechanisms underlying the association between liver enzyme levels and mortality.

INTRODUCTION

Serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) are highly sensitive to liver dysfunction and damage.[1] Because assays for these liver enzymes are cost-effective, they are widely used during general health check-ups worldwide.[2,3]

Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality in the general population remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality in the general population (reviewed by Kunstor et al.[3]). The association between liver enzyme levels and mortality might not be linear, and both higher and lower liver enzyme levels would be associated with higher mortality (i.e., non-monotonic dose-response associations). Although we expected the non-monotonic dose-response associations between liver enzyme levels and mortality, this possibility has only been investigated in limited number of studies.[3,7,8]

Liver enzyme levels can increase due to factors such as liver damage and injury and can decrease due to factors such as hepatic aging, frailty, and reduced hepatic blood circulation.[9,10] Therefore, changes (increases or decreases) in the levels of these enzymes over time may be associated with higher mortality independent of baseline liver enzyme levels. However, to our knowledge, no study has explored the possibility of non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality.

Therefore, in the present study, we hypothesized that not only higher baseline liver enzyme

levels but also lower baseline levels are associated with higher mortality. In addition, we hypothesized that the increase or decrease in liver enzyme levels over time is also associated with higher mortality independent of baseline liver enzyme levels. We evaluated these hypotheses using a longitudinal study design with a large-scale (n > 500,000) national health screening cohort in which serum liver enzyme levels were repeatedly measured.

METHODS

Study population

The present study used data from the National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS) of the Republic of Korea. Detailed information on the NHIS-HEALS has been presented elsewhere.[11] Briefly, the NHIS-HEALS is a de-identified cohort released to researchers by the NHIS for the purpose of public research. Several epidemiological studies have been published using this data.[12–14] The NHIS-HEALS contains the data of 514,866 individuals, representing a 10% random sample of all participants in the National Health Screening Program between 2002 and 2003 (data collected during this period comprise the baseline) who were followed up to December 31, 2013. In the Republic of Korea, all individuals aged ≥40 years are invited to participate at least every 2 year in this general, free-of-charge health screening program conducted at designated healthcare institutions that meet the quality standards set by the Framework Act on Health Examinations. The rate of participation in the National Health Screening Program was 43.2% in 2003, and increased gradually to 74.8% in 2014.[14] The prevalence rates of common diseases such as hypertension and type 2 diabetes

mellitus in the NHIS-HEALS are generally similar to those reported in a nationally representative sample.[11]

The NHIS-HEALS includes various types of information, such as sociodemographic factors, lifestyles, past medical histories, and family histories (collected from self-reported questionnaires during the national health screening), health screening results (from physical examinations and clinical laboratory tests during the national health screening), healthcare usage (from claims data), and dates and causes of death (from Statistics Korea).

In the present study, from a total of 514,866 individuals included in the NHIS-HEALS, we sequentially excluded those who died between 2002 and 2007 (n = 13,278, 2.6%), those without any liver enzyme data between 2002 and 2003 (n = 494, 0.1%), and those with hepatitis or chronic liver disease up to 2007 (n = 16,622, 3.2%). Therefore, 484,472 individuals (94.1%) were included in the final analysis. The Institutional Review Board (IRB) of Seoul National University Hospital reviewed and approved the study protocol (IRB no. E-1804-045-936). Because the present study used claims data released to researchers after de-identification process, which is performed by the NHIS, the IRB of Seoul National University Hospital waived the requirement of informed consent for study participation. All experiments were conducted in accordance with the relevant guidelines and regulations.

Patient and public involvement

Patients were not recruited or involved in this study.

Exposures

We used two exposure indices: 1) deciles of the baseline levels of ALT, AST, and GGT measured in 2002 or 2003 and 2) deciles of changes in the ALT, AST, and GGT levels over a 4-year period (2002–2006 or 2003–2007) (figure 1). The first exposure index was used in an analysis of the association between baseline liver enzyme levels and mortality, while the second was used in an analysis of the association between changes in liver enzyme levels and mortality. If both the earlier and later exposure indices were available, the latter (baseline liver enzyme levels in 2003 or changes in liver enzyme levels between 2003 and 2007) was assigned as the exposure index for each individual.

Outcomes

The outcomes of the present study were all-cause and cause-specific mortality occurring between January 1, 2008 and December 31, 2013 (figure 1). Information on the dates and causes of death was obtained from Statistics Korea and merged with other data by the NHIS via personal identification numbers. The various causes of death were coded according to the International Classification of Disease, 10th Revision (ICD-10). For this analysis, we considered deaths from cardiovascular disease (defined as ICD-10 codes I20–I25, I50, and I60–I70), cancer (C00–C97), diabetes mellitus (E10–E14), and liver disease (B15–B19 and K70–K77), according to previous studies.[7,8,15]

Covariates

Based on previous reports and existing biomedical knowledge,[3,7–9,15–17] we identified the following potential confounders and included them as covariates for further analyses: age (years), sex, household income (decile), smoking status (non-smoker, ex-smoker, or smoker), alcohol consumption (none, ≤1–2, or ≥3–4 times/week), physical activity (did not exercise, ≤1–2, or ≥3–4 times/week), body mass index (<18.5, 18.5–22.9, 23–24.9, 25–29.9, or ≥30 kg/m^2), systolic blood pressure (<120, 120–139.9, or ≥140 mmHg), diastolic blood pressure (<80, 80–89.9, or ≥90 mmHg), fasting glucose levels (<70, 70–99.9, 100–125.9, or ≥126 mmHg), history of heart disease (yes or no), history of stroke (yes or no), and history of cancer (yes or no). Information about these variables was collected from the National Health Screening Program at baseline (2002–2003). We created a missing indicator category for the missing values (0.03–3.4%) of the categorical variables (table 1).

Statistical analysis

We constructed Cox proportional hazard models adjusted for the above-mentioned covariates to investigate the associations of exposure indices (deciles of baseline liver enzyme levels and deciles of changes in liver enzyme levels) with all-cause mortality. Since the associations between changes in liver enzyme levels and mortality may differ according to the baseline liver enzyme levels, we also divided the study population into those with values above and below the

medians or quartiles of the baseline levels of ALT, AST, and GGT. We then assessed the associations between deciles of changes in the liver enzyme levels and all-cause mortality in each stratum.

We evaluated the non-linearity of the associations (non-monotonic dose-response or monotonic non-linear associations) by 1) visually inspecting the shapes of the associations in the analysis using categorized exposure indices and 2) testing the squared-terms of the log2-transformed continuous baseline or changes in liver enzyme levels that were added to Cox models including the log2-transformed baseline or changes in liver enzyme levels and the same covariates.

To evaluate the associations of deciles of the baseline and changes in liver enzyme levels with cause-specific mortality (i.e., deaths from cardiovascular disease, cancer, diabetes mellitus, and liver disease), we constructed cause-specific proportional hazard models adjusted for the same covariates. In these competing risk analyses, we estimated cause-specific hazards rather than sub-distribution hazards, because the hazard ratios (HRs) from cause-specific proportional hazard models would be easily interpretable and could be used to draw public health implications.[18,19]

In a sensitivity analysis, because distributions of liver enzyme levels were different by sex (U/L, ALT: median, 25, interquartile range [IQR], 16 among men; median, 18, IQR, 10 among women; AST: median, 25, IQR, 11 among men; median, 22, IQR, 9 among women; GGT: median, 33, IQR, 34 among men; median, 16, IQR, 12 among women), we repeated all analyses using sex-specific cut-off points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-off points, to confirm the robustness

of the results.[7] Detailed information on the sex-specific cut-off points is presented in the Supplementary Material. In addition, we also conducted analyses further excluding individuals with a history of cancer, history of type 2 diabetes mellitus, and those who drank alcohol ≥3–4 times/week, because these conditions can also impact liver enzyme levels.

In both the Cox and cause-specific proportional hazard models, the follow-up duration was calculated in months from January 1, 2008 to the date of death or December 31, 2013 (if death did not occur). For analyses using categorized exposure indices, we designated categories with the lowest betas for each outcome of interest in the association analysis as the referent categories. We used SAS version 9.4 (SAS Institute Inc.) for all analyses.

RESULTS

Of the 484,472 study participants, the mean age was 53.0 years (range: 40–80 years), and 53.3% were men. A larger proportion of participants had higher income levels (household income deciles 9–10, 33.3% vs. deciles 0–2, 15.9%). A majority of the study participants were non-smokers (65.5%), did not drink alcohol (56.1%), and did not exercise regularly (55.8%). Most participants had no history of heart disease, stroke, or cancer (table 1).

Compared with the 4th decile of baseline ALT levels (17–18 U/L), the risk of all-cause mortality was higher in both the higher and lower baseline ALT deciles (10th decile, ≥43 U/L: HR = 1.53, 95% confidence interval [CI]: 1.44, 1.62; 1st decile, ≤12 U/L: HR = 1.16, 95% CI: 1.10, 1.23). Similarly, compared with the 4th decile of baseline AST levels (21 U/L), the risk of all-cause

mortality was also higher in both the higher and lower baseline AST deciles (10th decile≥38 U/L: HR = 1.70, 95% CI: 1.59, 1.81; 1st decile, ≤16 U/L: HR = 1.15, 95% CI: 1.07, 1.24). When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile, ≥72 U/L: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). Because the normal range of ALT is generally considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9–85 U/L,[2] even within the normal range, lower or higher levels of ALT and AST and higher levels of GGT were associated with higher mortality risk (Table 2). We confirmed the non-linearity of these associations by testing the squared-terms of the log2-transformed ALT, AST, and GGT levels added to the Cox models (all *p*-values for the squared-terms < 0.0001).

We also assessed the associations of deciles of changes in the liver enzyme levels over a 4-year period with all-cause mortality. Compared with the 9th decile of ALT changes (8–13 U/L), the 6th decile of AST changes (1 U/L), and the 4th decile of GGT changes (-3 to -2 U/L), respectively, the risk of all-cause mortality was higher in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10th decile, ≥14 U/L: HR = 1.36, 95% CI: 1.24, 1.48, 1st decile, ≤-16 U/L: HR = 1.46, 95% CI: 1.34, 1.59 for ALT; 10th decile, ≥11 U/L: HR = 1.55, 95% CI: 1.40, 1.71, 1st decile, ≤-12 U/L: HR = 1.53, 95% CI: 1.38, 1.69 for AST; 10th decile, ≥21 U/L: HR = 1.71, 95% CI: 1.56, 1.88, 1st decile, ≤-19 U/L: HR = 1.67, 95% CI: 1.52, 1.84 for GGT) (table 3). We then tested the squared-terms of the log2-transformed changes in ALT, AST, and GGT and confirmed the non-linearity of the associations between the changes in liver enzyme levels and mortality (all *p*-values for the squared-terms < 0.0001).

When we performed stratified analyses by the medians or quartiles of the baseline liver enzyme levels, the same non-monotonic dose-response associations between the deciles of changes in liver enzyme levels and all-cause mortality remained in each stratum (figure 2; online supplementary figure 1), similar to the results of the non-stratified analysis (table 3).

In analyses of the associations between the deciles of baseline liver enzyme levels and cause-specific mortality (mortality due to cardiovascular disease, cancer, diabetes mellitus, and liver disease), we found non-monotonic dose-response associations of baseline ALT and AST levels with mortality due to cardiovascular disease, cancer, and diabetes mellitus, whereas we found monotonic non-linear associations in other cases (online supplementary figure 2). When we analyzed the associations between the deciles of changes in the liver enzyme levels and cause-specific mortality, we found non-monotonic dose-response associations in all cases (online supplementary figure 3).

In sensitivity analyses based on sex-specific deciles of the baseline and changes in liver enzyme levels, the results remained robust and did not change appreciably (data not shown). When we excluded individuals with a history of cancer (n = 2,732), those with a history of type 2 diabetes mellitus (n = 20,691), and those who drank ≥3–4 times/week (n = 53,350), the results did not change appreciably (data not shown).

DISCUSSION

In the present study using the large-scale national health screening cohort, we found non-linear

(non-monotonic dose-response or monotonic non-linear) associations of baseline liver enzyme levels and changes in these levels over time with both all-cause and cause-specific mortality. The non-monotonic dose-response associations between changes in the liver enzyme levels and mortality remained after stratifying the study population into subgroups according to the baseline liver enzyme levels.

Although some previous studies have reported inverse associations between ALT levels and all-cause mortality,[9,17,20] other studies have demonstrated positive associations.[21,22] Similarly, some studies have described positive associations between AST levels and all-cause mortality,[21,23,24] whereas other studies found no such associations.[25,26] This heterogeneity may be explained by the increased risk of mortality associated with both higher and lower baseline levels of ALT and AST (non-monotonic dose-response associations), as demonstrated in the present study.[3,7,8] Meanwhile, most previous studies have reported positive associations between baseline GGT levels and all-cause mortality.[15,17] The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.

It has been reported that serum ALT, AST, and GGT levels are associated with a higher risk of type 2 diabetes mellitus and body mass index even within the normal ranges.[27] In the present study, liver enzyme levels within normal ranges were also found to be associated with mortality. Because the liver is a central organ of glucose and lipid metabolism and critical for maintaining health,[28] re-evaluation of the relevance of current standards is warranted.

In the present study, larger changes (both increases and decreases) in the ALT, AST, and GGT

levels over a 4-year period were associated with a higher risk of all-cause mortality when compared with smaller changes. These non-linear associations remained even after the participants were stratified according to the baseline liver enzyme levels. These results suggest that changes in liver enzyme levels over time as well as baseline levels can be used to predict mortality and assess risk by clinicians and public health practitioners. To our knowledge, this is the first study to demonstrate non-monotonic dose-response associations between changes in liver enzyme levels and mortality; further studies are warranted to confirm the findings.

In a study based on a representative U.S. population data, lower baseline ALT levels were found to be associated with higher risks of cardiovascular disease, cancer, and liver disease mortality, whereas higher ALT levels were only associated with a higher risk of liver disease mortality.[7] In another study based on a representative sample of the U.S. population, higher ALT levels were associated with a higher risk of liver disease mortality, whereas higher GGT levels were associated with higher risks of cancer, diabetes mellitus, and liver disease mortality.[15] A study using data from a prospective elderly cohort reported an association of higher AST levels with an increased risk of cancer mortality, as well as an association of higher GGT levels with an increased risk of cardiovascular disease mortality.[8] Among patients with acute myocardial infarction who underwent percutaneous coronary intervention, ALT and AST levels were associated with stenosis diameter, an indicator of stenosis severity.[29] Although the results of the present study were generally consistent with those of previous studies,[7,8,15] some inconsistencies were also found (e.g., positive associations of baseline ALT levels with cardiovascular, cancer, and diabetes mellitus mortality in the present study but not in previous studies). These discrepancies might be attributable to factors such as differences in the accuracy

of cause of death data, study populations, adjusted covariates, and analytical methods. As the associations of baseline and changes in liver enzyme levels with cause-specific mortality have not been thoroughly investigated, additional studies are needed, particularly those using changes in liver enzyme levels as explanatory variables.

Serum liver enzyme levels may increase in response to various factors, including liver damage and cell destruction (e.g., hepatocytes, biliary epithelium, and other cells of the organs such as the heart, skeletal muscle, and kidney),[1,2] and may decrease in response to factors such as age and frailty-related reductions in liver size and blood circulation.[9,10,30] Therefore, changes (both increases and decreases) in liver enzyme levels over time may reflect these conditions related to the deterioration of liver function and could thus be associated with a higher risk of mortality.

The present study has some limitations. First, although the NHIS-HEALS provides information on serum ALT, AST, and GGT levels, data from other standard liver panel tests, such as albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels, are not available. This limits our ability to assess liver function comprehensively. Second, although the national health screening program was performed at designated health care institutions meeting the quality standards, liver enzyme levels were analysed by different laboratories with a different sensitivity and specificity, leading to potential information bias. Third, the present study used data that were not primarily collected for the purpose of research. Therefore, information on the sociodemographic factors, lifestyles, past medical histories, and family histories are insufficient, leading to the possibility of residual confounding. Fourth, although common liver disease, such

as nonalcoholic fatty liver disease, could increase liver enzyme levels [31] and increase mortality [32–34] as well, specific mechanisms underlying the association between liver enzyme levels and mortality could not be identified thoroughly.

However, the present study also has notable strengths. First, to our knowledge, this is the first study to report non-monotonic dose-response associations between changes in liver enzyme levels over time and mortality. Second, we used a large-scale cohort constructed from national administration data, which has a negligible follow-up loss. The large sample size (n = 484,472) of the present study allowed us to evaluate the potential associations with sufficient power. Third, we identified the dates and causes of death using a national database maintained by Statistics Korea, which covers all individuals residing in the Republic of Korea. The accuracy of the recorded causes of death in this database is considered high (>90%).[35]

CONCLUSIONS

We found non-linear associations of the baseline liver enzyme levels and their changes over time with risks of mortality. The results of the present study suggest that changes in liver enzyme levels, as well as the baseline levels, can be used to predict health outcomes such as mortality and to assess risk in clinical and public health settings.

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Author Contributions

KK, JJ, HKS, CHK, HK, and YJK contributed to conception and design of the study. KNK conducted statistical analyses and wrote the initial manuscript. JJ, HKS, CHK, HK, and YJK reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Disclaimer

The views in this study are those of the authors and not necessarily those of the Seoul National University Hospital or the National Health Insurance Service of the Republic of Korea.

Competing interests

The authors declare that they have no competing interests.

Patient consent

Not required.

Ethics approval

The study protocol was reviewed and approved by the Institutional Review Board of Seoul

National University Hospital (IRB no. E-1804-045-936).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

The National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS), which is used in the present study, is a de-identified cohort released to researchers by the NHIS for the purpose of public research. The data sets are provided to researchers after the study protocols are approved by the IRB of the researcher's institute and by the NHIS. Researchers can request the data sets through the website https://nhiss.nhis.or.kr/bd/ay/bdaya001iv.do.

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Table 1. Baseline sociodemographic characteristics and liver enzyme levels of the study participants (2002–2003) (n = 484,472)

Characteristics	Total	ALT (U/L)	AST (U/L)	GGT (U/L)
	n (%)	GM ± GSD		
Age (years)				
40–49	212,004 (43.8)	21.8 ± 1.7	23.5 ± 1.4	26.1 ± 2.2
50–59	140,742 (29.1)	22.8 ± 1.6	24.9 ± 1.4	26.8 ± 2.1
60–69	98,759 (20.4)	21.6 ± 1.6	25.4 ± 1.4	24.6 ± 2.0
70–80	32,967 (6.8)	19.5 ± 1.6	25.2 ± 1.4	21.6 ± 1.9
Sex				
Men	258,010 (53.3)	25.3 ± 1.7	26.1 ± 1.5	35.8 ± 2.1
Women	226,462 (46.7)	18.5 ± 1.6	22.6 ± 1.4	17.6 ± 1.8
Household income (deciles)				
0–2	76,994 (15.9)	20.8 ± 1.7	24.2 ± 1.4	23.7 ± 2.1
3–5	106,260 (21.9)	21.6 ± 1.7	24.7 ± 1.5	25.1 ± 2.1
6–8	139,763 (28.9)	22.4 ± 1.7	24.7 ± 1.4	26.6 ± 2.1
9–10	161,455 (33.3)	22.2 ± 1.7	24.0 ± 1.4	26.2 ± 2.1
Smoking status				
Non-smoker	317,199 (65.5)	20.5 ± 1.6	23.6 ± 1.4	21.4 ± 1.9
Ex-smoker	40,797 (8.4)	25.4 ± 1.7	25.8 ± 1.4	34.1 ± 2.0

Smoker	109,868 (22.7)	25.0 ± 1.7	26.0 ± 1.5	39.0 ± 2.1
Missing	16,608 (3.4)	22.0 ± 1.7	24.6 ± 1.4	25.4 ± 2.1
Alcohol consumption				
None	271,551 (56.1)	20.4 ± 1.6	23.2 ± 1.4	20.1 ± 1.8
≤1–2 times/week	151,559 (31.3)	23.4 ± 1.7	24.9 ± 1.4	31.4 ± 2.1
≥3–4 times/week	53,350 (11.0)	25.9 ± 1.7	28.9 ± 1.5	51.1 ± 2.3
Missing	8,012 (1.7)	20.9 ± 1.6	23.5 ± 1.4	21.0 ± 1.9
Physical activity				
Did not exercise	270,329 (55.8)	21.4 ± 1.7	24.3 ± 1.4	24.5 ± 2.1
≤1–2 times/week	112,413 (23.2)	23.0 ± 1.7	24.5 ± 1.4	28.5 ± 2.1
≥3–4 times/week	89,843 (18.5)	21.9 ± 1.6	24.4 ± 1.4	25.9 ± 2.1
Missing	11,887 (2.5)	21.7 ± 1.7	25.4 ± 1.4	25.2 ± 2.1
Body mass index (kg/m^2)				
<18.5	10,599 (2.2)	17.7 ± 1.6	24.6 ± 1.5	21.1 ± 2.2
18.5–22.9	171,108 (35.3)	19.1 ± 1.6	23.4 ± 1.4	21.9 ± 2.1
23–24.9	132,394 (27.3)	21.9 ± 1.6	24.0 ± 1.4	25.9 ± 2.1
25–29.9	156,051 (32.2)	25.1 ± 1.7	25.5 ± 1.4	30.1 ± 2.1
≥30	13,926 (2.9)	29.0 ± 1.8	27.6 ± 1.5	32.1 ± 2.1
Missing	394 (0.1)	21.4 ± 1.6	24.6 ± 1.4	22.2 ± 2.0
Systolic blood pressure				

(mmHg)				
<120	140,993 (29.1)	19.7 ± 1.6	22.8 ± 1.4	21.1 ± 2.0
120–139.9	214,824 (44.3)	22.3 ± 1.7	24.5 ± 1.4	26.4 ± 2.1
≥140	128,464 (26.5)	23.7 ± 1.7	26.0 ± 1.5	30.4 ± 2.2
Missing	191 (0.04)	21.1 ± 1.7	23.3 ± 1.5	24.4 ± 2.0
Diastolic blood pressure (mmHg)				
<80	191,221 (39.5)	20.1 ± 1.6	23.2 ± 1.4	21.7 ± 2.0
80–89.9	167,612 (34.6)	22.4 ± 1.7	24.6 ± 1.4	26.6 ± 2.1
≥90	125,372 (25.9)	24.2 ± 1.7	26.0 ± 1.5	31.5 ± 2.2
Missing	267 (0.1)	21.5 ± 1.7	23.4 ± 1.4	23.8 ± 2.0
Fasting glucose level (mg/dL)				
<70	8,510 (1.8)	20.3 ± 1.7	23.9 ± 1.4	23.2 ± 2.0
70–99.9	324,064 (66.9)	20.9 ± 1.6	23.9 ± 1.4	23.7 ± 2.0
100–125.9	114,597 (23.7)	23.6 ± 1.7	25.3 ± 1.5	29.1 ± 2.2
≥126	37,167 (7.7)	26.5 ± 1.7	26.1 ± 1.6	35.9 ± 2.3
Missing	134 (0.03)	19.7 ± 1.7	22.8 ± 1.7	20.7 ± 2.0
Past history of heart disease				
No	494,401 (98.6)	21.9 ± 1.7	24.4 ± 1.4	25.6 ± 2.1

Yes	7,187 (1.4)	22.4 ± 1.6	25.0 ± 1.4	26.3 ± 2.0
Past history of stroke				
No	499,352 (99.6)	21.9 ± 1.7	24.4 ± 1.4	25.7 ± 2.1
Yes	2,236 (0.5)	22.0 ± 1.6	24.6 ± 1.4	27.4 ± 2.0
Past history of cancer				
No	498,856 (99.5)	21.9 ± 1.7	24.4 ± 1.4	25.7 ± 2.1
Yes	2,732 (0.5)	20.7 ± 1.6	25.0 ± 1.4	21.2 ± 1.9

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; GM, geometric mean; GSD, geometric standard deviation

Table 2. Associations[a] between baseline liver enzyme levels (2002–2003) and all-cause mortality (2008–2013)

Decile[b]	Levels (U/L)	*n* (%)	HR	95% CI	*p*-value
ALT					
1	≤12	56,870 (11.7)	1.16	1.10, 1.23	<0.0001
2	13–14	41,904 (8.7)	1.11	1.04, 1.17	0.0014
3	15–16	46,115 (9.5)	1.07	1.01, 1.13	0.0292
4	17–18	46,272 (9.6)	Ref.	Ref.	Ref.
5	19–21	62,738 (13.0)	1.06	1.01, 1.12	0.0320
6	22–23	33,898 (7.0)	1.04	0.97, 1.11	0.2722
7	24–27	54,157 (11.2)	1.05	0.99, 1.11	0.1046
8	28–32	46,873 (9.7)	1.11	1.05, 1.18	0.0005
9	33–42	49,009 (10.1)	1.17	1.10, 1.24	<0.0001
10	≥43	46,601 (9.6)	1.53	1.44, 1.62	<0.0001
AST					
1	≤16	48,178 (10.0)	1.15	1.07, 1.24	0.0001
2	17–18	45,357 (9.4)	1.09	1.01, 1.18	0.0217
3	19–20	58,461 (12.1)	1.09	1.02, 1.17	0.0175
4	21	30,304 (6.3)	Ref.	Ref.	Ref.
5	22–23	58,761 (12.1)	1.03	0.96, 1.10	0.4151
6	24–25	52,190 (10.8)	1.06	0.99, 1.14	0.0896

7	26–27	41,805 (8.6)	1.09	1.01, 1.17	0.0185
8	28–31	58,055 (12.0)	1.08	1.01, 1.15	0.0257
9	32–37	44,515 (9.2)	1.20	1.12, 1.28	<0.0001
10	≥38	46,802 (9.7)	1.70	1.59, 1.81	<0.0001
GGT					
1	≤11	54,316 (11.2)	Ref.	Ref.	Ref.
2	12–13	36,355 (7.5)	1.01	0.94, 1.08	0.7713
3	14–16	55,121 (11.4)	1.07	1.00, 1.13	0.0412
4	17–19	49,756 (10.3)	1.10	1.04, 1.17	0.0019
5	20–23	53,476 (11.0)	1.12	1.06, 1.19	0.0002
6	24–27	41,003 (8.5)	1.16	1.09, 1.24	<0.0001
7	28–34	50,696 (10.5)	1.24	1.17, 1.32	<0.0001
8	35–45	48,116 (9.9)	1.31	1.23, 1.39	<0.0001
9	46–71	49,144 (10.1)	1.46	1.37, 1.55	<0.0001
10	≥72	46,450 (9.6)	2.05	1.93, 2.18	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

[b]Deciles for baseline levels of alanine aminotransferase, aspartate aminotransferase, and gamma

glutamyltransferase

Table 3. Associations[a] between changes in liver enzyme levels since the baseline survey (2002–2003) and all-cause mortality (2008–2013)

Decile[b]	Levels (U/L)	*n* (%)	HR	95% CI	*p*-value
ALT					
1	≤-16	28,441 (9.6)	1.46	1.34, 1.59	<0.0001
2	-15 to -9	29,670 (10.0)	1.23	1.13, 1.34	<0.0001
3	-8 to -5	34,065 (11.4)	1.11	1.02, 1.21	0.0137
4	-4 to -3	23,295 (7.8)	1.08	0.98, 1.18	0.1334
5	-2 to -1	27,637 (9.3)	1.12	1.03, 1.23	0.0127
6	0–1	28,738 (9.7)	1.11	1.02, 1.22	0.0226
7	2–4	38,771 (13.0)	1.02	0.94, 1.12	0.5916
8	5–7	27,754 (9.3)	1.01	0.92, 1.11	0.8602
9	8–13	29,713 (10.0)	Ref.	Ref.	Ref.
10	≥14	29,842 (10.0)	1.36	1.24, 1.48	<0.0001
AST					
1	≤-12	27,631 (9.3)	1.53	1.38, 1.69	<0.0001
2	-11 to -7	29,839 (10.0)	1.23	1.11, 1.36	0.0001
3	-6 to -4	33,587 (11.3)	1.09	0.98, 1.21	0.1081
4	-3 to -2	30,433 (10.2)	1.15	1.03, 1.28	0.0113
5	-1 to 0	35,094 (11.8)	1.10	0.99, 1.22	0.0773
6	1	17,637 (5.9)	Ref.	Ref.	Ref.

7	2–3	32,282 (10.8)	1.02	0.92, 1.14	0.7010
8	4–6	36,128 (12.1)	1.05	0.95, 1.17	0.3479
9	7–10	26,425 (8.9)	1.04	0.93, 1.16	0.4996
10	≥11	28,874 (9.7)	1.55	1.40, 1.71	<0.0001
GGT					
1	≤-19	28,402 (9.5)	1.67	1.52, 1.84	<0.0001
2	-18 to -9	28,240 (9.5)	1.15	1.05, 1.27	0.0043
3	-8 to -4	35,442 (11.9)	1.09	0.99, 1.20	0.0931
4	-3 to -2	22,232 (7.5)	Ref.	Ref.	Ref.
5	-1 to 1	41,200 (13.8)	1.01	0.92, 1.12	0.7703
6	2–3	26,555 (8.9)	1.00	0.90, 1.12	0.9599
7	4–6	31,623 (10.6)	1.10	1.00, 1.21	0.0602
8	7–10	26,769 (9.0)	1.07	0.96, 1.18	0.2187
9	11–20	28,416 (9.5)	1.22	1.11, 1.35	<0.0001
10	≥21	29,297 (9.8)	1.71	1.56, 1.88	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

[b]Deciles for changes in levels of alanine aminotransferase, aspartate aminotransferase, and

gamma glutamyltransferase during the 4 years since the baseline survey

Figure Legends

Figure 1. Schematic representation of the study design and period.

Figure 2. Associations[a] between the deciles of changes in liver enzyme levels over a 4-year period and all-cause mortality, stratified by the median value of each baseline liver enzyme level.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using Cox proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

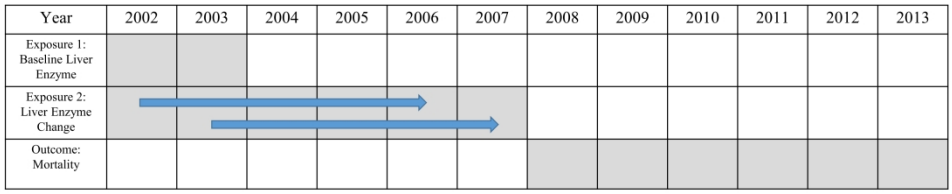

Figure 1

338x190mm (300 x 300 DPI)

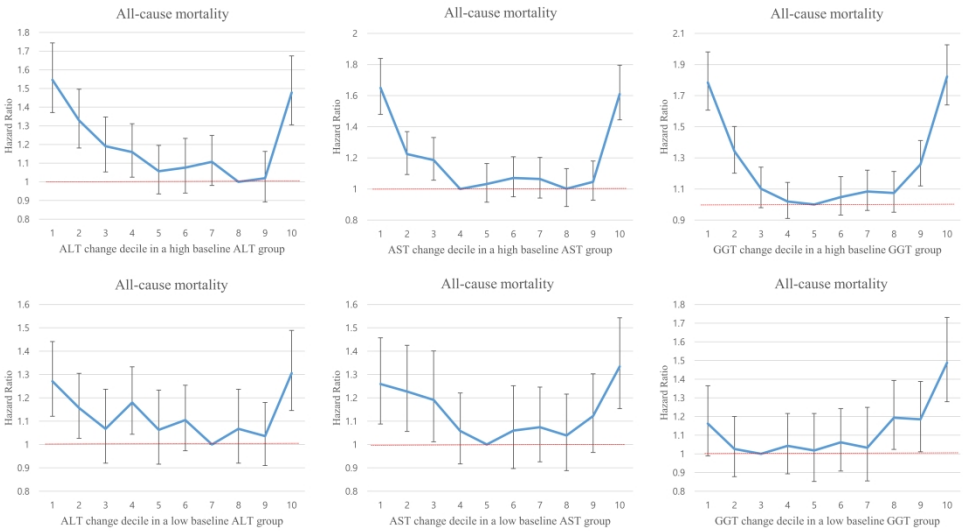

Figure 2

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Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study

Kyoung-Nam Kim, Jungmin Joo, Ho Kyung Sung, Chee Hae Kim, Haebin Kim, and Yong-Jin Kwon

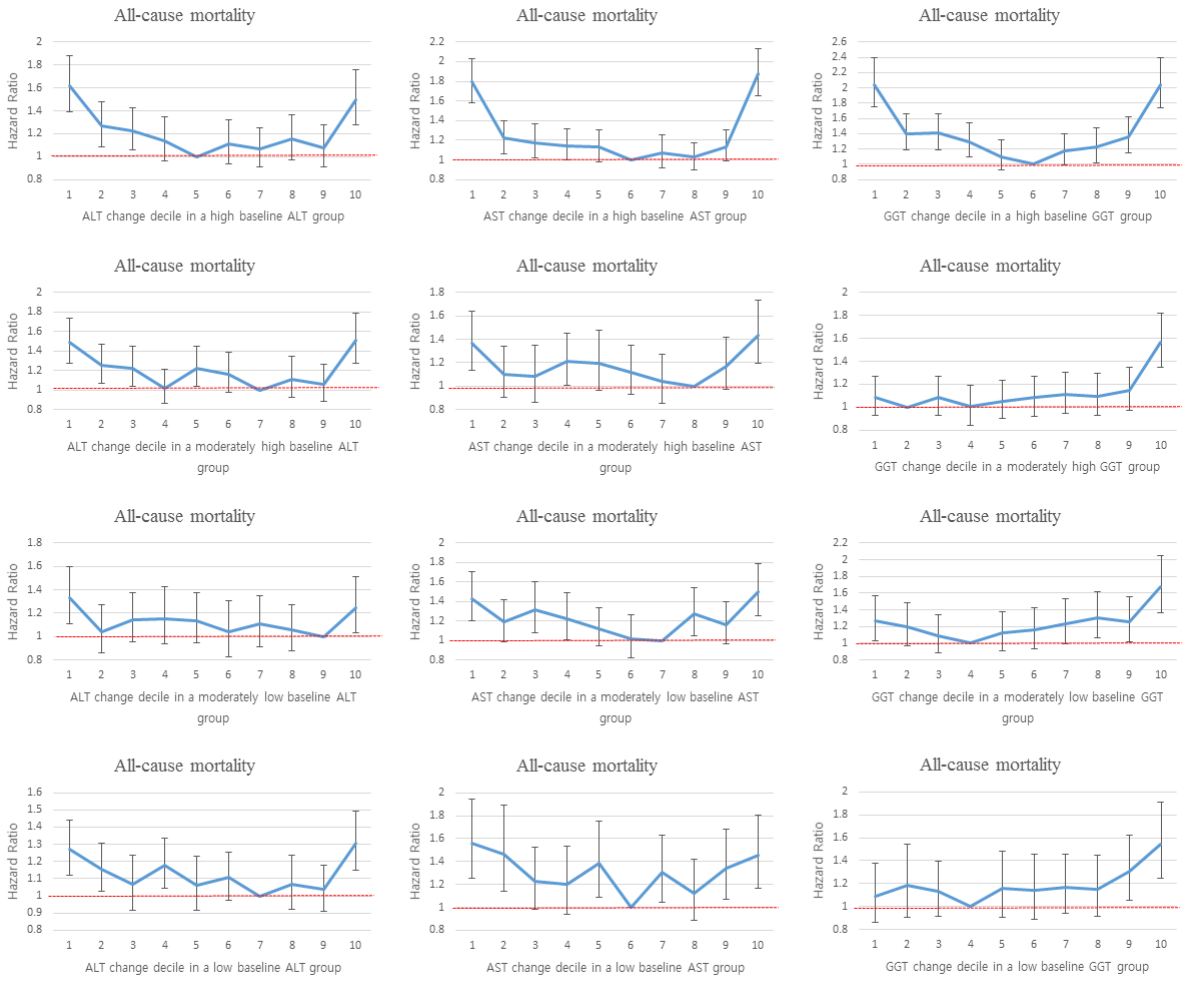
Table of Contents:

Supplementary Methods

In a sensitivity analysis, because distributions of liver enzyme levels were different by sex (U/L, ALT: median, 25, interquartile range [IQR], 16 among men; median, 18, IQR, 10 among women; AST: median, 25, IQR, 11 among men; median, 22, IQR, 9 among women; GGT: median, 33, IQR, 34 among men; median, 16, IQR, 12 among women), we repeated all analyses using sex-specific cut-off points for deciles of the baseline liver enzyme levels (U/L, ALT: ≤14, 15–16, 17–19, 20–21, 22–24, 25–27, 28–31, 32–37, 38–48, or ≥49 among men; ≤10, 11–12, 13–14, 15–16, 17, 18–19, 20–22, 23–26, 27–33, or ≥34 among women; AST: ≤17, 18–19, 20–21, 22–23, 24–25, 26–27, 28–29, 30–33, 34–40, or ≥41 among men; ≤15, 16–17, 18–19, 20, 21–22, 23, 24–25, 26–28, 29–33, or ≥34 among women; GGT: ≤15, 16–19, 20–23, 24–27, 28–32, 33–39, 40–48, 49–63, 64–95, or ≥96 among men; ≤9, 10–11, 12, 13–14, 15–16, 17–18, 19–21, 22–26, 27–35, or ≥36 among women) and changes in liver enzyme levels (U/L, ALT: ≤-19, -18 to -11, -10 to -7, -6 to -4, -3 to -1, 0–1, 2–4, 5–7, 8–14, or ≥15 among men; ≤-12, -11 to -7, -6 to -4, -3 to -2, -1 to 0, 1–2, 3–4, 5–7, 8–12, or ≥13 among women; AST: ≤-13, -12 to -8, -7 to -5, -4 to -3, -2 to -1, 0–1, 2–3, 4–6, 7–10, or ≥11 among men; ≤-10, -9 to -6, -5 to -3, -2, -1 to 0, 1–2, 3, 4–6, 7–10, or ≥11 among women; GGT: ≤-27, -26 to -13, -12 to -7, -6 to -3, -2 to 0, 1–3, 4–7, 8–13, 14–27, or ≥28 among men; ≤-10, -9 to -5, -4 to -3, -2 to -1, 0–1, 2, 3–4, 5–7, 8–13, or ≥14 among women), instead of the above mentioned sex-non-specific cut-off points, to confirm the robustness of the results.

Figure Legends

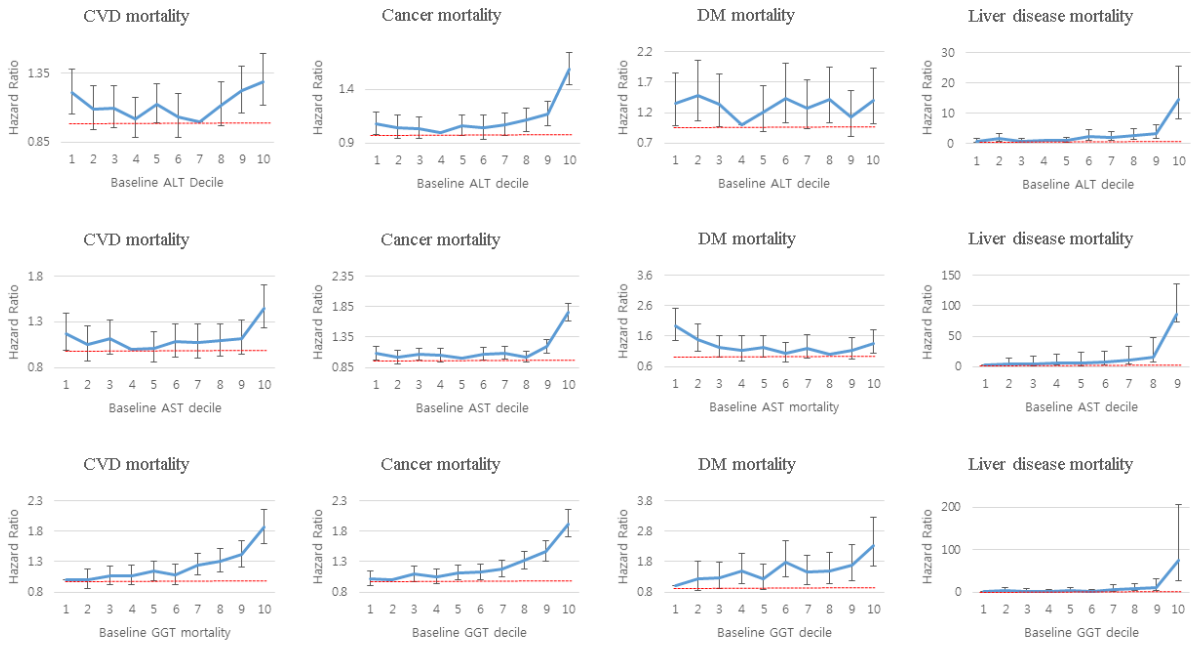
Supplementary Figure 1. Associations[a] between deciles of changes in liver enzyme levels over a 4-year period and all-cause mortality, stratified by the quartiles of each baseline liver enzyme levels.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using Cox proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

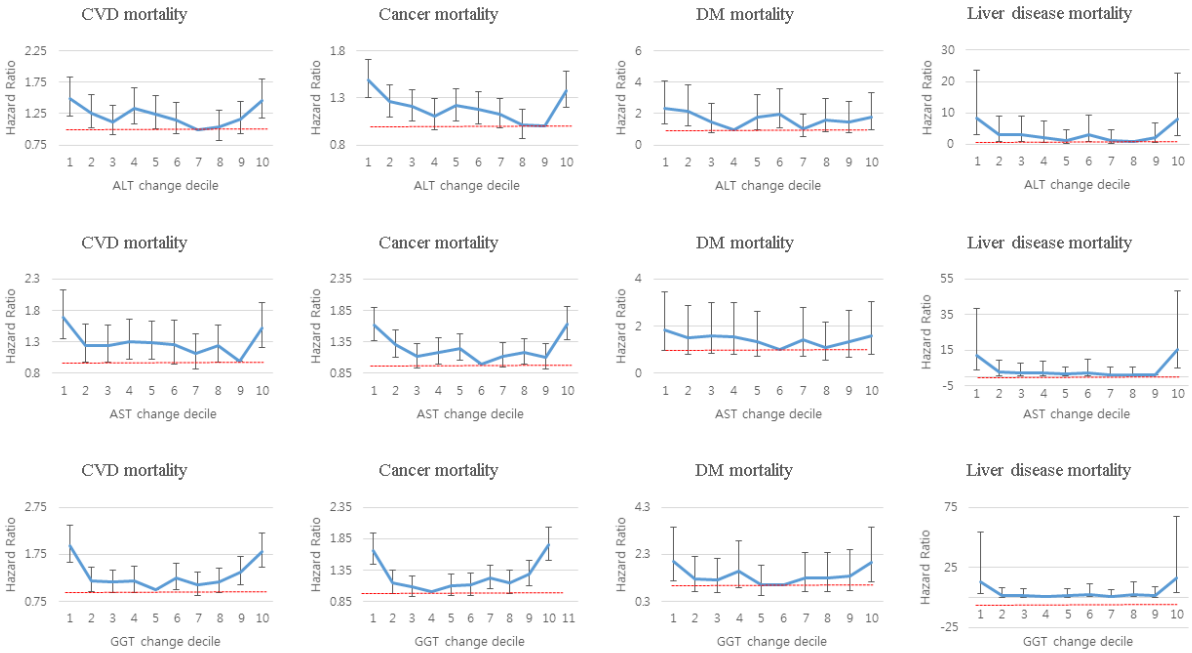
Supplementary Figure 2. Associations[a] between deciles of the baseline liver enzyme levels and cause-specific mortality.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using cause-specific proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

Supplementary Figure 3. Associations[a] between deciles of changes in liver enzyme levels over a 4-year period and cause-specific mortality.



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

[a]Hazard ratios were estimated using cause-specific proportional hazard models adjusted for age, sex, household income decile, smoking status, alcohol consumption, physical activity, body mass index, systolic and diastolic blood pressure, fasting glucose levels, and past history of heart disease, stroke, and cancer.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5, 6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	9, 10
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	10

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	23, 24
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11, 12
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 12
		(*b*) Report category boundaries when continuous variables were categorized	26, 27, 29, 30
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.