# Serum alanine aminotransferase activity is age- and sex-depended but independently predicted by body mass index in both sexes. The Tromsø study

**Svein Ivar Bekkelund** ( svein.ivar.bekkelund@unn.no )
The Arctic University of Norway

**Research**

**Keywords:** Alanine aminotransferase, Sex difference, Age, BMI, Population study

**Posted Date:** September 3rd, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-66055/v1

## Abstract

# Background

High and low levels of serum alanine aminotransferase (ALT) are associated with cardiovascular diseases (CVD) especially in elderly but the roles of sex and age are unexplained. This study investigated sex- and age-related variation of serum ALT and associations between ALT and CVD risk factors in men and women in a Caucasian population.

# Methods

This study used cross-sectional data from Tromsø 6 in 2555 men (mean age 60.4 years) and 2858 women (mean age 60.0 years). Associations were assessed by variance analysis and multivariable logistic regression of odds to have abnormal ALT.

# Results

Abnormal ALT was detected in 113 (4.4%) men and 188 (6.6%) women. ALT correlated negatively with age in men (r = -0.231, $p < 0.001$) and positively in women (r = 0.124, $p < 0.001$). A linear inversed association between age and ALT in men and a non-linear inversed U-trend in women with maximum level between 60–64 years were found. Age was independently associated with ALT in men only [OR 1.05 (95% CI 1.04, 1.07), $p < 0.001$] and body mass index (BMI) was independently associated with ALT in both sexes.

# Conclusion

The relationship between age and ALT was opposite directed in men compared to women, and linearly and independently in men only. Neither BMI as the strongest associated CVD risk factor in both sexes nor other risk components could explain this. Separate sex-analyses should be used in studies investigating the role of ALT as a disease marker.

## Background

Elevated levels of alanine aminotransferase (ALT) is associated with higher risk to develop cardiovascular disease (CVD) [1, 2], obesity [3, 4], insulin resistance[5], the metabolic syndrome and type 2 diabetes [6, 7]. The full spectre of underlying mechanisms is unresolved, but ALT-related non-alcoholic fatty liver disease (NAFLD) is an important condition involved, at least in men [8, 9]. Higher prevalence of metabolic abnormalities in men may explain sex-differences in associations between ALT and metabolic syndrome [10]. Low levels have also been reported to be unfavourable and may be explained by higher age, sarcopenia and hepatic ageing [11]. Accordingly, a J-shaped ALT-mortality curve is demonstrated

[12–14]. An American survey with measurement of body composition in 15028 inhabitants reported higher mortality risk in subgroups with ALT deciles 1–3 and an insignificant mortality increase in those with highest ALT levels (decile 10) [14]. The risk excess associated with lower ALT levels was probably due to decreased appendicular lean mass found in the same study fraction [14]. Furthermore, ALT-related coronary disease may be unrelated to presence of risk factors [15, 16]. Whether this may be explained in part by age and sex variations is unknown.

An inversed relationship between age and ALT is reported [17], which has been associated with male sex in particular [4]. An inverse U-shaped relationship between age and ALT is demonstrated in Israeli and Italian cohorts of men and women recruited from general practice populations [18, 19]. The purpose of the present study was to investigate the relationship between age and ALT by sex in a Scandinavian predominantly Caucasian general population and to identify CVD-related risk factor(s) most strongly associated with ALT.

## Methods

# Patients and ethics

The participants of this cross-sectional community study were recruited from the 6th Tromsø Study [20]. ALT was measured in 5413 participants (2555 men and 2858 women), aged 30–87 years, representing 42% of those attended. The ethnic origin included 87.3% Norwegians, 1.6% Sami and 1.3% of Finnish origin, 2.2% of other ethnicities, and 7.6% with unknown ethnicity. Written consent was obtained from all participants, and the Norwegian Committee for Medical and Health Research Ethics (REC) approved the study.

# Measurements

Clinical examinations are performed standardized in the Tromsø study. Accordingly, height and weight were measured with light clothing without shoes, and body mass index (BMI) calculated as weight divided by height squared ($kg/m^2$) to the nearest 0.1 cm and 0.1 kg using an automatic device. Also, blood pressure was recorded automatically by Dinamap Vital Signs Monitor 1846; Critikon Inc, Tampa, FL. Three readings were taken from the upper right arm at 1-minute intervals after an initial 2-minute rest in a sitting position. The average of the 2 last readings was then used in the analyses. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of antihypertensive medication. Coronary heart disease (reported previous heart attack) was registered via standard questionnaires in the Tromsø study and diabetes defined as $HbA_{1c} \geq 6.5\%$.

ALT was analysed photometrically using an enzymatic method (CK-NAC, Roche Diagnostics, Mannheim, Germany) and by an automated clinical chemistry analyzer (Modular P, Roche) by photometry. Serum ALT reference limits were 10–70 U/L for men and 10–45 U/L in women. The lower detected limit of ALT assay was 5.0 U/L and the analytical variation (Vka) 4.9%. The standard cut-off limits for ALT, aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) used in the Hospital are those

developed by the Nordic Reference Interval Project (NORIP) [21]. HbA1c was measured in EDTA whole blood based on an immunoturbidometric assay (UNIMATES, F. Hoffmann-La Roche AG) and $HbA_{1c}$ (%) was calculated from the $HbA_{1c}$ /Hb ratio. Serum-glucose was obtained by a non-fasting procedure. An enzymatic colorimetric method using a commercially available kit (CHOD-PAP, Boehringer-Mannheim, Mannheim, Germany) was used to measure serum total cholesterol. High-density lipoprotein (HDL) cholesterol was obtained after precipitation of lower-density lipoproteins (LDL) with heparin and manganese chloride. Total cholesterol/HDL cholesterol ratio was used in the statistical analyses. High-sensitive C-reactive protein (hs-CRP) was analyzed with a particle-enhanced immunoturbidimetric assay on a Modular P (Roche Hitachi, Mannheim, Germany) in thawed aliquots after storage at − 20 °C. The lower detection limit of the hs-CRP assay was 0.03 mg /L and measurements of hs-CRP lower than 0.03 mg /L were set at this value. The analytical coefficient of hs-CRP variation between 0.1 mg /L and 20 mg /L was < 4%. Department of Clinical Biochemistry, University Hospital of North Norway, Tromsø performed all analyses.

# Statistical analysis

ALT values showed left-sided skewness by histograms and therefore log-transformed in the analyses. Additionally, AST, GGT, hs-CRP, $HbA_{1c}$ and glucose-values were log-transformed due to right-sided skewness. Descriptive data are presented as mean and standard deviations (SD) for continuous variables or numbers and frequencies for dichotomous data. Pearson´s correlation coefficient was calculated for associations between continuous variables. Two-sided student´s t-test was used to compare means respectively $\chi^2$- test between frequencies of data. ANOVA was used to test differences between means of ALT vs. age quartiles and independent variables (social, demographic and metabolic) vs. ALT quartiles while associations between age and ALT adjusted for covariates was done by ANCOVA. By multiple logistic regression analysis, possible confounders were tested and adjusted for with abnormal ALT as dependent variable and age, BMI, waist-to-hip-ratio, diastolic blood pressure, total/HDL cholesterol ratio, triglycerides and log CRP as independent variables; i.e. those variables found statistically significantly associated with ALT in the variance analyses. Systolic blood pressure is not included in the female model due to high correlation with diastolic blood pressure (r = 0.651, $p < 0.001$). $P < 0.05$ was considered statistically significant and SPSS software (Statistical Package for Social Science INC, Chicago, Illinois, USA), version 26) was used in the statistical analyses.

## Results

# Alanine aminotransferase, supplementary blood samples and clinical characteristics in men and women

Table 1 shows characteristics of the subjects in the population. Men had higher levels of liver markers and CVD risk markers including BMI and waist-to-hip-ratio but lower total cholesterol, LDL and HDL levels than women (Table 1). Accordingly, 580 (20.3%) women used lipid lowering drugs compared to 185

(7.2%) in the mail group, $p < 0.001$. The rate of hypertension was high in the total group, but relatively higher in men (Table 1).

# Alanine aminotransferase is associated with cardiovascular risk factors

Tables 2 and 3 show positive and significant relationships between ALT and BMI, waist-to-hip-ratio and most CVD-related variables when comparing lowest and highest quartiles of variables. Exceptions are insignificant associations with diastolic blood pressure and CRP in men (Table 2). Thus, CRP-levels were elevated in 1st ALT-quartiles in both sexes and thereby slightly J-shaped. Consequently, the difference between means of ALT in 2. and 4. quartiles in men was significant ($p = 0.03$).

**Age- and sex are differently associated with serum alanine aminotransferase levels** Age correlated positively with ALT values in women and negatively in men (Fig. 1). A negative linear association between age quintiles and log ALT was found for men and a non-linear inversed U-shaped relationship was observed in women in a variance analysis adjusted for covariates (Tables 4 and 5). Mean log ALT changed from 1.51 U/L from the youngest group to 1.37 U/L in the oldest (9.3% decline). Age and log ALT were positively associated until a maximum level was reached at age group 60–63 years in women, whereupon it declined (Table 5). Thus, age correlated positively with ALT in females < 60 years ($r = 0.129$, $p = 0.001$) and negatively in those ≥ 60 years ($r = -0.161$, $p < 0.001$). A similar pattern is seen in Table 3 where mean age in women increased through quartiles 1 to 3 of log ALT until it decreased. Also, age correlated contradictive with BMI in men and women (men: $r = -0.045$, $p = 0.024$; women: $r = 0.095$, $p < 0.001$). Except alcohol use, none of the CVD risk parameters showed an inversed U-form in comparison with ALT quartiles (Tables 4 and 5).

# BMI independently predicts ALT in men and women

Variables included in the multivariate analysis are those significantly associated with log ALT by ANOVA (Tables 2 and 3) except systolic blood pressure in women. BMI was the only predictor independently associated with log ALT in both sexes after adjusting for age, lipids, glucose, $HbA_{1C}$ (%) (men and women) and additionally, log CRP in women (Table 6). Age was significantly and independently associated with log ALT in men, but not in women.

## Discussion

The relationship between age and ALT as an inversed linearly trend or formed as an inverted U-wave along quantiles of age-groups is usually reported as representative for the entire study population but is recognized in the present study as confined to either sex group, respectively men and women. Furthermore, ALT is associated with metabolic components and clinical CVD risk factors, but BMI was the only one independently associated with ALT in both sexes in a multivariate analysis.

A linear and inversed relationship between age and ALT in both sexes is reported in cross-sectional and longitudinal studies [17, 22]. Although the present study participants were younger in comparison, it confirmed a linear age-ALT trend that persisted after adjusting for covariates, but in men only. An Australian community study in 1673 elderly men (≥ 70 years) designed as a survival analyses, showed an inversed relationship between age and ALT like the findings in the male group here [23]. That study found low ALT to be associated with reduced survival partly due to high age, but the mechanisms is otherwise largely unknown [23]. However, obesity without accompanied CVD has been related to increased survival (the obesity paradox) [24]. Like here, age and BMI were negatively correlated in men contrasting the positive relationship in women. Accordingly, the third U.S. National Health and Nutrition Examination Survey ($n$ = 5724) found positive associations between age and male sex in relation to ALT which abolished in a multivariate analysis. Also, BMI remained significantly associated with ALT [4]. Age, sex and BMI also influenced transaminase levels in children and adolescents. In girls, ALT declined from infancy until 4 years of age, then increased to peak at 16 years. The same U-form was found for boys although a little delayed [25]. Boys had higher ALT levels than girls, and BMI correlated stronger with ALT in boys [25].

Despite different age-ALT curves between men and women in the present study, the relationships between ALT and CVD risk factors including glucose were approximately the same. In women, an inversed U-form with significant and opposite directed age-ALT correlations on both sides of the 60-year cut-off is demonstrated. A similar pattern with ALT-maximum at 65-year of age was found equal for both sexes in a recent cross-sectional study in 10,000 non-diabetic subjects [11]. By showing a similar trend for age vs. fasting glucose levels, glucose was suggested to entail a joint effect on the ALT levels across ages [11]. The same phenomenon is demonstrated in a study that included subjects living in aged home and participants recruited from three general practices ($n$ = 335). ALT values was distributed along an inversed U-shaped curve with a peak level between 40–55 years [19]. In parallel, ALT increased until the third decade in men and the fifth decade in women in a large Italian study where ALT additionally associated positively with BMI, glucose and lipids [18].

The sex-difference in ALT vs. age in the present study is not shown in previous studies, and the data otherwise do not point to any certain mechanism. A study in an obese cohort from the same area, showed an association between ALT and the muscular component of body composition in both sexes, but ALT was associated with fat mass in men only [26]. Furthermore, ALT was positively correlated with glucose, glycated haemoglobin and cholesterol in obese women [26]. ALT is higher in men than women but whether this is due to different body composition, sex hormones or diverging effect of confounders is unknown [27, 28]. Thus, ALT predicted prevalent coronary heart disease in men in an European-American study [29]. Although the Tromsø study provides robust data, the evidence of the present cross-sectional study confines to associations between data, which itself might contribute to differences in comparisons with others.

# Perspectives and significance

These data confirms an independent and equal association between ALT and BMI in both sexes [30]. The sex-specific variations in ALT levels seem to be unrelated to CVD-related risk factors and is largely unexplained. Data from studies on ALT should be analysed sex-stratified.

# Conclusion

Biological mechanisms explaining how age and sex influence ALT levels differently should be further investigated.

# Declarations


### Acknowledgement

I am indebted to the Norwegian Institute of Public Health for their participation in the data collection in the sixth survey of the Tromsø Study.

### Funding

The publication charges for this article have been funded by a grant from the publication fund of UiT, The Arctic University of Norway.


### Availability of data and materials

The data files are the property of The Tromsø Study which can be contacted by E-mail (tromsous@uit.no).

### Authors´ contribution

SIB designed the study, analyzed and interpreted the data and wrote the manuscript.

### Ethics approval and consent to participate

The study was approved by the Regional Ethical Committee for research. All participants in The Tromsø Study have given informed written consent.

### Consent to publication

Not applicable.

### Competing interests

The author declares that there is no conflict of interest related to the study.

# References

1. Yokoyama M, Watanabe T, Otaki Y, Takahashi H, Arimoto T, Shishido T, et al. Association of the Aspartate Aminotransferase to Alanine Aminotransferase Ratio with BNP Level and Cardiovascular Mortality in the General Population: The Yamagata Study 10-Year Follow-Up. Dis Markers. 2016;2016:4857917. doi:10.1155/2016/4857917.
2. Schindhelm RK, Dekker JM, Nijpels G, Bouter LM, Stehouwer CD, Heine RJ, et al. Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. Atherosclerosis. 2007;191(2):391–6. doi:10.1016/j.atherosclerosis.2006.04.006.
3. Klein M, Iazzettii L, Speiser P, Carey D, Shelov S, Accacha S, et al. Alanine transferase: An independent indicator of adiposity related comorbidity risk in youth. J Diabetes. 2015;7:5:649–56. doi:10.1111/1753-0407.12221.
4. Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology. 2003;124:1. doi:10.1053/gast.203.50004. 71 – 9.
5. Hanley AJ, Wagenknecht LE, Festa A, D'Agostino RB Jr, Haffner SM. Alanine aminotransferase and directly measured insulin sensitivity in a multiethnic cohort: the Insulin Resistance Atherosclerosis Study. Diabetes Care. 2007;30:7:1819–27. doi:10.2337/dc07-0086.
6. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002;51:6:1889–95.
7. Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;31:5. doi:10.1111/jgh.13264. 936 – 44.
8. Chang Y, Ryu S, Sung E, Jang Y. Higher concentrations of alanine aminotransferase within the reference interval predict nonalcoholic fatty liver disease. Clin Chem. 2007;53:4. doi:10.1373/clinchem.2006.081257. 686 – 92.
9. Martin-Rodriguez JL, Gonzalez-Cantero J, Gonzalez-Cantero A, Arrebola JP, Gonzalez-Calvin JL. Diagnostic accuracy of serum alanine aminotransferase as biomarker for nonalcoholic fatty liver disease and insulin resistance in healthy subjects, using 3T MR spectroscopy. Medicine. 2017;96:17:e6770. doi:10.1097/md.0000000000006770.
10. Chen KW, Meng FC, Shih YL, Su FY, Lin YP, Lin F, et al. Sex-Specific Association between Metabolic Abnormalities and Elevated Alanine Aminotransferase Levels in a Military Cohort: The CHIEF Study. Int J Environ Res Public Health. 2018;15:3. doi:10.3390/ijerph15030545.
11. Preuss HG, Kaats GR, Mrvichin N, Bagchi D, Preuss JM. Circulating ALT Levels in Healthy Volunteers Over Life-Span: Assessing Aging Paradox and Nutritional Implications. J Am Coll Nutr. 2019;38:8:661–9. doi:10.1080/07315724.2019.1580169.
12. Deetman PE, Alkhalaf A, Landman GW, Groenier KH, Kootstra-Ros JE, Navis G, et al. Alanine aminotransferase and mortality in patients with type 2 diabetes (ZODIAC-38). Eur J Clin Invest.

2015;45:8:807–14. doi:10.1111/eci.12474.

13. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Blokzijl H, Gansevoort RT, Dullaart RP. Inverse linear associations between liver aminotransferases and incident cardiovascular disease risk: The PREVEND study. Atherosclerosis. 2015;243:1. doi:10.1016/j.atherosclerosis.2015.09.006. 138 – 47.
14. Ruhl CE, Everhart JE. The association of low serum alanine aminotransferase activity with mortality in the US population. Am J Epidemiol. 2013;178:12:1702–11. doi:10.1093/aje/kwt209.
15. Ndrepepa G, Holdenrieder S, Colleran R, Cassese S, Xhepa E, Fusaro M, et al. Inverse association of alanine aminotransferase within normal range with prognosis in patients with coronary artery disease. Clin Chim Acta. 2019;496:55–61. doi:10.1016/j.cca.2019.06.021.
16. Afarideh M, Aryan Z, Ghajar A, Noshad S, Nakhjavani M, Baber U, et al. Complex association of serum alanine aminotransferase with the risk of future cardiovascular disease in type 2 diabetes. Atherosclerosis. 2016;254:42–51. doi:10.1016/j.atherosclerosis.2016.09.009.
17. Dong MH, Bettencourt R, Barrett-Connor E, Loomba R. Alanine aminotransferase decreases with age: the Rancho Bernardo Study. PloS One. 2010;5:12:e14254. doi:10.1371/journal.pone.0014254.
18. Vespasiani-Gentilucci U, Gallo P, Piccinocchi G, Piccinocchi R, Schena E, Galati G, et al. Determinants of alanine aminotransferase levels in a large population from Southern Italy: relationship between alanine aminotransferase and age. Dig Liver Dis. 2014;46:10:909–15. doi:10.1016/j.dld.2014.05.021.
19. Elinav E, Ben-Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. Am J Gastroenterol. 2005;100:10:2201–4. doi:10.1111/j.1572-0241.2005.41822.x.
20. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. Int J Epidemiol. 2012;41:4:961–7. doi:10.1093/ije/dyr049.
21. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest. 2004;64:4. doi:10.1080/00365510410006324. 271 – 84.
22. 10.1016/j.cgh.2011.10.014
    Dong MH, Bettencourt R, Brenner DA, Barrett-Connor E, Loomba R. Serum levels of alanine aminotransferase decrease with age in longitudinal analysis. Clin Gastroenterol Hepatol. 2012;10:3:285 – 90.e1; doi:10.1016/j.cgh.2011.10.014.
23. Le Couteur DG, Blyth FM, Creasey HM, Handelsman DJ, Naganathan V, Sambrook PN, et al. The association of alanine transaminase with aging, frailty, and mortality. J Gerontol A Biol Sci Med Sci. 2010;65:7:712–7. doi:10.1093/gerona/glq082.
24. Myers J, Lata K, Chowdhury S, McAuley P, Jain N, Froelicher V. The obesity paradox and weight loss. Am J Med. 2011;124:10:924–30. doi:10.1016/j.amjmed.2011.04.018.
25. Bussler S, Vogel M, Pietzner D, Harms K, Buzek T, Penke M, et al. New pediatric percentiles of liver enzyme serum levels (alanine aminotransferase, aspartate aminotransferase, gamma-

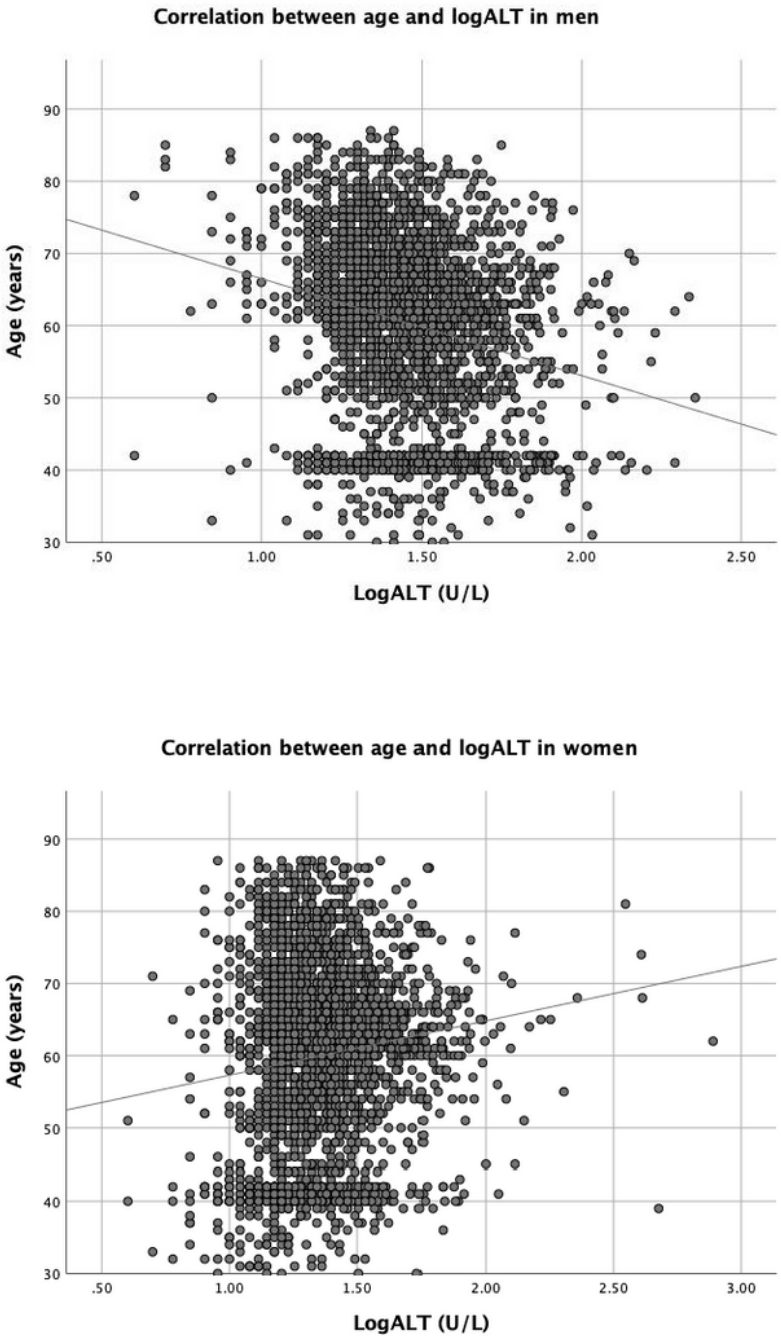
glutamyltransferase): Effects of age, sex, body mass index, and pubertal stage. Hepatology. 2017. doi:10.1002/hep.29542.

26. Bekkelund SI, Jorde R. Alanine Aminotransferase and Body Composition in Obese Men and Women. Dis Markers. 2019;2019:1695874. doi:10.1155/2019/1695874.
27. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137:1:1–10.
28. Poustchi H, George J, Esmaili S, Esna-Ashari F, Ardalan G, Sepanlou SG, et al. Gender differences in healthy ranges for serum alanine aminotransferase levels in adolescence. PloS One. 2011;6:6:e21178. doi:10.1371/journal.pone.0021178.
29. Feitosa MF, Reiner AP, Wojczynski MK, Graff M, North KE, Carr JJ, et al. Sex-influenced association of nonalcoholic fatty liver disease with coronary heart disease. Atherosclerosis. 2013;227:2:420–4. doi:10.1016/j.atherosclerosis.2013.01.013.
30. Tilg H, Moschen A. Weight loss: cornerstone in the treatment of non-alcoholic fatty liver disease. Minerva Gastroenterol Dietol. 2010;56:2:159–67.

# Tables

Due to technical limitations, table 1 to 6 are only available as a download in the Supplemental Files section.

# Figures




## Figure 1

Correlations between age and serum alanine aminotransferase (ALT) in men and women. Spearman´s rank correlation coefficient was inversed in the group of 2555 men (r = -0.231, p < 0.001) and positive in 2858 women (r = 0.124, p < 0.001)

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TABLES.biol.doc