Sex-influenced Association of Nonalcoholic Fatty Liver Disease with Coronary Heart Disease

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Abstract

Objective—This study investigated whether nonalcoholic fatty liver disease (NAFLD) predicts prevalent coronary heart disease (CHD).

Methods—Epidemiologic studies have used various definitions for NAFLD. Here, we considered both liver fat burden measured by CT (FL) and the non-specific measure of hepatic inflammation –alanine aminotransferase (ALT). The association of FL and ALT with CHD (self report of coronary bypass, myocardial infarction, or percutaneous transluminal coronary angioplasty) was investigated in 2,756 European-American participants of the Family Heart Study.

Results—FL (p=0.0084) and ALT (40U/L, p=0.014) were each individually associated with prevalent CHD. However, when accounting for traditional metabolic risk factors in a multivariate model FL had no predictive value for CHD in either men or women; whereas ALT was a significant predictor of CHD in men, and the association strengthened among non-diabetic men. In non-diabetic women, neither FL nor ALT was associated with CHD.

Conclusions—ALT (40U/L) was a predictor of prevalent CHD in men but not in women, while CT measured FL was not significant in either sex. The failure to account for traditional risk factors, heterogeneity by sex, and varying definitions of NAFLD may account for some of the conflicting evidence in the literature regarding the association between NAFLD and coronary disease.

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5. Disclosures
None.

Supplementary material
Supplementary data related to this article can be found online

Keywords

cardiovascular disease; nonalcoholic steatohepatitis; sex-specific association; insulin resistance; glucose metabolism

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by increased triglyceride deposition in the liver in the absence of excessive alcohol intake or other known causes of liver disease [1–3]. The clinico-pathologic spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) to cirrhosis [1, 4]. While liver biopsy is the diagnostic reference standard for NAFLD, it is invasive and used infrequently in epidemiologic research. Non-invasive imaging such as unenhanced computerized tomography (CT) can accurately measure liver fat burden with low attenuation in the hepatic tissue, with 82% sensitivity and 100% specificity for detecting >30% hepatic steatosis [5–6]. Alternatively, liver function tests such as alanine transaminase (ALT) levels are sometimes used to screen for liver function abnormalities. Elevated serum levels of ALT are indicative of liver damage, and have been used as a biochemical marker of hepatic inflammation to monitor the course and severity of NAFLD [4,7–8] and as a surrogate marker of NAFLD in several epidemiologic studies [4,7–8].

NAFLD is the most common cause of abnormal liver function, ranging in prevalence from 10 to 40% in the general population of various countries and is far more prevalent among individuals with obesity and type 2 diabetes, occurring in 70%–90% in such patients [1–3, 9]. NAFLD is closely related to insulin resistance and can be considered part of the 'metabolic syndrome' [10–11]. The pathogenesis of NAFLD includes fatty acid lipotoxicity from lipolysis of dietary or visceral fat, insulin resistance, increased inflammation, oxidative stress and lipid peroxidation. Coronary heart disease (CHD) is related to several of these factors, including abdominal and overall obesity and insulin resistance, and it shares several additional risk factors with NAFLD including hypertension and dyslipidemia [11–14]. What is not clear is whether NAFLD independently influences risk of CHD. Using ALT levels as a surrogate measure of NAFLD, some studies found an association with cardiovascular risk [4, 8, 12], while other studies failed to detect an independent association with cardiovascular outcomes [15]. ALT levels have low specificity [1, 4, 8] to diagnose NAFLD which may have contributed, at least in part, to these discrepant conclusions.

A growing number of studies have addressed the relationship of NAFLD to prevalent and incident clinical coronary artery disease in subjects of European ancestry, though the evidence is mixed (reviewed in [1, 4, 12, 14, 16]). Differences in definition or diagnostic criteria for NAFLD and study patient characteristics (including presence of type 2 diabetes and other components of metabolic syndrome) may account for some of the conflicting results among studies. Also, the increased cardiovascular risk in patients with NAFLD might reflect the coexistence of underlying metabolic syndrome risk factors, which makes it difficult to distinguish the pathologic factors underlying the increased risk of coronary artery disease with NAFLD. Alternatively, NAFLD itself might confer a cardiovascular risk above and beyond that associated with other traditional risk factors, such as the components of metabolic syndrome.

In this study, we investigate whether NAFLD is an independent predictor of CHD. To address this question we test: (i) whether liver fat burden measured by CT, and hepatic inflammation measured by elevated ALT levels (40 U/L) are associated with prevalent CHD; (ii) whether NAFLD is a predictor of CHD independent of their shared metabolic risk

factors; and (iii) whether association modification by sex and/or diabetic status exists. We carried out this study in 2,756 European American (EA) participants of the NHLBI Family Heart Study (FamHS).

2. Material and Methods

2.1. Study Design and Sample

The Family Heart Study (https://dsgweb.wustl.edu/fhscc/) recruited 1,200 families, half randomly sampled, and half selected because of an excess of CHD or risk factor abnormalities as compared with age- and sex-specific population rates [17], from four population-based parent studies: the Framingham Heart Study, the Utah Family Tree Study, and two centers for the Atherosclerosis Risk in Communities study (Minneapolis, and Forsyth County, NC). Study participants belonging to the largest pedigrees were invited to undergo CT imaging for coronary artery calcification; and scans were further assessed to quantitative hepatic and abdominal fat burden. The description of CT scan measurements is presented in Supplemental Material available online.

A total of 2,756 subjects in 510 extended random and high CHD risk families were studied. Informed consent was obtained from all participants, and this project was approved by the Institutional Review Boards of all participating institutions.

2.2. Phenotypes and Covariates

Prevalent CHD was obtained by self report of coronary bypass, myocardial infarction, and/ or any of the following: coronary angioplasty, balloon angioplasty, atherectomy, stent, percutaneous transluminal coronary angioplasty, or percutaneous coronary intervention; the information was validated by hospital records.

The CT liver attenuation (LA) was measured by three round regions of interest (ROI's) in two slices of the superior right lobe of the liver. The six ROI's values (three from each slice) were averaged. Instead of a spleen standard, we used a standardized phantom at a density of water (HU=0) as well as increasing densities of calcium for standardization. Lower LA values indicate higher fat content. If the liver attenuation on unenhanced CT scans is 40 HU, the predicted hepatic steatosis is approximately 30% [1, 5]. We inverted the scale of LA by multiplying each value by negative one to produce a phenotype of fatty liver (FL) for which higher values are associated with greater liver fat.

Hepatic inflammation was assessed by levels of alanine transaminase (ALT in units of U/L). ALT levels were measured by reaction rate assay based on the conversion of NADH to NAD using the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc. Rochester NY 14650). We discretized ALT 40 U/L to indicate hepatic inflammation in our models [18]. Insulin resistance was assessed by HOMA-IR (Homeostatic Model Assessment: fasting glucose (mmol/L)*fasting insulin (mU/L)/22.5), and diabetes was defined as taking antihyperglycemic medication and/or fasting glucose 126 ml/dl. Overall adiposity was characterized by body mass index (BMI, kg/m^2). Lipids were assessed by standard ultracentrifugation, and low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [19]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken three times using a random zero sphygmomanometry in a sitting position and the mean of the second and the third measurements was used. A log transformation of HOMA-IR was taken to better approximate normality.

Information on antihyperglycemic, antihypertensive and lipid-lowering medications, current cigarette smoking (all measures as: 0 = No, 1 =Yes) and on habitual alcohol intake were obtained by questionnaire. The clinic interviewer asked whether the subject "presently

drinks alcoholic beverages at all (alcohol intake: 0 = No, 1 =Yes)" and if so, the number of 1.5 oz cocktails, 12 oz glasses (or can) of beer, and 4 oz glasses of wine they consumed per day in 1 week. Based on these responses, total alcohol intake (g/day) was calculated. Subjects were excluded from the analyses if: (i) alcohol consumption exceeded 21 drinks/week for men and >14 drinks/week) for women; (ii) if taking Amiodarone, or (iii) if positive for hepatitis C antibodies. In all, 88 subjects for FL and 124 subjects for ALT were excluded.

2.3. Statistical analysis

Groups were compared using t-test for continuous variables and χ^2 test for categorical variables. For association analysis, we employed a mixed model (proc glimmix using SAS®) to account for the non-independence of family members according to their kinship using a logistic link function. Age, age^2, sex, current cigarette smoking (No/Yes), alcohol intake (No/Yes) and clinical centers were included in each model of CHD. A p value of less than 0.05 for covariates was considered statistically significant in the association model.

3. Results

3.1. Characteristics of the Study Population by CHD Status

Table 1 shows the descriptive statistics of European American participants in the FamHS with and without CHD. The prevalence of CHD was significantly higher in men (18.9%) as compared to women (6.2%). On average, the subjects with CHD were 12.5 years older than unaffected subjects. The mean levels of FL, BMI, HOMA-IR and SBP were significantly higher with among those with CHD compared to those without CHD (Table 1). The mean levels of LDL-C and DBP were lower in subjects with CHD compared to those without, which may be attributable to the differential proportion of subjects taking medications: 62% of CHD subjects (*vs.* 27% in non-CHD) were taking antihypertensive medications and 67% took lipid-lowering medications (*vs.* 18% in non-CHD).

3.2. Univariate Associations with Prevalent CHD

First, we tested whether FL or ALT levels (40 U/L) individually were associated with prevalent CHD (Table 2). Both FL (p=0.0084) and ALT levels (40 U/L, p=0.014) were predictors of CHD. We also found the expected associations between CHD and traditional risk factors, such as diabetes (p<0.0001), HOMA-IR (p<0.0001), BMI (p=0.012), LDL-C (p<0.0001), SBP (p=0.047) and DBP (p<0.0001).

3.3. Multivariate Associations of Prevalent CHD with Metabolic Traits

We sought to determine whether NAFLD has an independent association above and beyond that explained by traditional risk factors, namely, obesity, insulin resistance, and diabetes. We fit a multivariate model including FL and ALT with these other CHD risk factors. When accounting for the metabolic factors, which increase risk for both NAFLD as well as CHD, the marginal association of FL (p=0.598) was no longer significant (Table 3), suggesting that the association observed in the univariate model could be explained by these common underlying factors. However, ALT (40 U/L, p=0.042) remained associated with prevalent CHD. Since the prevalence of CHD was much higher among men (18.8%) compared to women (6.2%), we further investigated whether the association results differed by sex (Table 3). An independent association with ALT (40 U/L, p=0.044) was noted but for men only.

Since diabetic status and use of hyperglycemic medications represent a potential source of heterogeneity, we tested the robustness of these associations while restricting the analysis to non-diabetic subjects (Supplementary Table 1). The same pattern of results was obtained.

However the significance of ALT (40 U/L, p=0.007) as a predictor in men was enhanced, lending additional credibility to the conclusion that this indicator of hepatic inflammation is an independent predictor of CHD risk in men; we continued to note no association in women.

We then expanded the predictive model to include several other variables that are utilized in the Framingham risk score for CHD (http://www.framinghamheartstudy.org/risk/coronary.html) such as lipids (LDL-C) and blood pressure measurements (SBP and DBP). Since the effects of lipid-lowering medications and antihypertensive medications may influence the results, we also included these indicators as covariates in the multivariate model (Supplementary Table 2). The results were qualitatively robust and similar to the previous analysis (Supplementary Table 1), again showing a sex-specific association of ALT (40 U/L) with CHD (p=0.031) independent of traditional CHD risk factors in non-diabetic men.

4. Discussion

Diverse findings for the relationship of NAFLD with atherosclerosis and clinical coronary artery disease have been reported in the literature, some studies supporting NAFLD as a cardiovascular risk factor [14, 20–22], others not [23, reviewed in 4, 8, 14–16]. There may be several reasons that could affect the different conclusions reached by these studies. On the phenotypic level, NAFLD represents a wide spectrum of liver damage, and differences in definition or diagnostic criteria for NAFLD may contribute to conflicting results; notably, some studies defined NAFLD on the basis of ALT levels alone [e.g., 8, 21]. Also, the coexistence of underlying metabolic risk factors in subjects with NAFLD (e.g., diabetes and insulin resistance) makes it difficult to distinguish whether NAFLD *per se* is a predictor of CHD, or whether the common metabolic risk factors are responsible for the association with CHD. Few previous studies examined the heterogeneity of NAFLD by sex. In this study, we set out to investigate whether NAFLD, characterized by either FL or ALT levels (40 U/L), is an independent predictor of prevalent CHD, while simultaneously modeling the common metabolic risk factors and allowing for sex differences. Even though elevated ALT levels are not diagnostic of NAFLD in practice, they are often regarded as a harbinger of liver disease or a marker of severity. In this context, our data provides support for elevated ALT levels (indicative of non-specific liver damage) being associated with increased risk of CHD, at least in men, which is more readily appreciated when considering non-diabetic subjects. Thus, there are aspects of liver disease that do appear to independently exacerbate risk for CHD, although those factors differ in men and women, and appear to be confounded by underlying insulin resistance and diabetes.

Much remains unknown about the complex mechanisms underlying the association of NAFLD with atherosclerosis and coronary artery disease, though insulin resistance, oxidative stress, inflammatory markers (e.g., IL-6, TNF-α and CRP), obesity-related ectopic fat (in nonadipose tissues) and lipotoxicity appear to be important interacting factors [12, 24]. Here, we observed that the association between CT measured FL with CHD was attenuated by adjustment for insulin resistance and diabetes in the multivariate model. This suggests that the association of FL with CHD may be mediated via insulin resistance and diabetes. In a study of diabetic patients, liver fat measured by ultrasound was a predictor of prevalent cardiovascular disease independent of traditional risk factors [25]. NAFLD is very common in diabetic patients [25], consistent with the idea that insulin resistance is an early manifestation common to both the accumulation of liver fat and to diabetes, and a strong CHD risk factor.

Hepatic inflammation, indicated by elevated ALT levels, may influence risk of coronary artery disease. Our study showed that ALT (40 U/L) predicts prevalent CHD independent of traditional cardiovascular risk factors, such as BMI, insulin resistance, diabetes, LDL-C, SBP and DBP in men, but not in women. A similar observation was made in the Hoorn Study, in which association between ALT and CHD remained significant for subjects in the third tertile relative to those in the first tertile after adjustment for traditional risk factors [8]. Another study found that increased ALT activity was associated with risk of CHD as estimated using the Framingham risk score in both sexes; however, the ALT threshold for an increased risk of CHD was higher in men (> 43 IU [26]). In addition, serum gamma-glutamyltransferase levels, which have been used as an index of liver dysfunction, were associated with all-cause cardiovascular mortality in men (but not in women), independent of other cardiometabolic risk factors [22]. Previous literature has shown that increased ALT was a predictor of coronary calcification [20] and related with the risk of carotid intima medial thickness in patients with NAFLD [21], independent of risk factors for atherosclerosis and metabolic syndrome. Moreover, severity of liver histopathology (degree of hepatic steatosis, necroinflammation, and fibrosis) was strongly associated with early carotid atherosclerosis, even controlling for traditional risk factors, in subjects with NAFLD [14].

Systemic up-regulation of inflammatory activity may simultaneously influence NAFLD, coronary atherosclerosis and CHD [24]. Indeed, patients with these diseases have higher plasma markers of oxidative stress and inflammation [12, 16, 24]. One possible mechanism of up-regulation of inflammatory activity is via hepatic insulin resistance [24], which is associated with NAFLD [12, 16, 24]. Hepatic insulin resistance is characterized by a reduced insulin-suppressing effect on glucose production in the liver, which exacerbates peripheral insulin resistance and hyperinsulinemia [24]. This leads to increased hepatic free fatty acid synthesis and decreased synthesis of apolipoprotein B-100, resulting in accumulation of triglycerides in the liver [12, 24]. It has been suggested that hepatic insulin resistance is mediated by free fatty acids and tumor necrosis factor-alpha (TNF-α) through JNK (c-Jun N-terminal kinase)-dependent insulin receptor substrate-1 phosphorylation. The nuclear factor-kappa B (NF-κB) activated by IKK-B (IκB kinase) is implicated in transcription of pro-inflammatory mediators (e.g., interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF-α). NF-κB dependent inflammatory mediators produced in hepatocytes down-regulate insulin sensitivity and trigger liver injury [12, 24]. These same proinflammatory cytokines and pathways are activated during the process of atherosclerotic plaque formation [12, 16, 24], which may play a dual role in the development of NAFLD and coronary disease.

This study has several relevant strengths and limitations. It is a large population-based study in which imaging data characterizing liver attenuation was available along with measurements of all other major cardiovascular risk factors. While it is not possible to characterize subjects with histology requiring liver biopsies in otherwise healthy subjects, we have an unprecedented opportunity to investigate associations among these conditions in a large epidemiologic study. CT imaging of the liver provides a non-invasive procedure that produces an accurate characterization of fat burden [1, 4, 5]. ALT levels have been the most commonly used variable for assessment of liver disease [18]. However, elevated ALT levels are not specific to NAFLD and can occur in response to many other conditions and, in that sense, they are not an adequate measure of NAFLD and they do not reflect the severity or the outcome of NAFLD [1, 4]. ALT measurement often fails to identify patients with minimal to mild necroinflammatory activity and normal liver enzymes may be observed in NAFLD [4]. The sensitivity of ALT for the diagnosis of NASH in large cohort studies was found to be only 40% [4], and there is no consensus on a standard cut-off value for detecting the presence of NASH [8]. However, several previous epidemiological studies have used

ALT levels to define NAFLD [7, 8]. Even if ALT levels do not specifically indicate NAFLD, it appears that the level of hepatic inflammation indicated by ALT (for whatever reason) predict prevalent CHD. We have used HOMA-IR as a measure of insulin resistance, which correlates reasonably well (r^2=−0.67, p<0.001 [27]) with the results of hyperinsulinemic euglycemic glucose clamp technique. Although this technique is a gold standard for assessment of insulin sensitivity, the complexity of this methodology limits its use in epidemiologic studies [27]. We used prevalent CHD as an outcome, and prospective data would be preferable in this context. The lack of association between NAFLD traits and CHD in the FamHS women may be due to the relatively young age of women and perhaps they have not had sufficient time to manifest CHD. It is worth mentioning that the presentation of coronary artery disease in women tends to be 10 years later than its presentation in men [28]. Nevertheless, other larger populations should investigate whether NAFLD predicts clinical cardiovascular events in women. Lastly, because half of the families were selected by an excess of CHD our findings should be considered in similar populations.

In summary, advanced hepatic inflammation may have an independent effect promoting CHD. A mechanism involving markers of oxidative stress and inflammation [12, 15, 16, 24] may play a crucial role in the progression of FL to histological changes associated with steatohepatitis and simultaneously in advancing development of coronary artery disease. Our findings suggest that, ALT levels (40 U/L) predict CHD in men, while association of FL with CHD may be mediated via insulin resistance and diabetes. The sex-specific associations observed here are novel and reinforce the idea that the metabolic profiles of men and women influence risk of CHD differently. The mechanisms underlying these associations merit further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research highlights

We investigated whether NAFLD is a predictor of CHD independent of traditional risk factors.

NAFLD was assessed by using CT measured fatty liver and elevated levels of alanine aminotransferase levels (ALT 40 U/L).

We investigated whether association modification by sex exists between NAFLD traits and prevalent CHD.

Elevated ALT levels (40 U/L) are associated with increased risk of CHD in men only, and the significance of ALT as a predictor is enhanced in non-diabetic subjects.

Association of liver fat burden measured by CT with CHD may be mediated via insulin resistance and diabetes.

Table 1

Characteristics of European American participants by Presence or Absence of CHD in the FamHS

	with CHD	without CHD	P value
Sample size	327	2,429	
Men	233	1003	<.0001
Age (years)	68.1 ± 9.1	55.6 ± 13.1	<.0001
FL (HU)	−57.5 ± 11.6	−59.4 ± 11.1	0.0047
FL −40 HU *(N$_{Total}$; % of affected)	25 (311; 8.0)	152 (2,328; 6.5)	0.056
ALT 40U/L *(N$_{Total}$; % of affected)	24 (312; 7.7)	138 (2,314; 6.0)	0.046
BMI (kg/m^2)	29.8 ± 5.1	28.7 ± 5.7	0.0004
HOMA-IR	4.10 ± 4.85	2.64 ± 2.46	<.0001
Diabetes (N$_{Total}$; % of affected)	103 (327; 31.5)	263 (2,427; 10.8)	<.0001
LDL-C (mg/dL)	92.1 ± 30.3	113.3 ± 33.4	<.0001
Lipid-lowering medication (N$_{Total}$; % of affected)	207 (308; 67.2)	420 (2,334; 18.0)	<.0001
SBP (mm Hg)	125.6 ±22.4	120.2 ±19.6	<.0001
DBP (mm Hg)	68.3 ± 10.1	70.0 ± 9.8	0.0055
Antihypertensive medication (N$_{Total}$; % of affected)	203 (327; 62.1)	655 (2,425; 27.0)	<.0001

Variables are shown as number (N), and/or frequency (%), or mean ± standard deviation;

P values refer to t-test for continuous variables and χ^2 test for categorical variables;

FL: Fatty liver (liver attenuation * −1); ALT: levels of alanine transaminase; BMI: body mass index; HOMA-IR: Homeostatic Model Assessment (fasting glucose (mmol/L)*fasting insulin (mU/L)/22.5); Diabetes: taking hyperglycemic medication and/or fasting glucose 126 ml/dl); LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; SBP: diastolic blood pressure;

*Fisher's Exact Test (table probability)

Table 2

Univariate Association of CHD with NAFLD and Other Metabolic Traits

Independent Trait	Odds Ratio Estimate (95% CI)	P value	N Total (CHD)
FL	1.116 (1.043; 1.329)	0.0084	2639 (311)
ALT 40 U/L	2.014 (1.156; 3.509)	0.014	2626 (312)
BMI	1.033 (1.007; 1.060)	0.012	2753 (327)
HOMA-IR	1.109 (1.062; 1.157)	<.0001	2693 (318)
Diabetes	2.582 (1.870; 3.566)	<.0001	2754 (327)
LDL-C	0.979 (0.973; 0.984)	<.0001	2642 (308)
SBP	0.993 (0.986; 1.015)	0.047	2752 (327)
DBP	0.961 (0.947; 0.976)	<.0001	2752 (327)

ALT   40 U/L defines abnormality.

Table 3

Multivariate Association of CHD with NAFLD and Other Metabolic Traits by Sex-Combined and Stratified by Sex

	Dependent Trait: CH D					
	Combined (N_{CHD}=299, $N_{Non-CHD}$= 2251)		Men (N_{CHD}=211, $N_{Non-CHD}$= 913)		Women (N_{CHD}=88, $N_{Non-CHD}$= 1338)	
Independent Trait	Odds Ratio Estimate (95% CI)	P value	Odds Ratio Estimate (95% CI)	P value	Odds Ratio Estimate (95% CI)	P value
FL	0.996 (0.980; 1.012)	0.598	0.996 (0.977; 1.014)	0.648	0.998 (0.971; 1.025)	0.886
ALT 40 U/L	1.898 (1.024; 3.520)	0.042	1.962 (1.019; 3.775)	0.044	0.942 (0.169; 5.248)	0.946
BMI	1.003 (0.971; 1.036)	0.867	0.990 (0.948; 1.034)	0.660	1.020 (0.972; 1.071)	0.422
HOMA-IR	1.067 (1.010; 1.128)	0.021	1.079 (1.014; 1.149)	0.016	1.030 (0.923; 1.149)	0.597
Diabetes	2.090 (1.446; 3.022)	<.0001	1.863 (1.219; 2.847)	0.004	2.412 (1.256; 4.631)	0.008
Smoke	2.147 (1.544; 2.986)	<.0001	2.545 (1.710; 3.788)	<.0001	1.798 (1.013; 3.192)	0.045
Alcohol Intake	0.996 (0.961; 1.033)	0.841	0.980 (0.944; 1.017)	0.291	1.030 (0.942; 1.126)	0.514
Age	9.819 (2.270; 42.48)	0.002	17.752 (3.109; 101.36)	0.001	5.937 (0.441; 80.005)	0.179
Age^2	0.897 (0.800; 1.006)	0.064	0.851 (0.741; 0.977)	0.022	0.943 (0.774; 1.149)	0.558
Sex	0.302 (0.222; 0.411)	<.0001				