Edinburgh Research Explorer

Nonalcoholic fatty liver disease and risk of incident young-onset hypertension

1   **Nonalcoholic fatty liver disease and risk of incident young-onset hypertension: effect**

2   **modification by sex**

3   **Running title:** Sex dimorphism, NAFLD and hypertension

4   Yejin Kim, MHS[1*], Yoosoo Chang, MD, PhD[1,2,3*], Seungho Ryu, MD, PhD[1,2,3**], Soyoung Park, MD,

5   PhD[2,4**], Yoosun Cho, MD, PhD[1, 4], Won Sohn, MD, PhD[5], Jeonggyu Kang, MD[1], Sarah H. Wild,

6   MB, BChir, PhD[6]; Christopher D Byrne, MB, BCh, PhD[7,8]

7

8   [1]Center for Cohort Studies, Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan

9   University School of Medicine, Seoul, Republic of Korea

10   [2]Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital,

11   Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

12   [3]Department of Clinical Research Design & Evaluation, SAIHST, Sungkyunkwan University, Seoul,

13   Republic of Korea

14   [4]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University School of

15   Medicine, Seoul, Republic of Korea

16   [5]Division of Gastroenterology, Department of Internal Medicine, Kangbuk Samsung Hospital,

17   Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

18   [6] Usher Institute, University of Edinburgh, Edinburgh, U.K.

19   [7] Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton, U.K.

20   [8] National Institute for Health and Care Research Southampton Biomedical Research Centre,

21   University Hospital Southampton, Southampton, U.K.

22

23   [*] **These authors share co-first authorship.**

24

25   **Author email addresses**

26   Yejin Kim: reenya273@gmail.com

27   Yoosoo Chang: yoosoo.chang@gmail.com

28 Seungho Ryu: sh703.yoo@samsung.com

29 Soyoung Park: syoem.park@samsung.com

30 Yoosun Cho: misslonghorn46@gmail.com

31 Won Sohn: hand0827@naver.com

32 Jeonggyu Kang: kamgjk@gmail.com

33 Sarah H. Wild: sarah.wild@ed.ac.uk

34 Christopher D Byrne: c.d.byrne@soton.ac.uk

35

36 **** Address correspondence to**

37 **Seungho Ryu, MD, PhD,** Department of Occupational and Environmental Medicine, Kangbuk

38 Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250,

39 Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

40 Tel: +82-2-2001-5137; fax: +82-2-757-0436; e-mail: sh703.yoo@samsung.com

41 **Soyoung Park, MD, PhD,** Department of Occupational and Environmental Medicine, Kangbuk

42 Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250,

43 Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

44 Tel: +82-2-2001-5139; fax: +82-2-757-0436; e-mail: syoem.park@samsung.com

45

46 **Word count:** 3,673 words (text); 230 (abstract)

47 **Number of tables and figure:** 4 tables; 1 figure

48 **Number of references:** 47 references

49 **Abbreviation list**

50 ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood

51 pressure; CI, confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyl

52 transpeptidase; HR, hazard ratio; HDL-C, high-density lipoprotein cholesterol; HOMA-IR,

53 homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein;

54 LDL-C, low-density lipoprotein cholesterol; PY, person-years; RAS, renin-angiotensin system; T2D,

55 type 2 diabetes

56

Data availability statement

The data are not available to be shared publicly as we do not have IRB permission for distributing the
data. However, data is available from the corresponding author on reasonable request.

ABSTRACT

Background and aims: Although nonalcoholic fatty liver disease (NAFLD) and hypertension are increasingly common among young adults, it is uncertain if NAFLD affects incidence of young-onset hypertension, and if the association is modified by sex. We investigated potential effect modification by sex on the association between NAFLD and incident hypertension in young adults (<40 years).

Method and results: This cohort study comprised 85,789 women and 67,553 men aged <40 years without hypertension at baseline. Hepatic steatosis was assessed by liver ultrasound and classified as mild or moderate/severe. Hypertension was defined as blood pressure (BP) $\geq$130/80 mmHg; self-reported history of physician-diagnosed hypertension; or current use of BP-lowering medications. Cox proportional hazard models were used to estimate hazard ratios (HRs; 95% confidence intervals [CIs]) for incident hypertension by NAFLD status (median follow-up 4.5 years). A total of 25,891 participants developed incident hypertension (incidence rates per 10^3 person-years: 15.6 for women and 63.5 for men). Multivariable-adjusted HRs (95% CIs) for incident hypertension comparing no NAFLD (reference) with mild or moderate/severe NAFLD were 1.68 (1.56–1.80) and 1.83 (1.60–2.09) for women and 1.21 (1.17–1.25) and 1.23 (1.17–1.30) for men, respectively. Stronger associations were consistently observed between NAFLD and incident hypertension in women, regardless of obesity/central obesity (all *p*-values for interaction by sex <0.001).

Conclusions: NAFLD is a potential risk factor for young-onset hypertension with a relatively greater impact in women and in those with more severe hepatic steatosis.

Keywords: Nonalcoholic fatty liver disease, sex dimorphism, sex difference, young-onset hypertension, cohort study

INTRODUCTION

Hypertension in young adults (<40 years of age) is estimated to occur in about 1 in 8 adults between 20 and 40 years of age [1]. Increasing prevalence of young-onset hypertension parallels that of obesity and other lifestyle- or metabolic-related diseases [1]. Hypertension at a young age can have detrimental consequences, including increased cardiovascular (CV) risks in middle age, early end-organ damage, as well as mortality [2-5]. With age being one of the strongest risk factors in the traditional model for estimating cardiovascular risk, the risk of uncontrolled blood pressure (BP) in young people is often underestimated, leading to delays in adequate management.

An approximately 7-fold increase in the incidence of nonalcoholic fatty liver disease (NAFLD) has been observed in young adults aged 18-39 years over a 20-year period [6]. Given the strong link between NAFLD and a wide range of CV complications including hypertension [7-9], the recent increase in NAFLD incidence is likely to be contributing to the recent rise in cardiovascular disease (CVD) incidence among young individuals [9]. However, previous literature investigating risk factors for hypertension has mainly focused on middle-aged or older adults, and the role of NAFLD on the risk of hypertension in young adults remains unclear.

Both NAFLD and BP show sexually dimorphic traits and prevalence of these conditions varies by sex across the lifespan [10, 11]. It is well established that women are protected from cardiometabolic risk due to female sex- and sex-related factors [12]. However, whether disparities by sex also apply to the association between NAFLD and hypertension in young adults aged <40 years has not been investigated. Given fundamental etiological differences in vascular physiology between men and women [13, 14], an accurate understanding of the role of sex as a risk modifier is essential in risk stratification, disease prevention, and optimizing therapeutic approaches to hypertension in young adults.

The present study aimed to investigate in young adults: a) the association between NAFLD (specifically mild and moderate/severe steatosis) and risk of incident hypertension and b) whether there was effect-modification by sex on these associations.

METHODS

Study participants

This cohort study is part of the Kangbuk Samsung Health Study, consisting of Korean men and women aged ≥18 years who underwent comprehensive annual or biennial examinations at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea, as previously described [15]. The participants under 40 years of age who underwent a comprehensive health examination between January 2011 and December 2019 and had at least one follow-up visit before December 31, 2020 (n = 235,193) were initially included. After applying exclusion criteria (see Supplementary Materials), the final sample yielded 153,342 participants, comprising 85,789 women and 67,553 men.

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2022-04-058), which waived the need for informed consent owing to the use of de-identified retrospective data from routine health screening. All procedures performed in the study were in accordance with the Declaration of Helsinki regarding ethical standards for research involving human subjects.

Data collection

Standardized, self-administered questionnaires, physical measurements, abdominal ultrasonography results, and serum biochemical measurements were collected at each visit during the basic health check-up program [15] (see Supplementary Materials for further details).

Sitting BP, height, weight, and waist circumference were measured by trained nurses. Waist circumference was measured by trained personnel in the horizontal plane around the unclothed abdomen to the nearest 0.1 cm at the midpoint between the bottom of the rib cage and the top of the iliac crest, with the subjects standing with their weight equally distributed on both feet, their arms at their sides, and head facing straight forward. Abdominal obesity was defined as waist circumference ≥90 cm for men and ≥85 cm for women, which are specific for Korean populations [16, 17]. BP was measured using an automated oscillometric device (Model 53000; Welch Allyn, New York, NY) while participants were in a seated position with the arm supported at heart level. Three BP readings were

136 recorded for each participant, and the average of the second and third readings was used in the analyses

137 to minimize measurement error. Hypertension was defined as systolic BP ≥130 mmHg, diastolic BP

138 ≥80 mmHg (using the threshold for diagnosis of stage 1 hypertension), self-reported history of

139 physician-diagnosed hypertension, or current use of antihypertensive medication, on the basis of the

140 2017 American College of Cardiology and American Heart Association Hypertension guidelines [18,

141 19]. A higher threshold of ≥140/90 mmHg was also used for supplementary analyses [18, 19].

142 Overweight was defined according to the Asian-specific criteria [20]: body mass index (BMI)

143 of ≥23 kg/m^2.

144 Blood specimens were collected after at least 10 h of fasting. Levels of lipid profiles, liver

145 enzymes, glucose, and high sensitivity C-reactive protein (hsCRP) were measured (see Supplementary

146 Materials for further details).

147

148 ***Diagnosis of NAFLD***

149 NAFLD was defined as the presence of fatty liver in the absence of excessive alcohol use (<20

150 g/day and <30 g/day for women and men, respectively) or any other identifiable cause [21]. Fatty liver

151 was diagnosed on the basis of an abdominal ultrasound performed by experienced radiologists who

152 were unaware of the study aim, using standard criteria, including a diffuse increase in fine echoes in the

153 liver parenchyma in comparison with the kidney or spleen, deep beam attenuation, and bright vessel

154 walls [22]. Radiologists graded hepatic steatosis as mild, moderate, or severe [23]. Mild hepatic

155 steatosis was identified by a slight increase in liver echogenicity. Moderate hepatic steatosis was

156 identified by a slightly impaired image of the intrahepatic vasculature and diaphragm, accompanied by

157 increased liver echogenicity. Severe hepatic steatosis was identified by a marked increase in liver

158 echogenicity, impaired penetration of the posterior segment of the right lobe, and poor or no image of

159 the intrahepatic vasculature and diaphragm [24]. Degree of hepatic steatosis was categorized as mild or

160 moderate/severe. The inter- and intra-observer reliability values for diagnosis of hepatic steatosis were

161 substantial (kappa statistic of 0·74) and excellent (kappa statistic of 0·94), respectively [15].

162

163   *Statistical analyses*

164   The participants' characteristics according to the presence of NAFLD separately for women

165   and men were summarized using descriptive statistics.

166   The primary exposure was any NAFLD and ultrasound-based assessment of severity of hepatic

167   steatosis at baseline. The primary endpoint was incident hypertension during follow-up: (1)

168   hypertension based on the threshold for stage 1 hypertension defined as ≥130/80 mmHg (details of the

169   definition described earlier); and (2) hypertension based on stage 2 hypertension defined as ≥140/90

170   mmHg [18, 19] for the sensitivity analyses.

171   The follow-up duration for each participant was extended from the baseline examination until

172   the development of the endpoint or the last health examination conducted prior to December 31, 2020,

173   whichever came first. Incidence rates were calculated as the number of incident cases divided by follow-

174   up person-years (PY). Cox proportional hazard models were used to estimate the hazard ratios (HRs)

175   with 95% CIs for the development of incident hypertension. Initially, we adjusted for age. In

176   multivariable-adjusted model, further adjustment was made for the study center (Seoul, Suwon), year

177   of the screening examination, alcohol consumption, smoking status, physical activity, education level,

178   lipid-lowering medication, and BMI (continuous). To evaluate the effects of NAFLD status changes

179   between baseline and follow up, and also change in other covariates during the follow-up period, we

180   performed additional analyses by introducing NAFLD status and other covariates/potential

181   confounding factors, as time-varying covariates in the models (time-dependent models). The

182   proportional hazards assumption was assessed via estimated log (-log) survival curves, and no violation

183   of the assumption was found.

184   To assess the interaction effect by sex, the multivariable model included the presence of

185   NAFLD, sex and the product term, as well as the potential confounders in the multivariable model. We

186   calculated stratum-specific effect estimates with confidence intervals using the *–lincom* command in

187   STATA after performing multivariable analysis. Since NAFLD is strongly associated with general and

188   abdominal obesity, we also performed stratified analyses based on binary categories of overweight

189   (defined as BMI of <23 and≥23 kg/m^2)[20] and abdominal obesity (waist circumference of <90 and

190 ≥90 cm in men and <85 and ≥85 cm in women[25]). To account for potential confounding effects of

191 metabolic comorbidities, additional analyses restricted to metabolically health individuals were also

192 performed. The interactions between NAFLD status and sex on the risk of hypertension were assessed

193 using likelihood ratio tests, comparing models with and without multiplicative interaction terms.

194   Statistical analyses were performed using STATA version 16.0 (StataCorp LP, College Station,

195 TX, USA). Statistical significance was set at $p < 0.05$.

196

197 **RESULTS**

198   The baseline characteristics of the study population stratified by sex and NAFLD status are

199 presented (**Table 1**). The mean (SD) age of women and men were 31.9 (3.7) and 32.0 (3.2) years,

200 respectively. Age, lipid-lowering medication usage, obesity parameters (BMI, obesity, waist

201 circumference), BP, glycemic parameters (glucose, HbA1c), total cholesterol, triglycerides, low-density

202 lipoprotein cholesterol (LDL-C), liver enzymes (gamma-glutamyl transpeptidase [GGT], alanine

203 aminotransferase [ALT], and aspartate aminotransferase [AST]), hs-CRP levels, and HOMA-IR were

204 higher in the NAFLD groups than in the non-NAFLD group, while high-density lipoprotein cholesterol

205 (HDL-C) was higher in the non-NAFLD group than NAFLD groups both in men and women.

206   **Table 2** presents the absolute and relative risks of incident hypertension based on NAFLD

207 status and sex. Within over 700,000 person-years of follow-up (median follow-up 4.5 years), 25,891

208 subjects developed incident hypertension (incidence rates per 10^3 person-years were 35.3 [95% CI,

209 35.1-36.1] overall; 15.6 [95% CI, 15.2-15.9] for women and 63.5 [95% CI, 62.6-64.4] for men. In the

210 age-adjusted model, NAFLD was positively associated with incident hypertension in both men and

211 women, and hazard ratios were significantly higher for women than men. After further adjustments for

212 sex, center, year of screening, alcohol consumption, smoking status, physical activity, education level,

213 lipid-lowering medication, and BMI, the multivariable-adjusted HRs (95% CIs) for incident

214 hypertension comparing NAFLD to no NAFLD were 1.67 (1.56–1.80) in women and 1.24 (1.20–1.28)

215 in men (*p* for interaction by sex <0.001). The association was virtually unchanged in both groups when

216 changes in status of NAFLD or other covariates during the follow-up period were treated as time-

217   varying covariates. Similar findings were observed in the sensitivity analyses where the risk of incident

218   stage 2 hypertension was assessed as an outcome (**eTable 1**) and when HOMA-IR was further adjusted

219   for (**eTable 2**).

220   The risk of incident hypertension according to NAFLD and its severity (assessed by the

221   ultrasound) were also investigated (**Table 3**). In the age-adjusted model, the degree of NAFLD severity

222   was positively associated with incident hypertension in a dose-response manner among men and women.

223   After further adjustments for confounders, these associations were attenuated, but the trends persisted

224   in both sexes; the multivariable-adjusted HRs (95% CIs) for incident hypertension comparing mild

225   NAFLD and moderate/severe NAFLD to no NAFLD group as the reference were 1.67 (1.54–1.80) and

226   1.78 (1.52–2.08) in women and 1.23 (1.19–1.27) and 1.28 (1.22–1.35) for men, respectively. These

227   associations remained similar when the covariates were treated as time-dependent variables (as reported

228   in the final column of **Table 3**). After further adjustment for HOMA-IR (**eTable 3**), increased excess

229   risks of hypertension was found in women with mild NAFLD. However, the associations were no longer

230   significant in women with moderate/severe NAFLD. When risks of stage 2 hypertension were assessed

231   based on NAFLD severity (**eTable 4**), the associations between mild and moderate/severe NAFLD at

232   baseline and incident stage 2 hypertension were even stronger compared with corresponding risks of

233   stage 1 hypertension. In a model with time-dependent variables, HRs (95% CIs) for incident

234   hypertension defined by the higher threshold ≥140/90 mmHg, comparing no NAFLD (reference) to

235   mild, or moderate/severe NAFLD were 2.01 (1.63–2.48) and 2.45 (1.73–3.45) for women; and 1.45

236   (1.28–1.64) and 1.55 (1.31–1.82) for men.

237   **Table 4** presents the association between NAFLD and stage 1 hypertension in BMI strata. In

238   both overweight and non-overweight groups with NAFLD, HRs for hypertension were higher in women

239   than in men. Similar patterns of associations were consistently observed when participants were

240   stratified based on abdominal obesity instead of overweight status (all *p*-values for interaction by sex

241   <0.001).

242   When we performed analyses restricted to metabolically healthy individuals (n = 91,628), the

243   association between NAFLD and development of stage 1 hypertension remained similar to the original

244 analyses both in women and men (**eTable 5**). Similarly, overall stronger relative excess risks of stage 1

245 hypertension were found in women with increasing severity of NAFLD compared with men.

246   In the analyses evaluating the risks of hypertension by NAFLD severity based on NFS (**eTable**

247 **6**) and FIB-4 (**eTable 7**), overall similar results were observed, with stronger excess risks among women

248 compared with men. However, significance was not detected for the groups with intermediate/high FIB-

249 4.

250

251   **DISCUSSION**

252   In this cohort study of 153,342 Korean young adults with a median follow up of almost five

253 years, our novel data shows that NAFLD is a potential risk factor for young-onset hypertension. The

254 relative impact of NAFLD as a risk factor is greater in women, and moderate/severe hepatic steatosis

255 is associated with a greater risk of developing incident hypertension than mild liver steatosis.

256   Previous epidemiological studies have shown that NAFLD and its severity are associated with

257 prevalent and incident hypertension in general populations [8, 26, 27]. A recent meta-analysis of 11

258 cohort studies suggests that NAFLD is associated with approximately a 1.6-fold increased risk of

259 incident hypertension [28]. However, limited data are available on the role of NAFLD in the

260 development of hypertension in younger adults under 40 years. Most of the existing studies to date have

261 also not considered sex-specific effects of NAFLD, which is known to be crucial in understanding the

262 incidence, progression and management of cardiometabolic diseases [11, 29-31]. Our study, to the best

263 of our knowledge, is the first to demonstrate that NAFLD is associated with increased risk of incident

264 hypertension in young adults. In addition, our study has revealed that the NAFLD-hypertension

265 relationship differs by sex, thus underscoring the need to consider the role of sex in estimating risk of

266 cardiovascular and other outcomes associated with NAFLD in young people.

267   Our data are consistent with the notion that cardiometabolic protection in women is diminished

268 in the presence of an underlying metabolic condition, as consistently reported in other studies conducted

269 in the context of obesity, type 2 diabetes (T2D), or metabolic syndrome [32-34]. In our study, the

270 absolute incidence of hypertension in women with NAFLD was similar to that of men without NAFLD.

271 The finding aligns with several lines of evidence including a previous systematic review that found no

272 differences in prevalence of NAFLD between men and women with T2D, in contrast to the general

273 population, in which men are more frequently affected than women [35]. Other studies have shown that

274 the presence of NAFLD attenuated protection against CVD in premenopausal women [32, 33]. Similar

275 patterns were also observed in our recent work demonstrating sex-specific associations between

276 NAFLD and T2D [36]. Taken together, in young women, whose cumulative exposure to metabolic risks

277 is relatively low, NAFLD may represent an increased cardiometabolic burden in these individuals that

278 may directly or indirectly contribute to higher BP, which needs to be further confirmed in additional

279 work.

280 Previously, the extent to which NAFLD is associated with increased risk of incident

281 hypertension beyond obesity or diabetes mellitus [9] has been uncertain. Our study addressed this issue

282 by restricting the study sample to individuals without diabetes or other known comorbidities at baseline

283 as well as evaluating the association in lean and overweight people using both general and abdominal

284 measures of obesity. In the stratified analyses higher hazard ratios of NAFLD for hypertension were

285 consistently observed in women than men regardless of overweight and abdominal obesity. The

286 significant excess risks associated with NAFLD in groups without abdominal obesity suggest that the

287 effect of NAFLD on blood pressure is not solely attributed to overall or central obesity [37]. Moreover,

288 in our analyses of a sample with metabolically healthy individuals only, the associations did not change.

289 These data suggest that, although the NAFLD group had a greater number of individuals with a range

290 of unfavorable metabolic abnormalities, these cardiometabolic comorbidities may not fully account for

291 the observed association. Further exploration is required to elucidate whether there are potentially

292 independent effects of NAFLD on hypertension development beyond central obesity.

293 While pathophysiological links between NAFLD and hypertension in the general population

294 have been relatively well described in previous literature [9], with some of the key mediators involving

295 insulin resistance, altered adipokine profiles, sympathetic nerve activation, and renin-angiotensin

296 system (RAS), mechanisms underlying sex dimorphism in the association between NAFLD and

297 hypertension are less clear and complex. NAFLD is associated with increased leptin levels [37-39].

298   Leptin may act in a sexually dimorphic fashion by promoting sympathetic activation in males and

299   stimulates aldosterone production in females [37]. Another mechanism may be related to the activation

300   of RAS by systemic inflammation in NAFLD [9]. RAS components such as renin are responsive to the

301   altering levels of estradiol [40], and there are also sex differences in the basal levels of several key

302   molecules involved in RAS activation (e.g., renin, angiotensin-converting enzyme) [14], suggesting the

303   effects of NAFLD on RAS may be different between sexes. In addition, while NAFLD may increase

304   vasoconstriction by decreasing the production of nitric oxide (NO) [41], estrogen may counteract this

305   effect by increase NO bioavailability by upregulating endothelial NOS. Moreover, inherent sex

306   differences in the balancing of sympathetic nerve activity [42] and responses to oxidative stress [43,

307   44], which are implicated as potential mechanisms linking NAFLD and hypertension [9], have been

308   reported. Further studies are necessary to determine whether there is a role of hepatic steatosis and its

309   severity in the pathogenesis of hypertension and whether there are inherent differences by sex in the

310   mechanisms.

311   There are some limitations to our study. First, NAFLD and its severity were assessed based on

312   ultrasound, instead of liver biopsy. The use of liver biopsy, however, for the purpose of routine

313   screening is considered unethical and not feasible. Moreover, liver ultrasound is widely accepted tools

314   in epidemiologic studies and reliably identifies NAFLD [45]. That said, further studies using diagnostic

315   tools with improved accuracy are needed to confirm our findings. Second, the determination of BP was

316   based on a single-day measurement, although it should be noted three readings were taken in our study.

317   This approach may lead to a misclassification of BP categories, possibly underestimating true

318   associations between NAFLD and incident hypertension. Third, causality cannot be determined owing

319   to the observational nature of our study, and a possibility of residual confounding remains due to

320   unmeasured confounders. Fourth, although we excluded postmenopausal women including those with

321   surgical/radiation-induced menopause, we did not exclude women with other potential causes of

322   menstrual irregularities, such as synthetic hormone use, intrauterine devices, or premature menopause

323   during follow-up, which may lead to a possibility of residual confounding. Considering these

324   reproductive factors would be important since the extent of cardioprotection in women can be dictated

325   by reproductive hormonal status in women. However, in our sample, none of the women were taking

326   oral contraceptives or using intrauterine devices. In addition, only 1.1% of the women reached

327   menopause during follow-up. Thus, it is unlikely that these factors had a substantial impact on our

328   results. Other conditions, such as polycystic ovary syndrome (PCOS), that may affect the menstrual

329   cycle as well as metabolic status [46] were not considered owing to the lack of data. Lastly, our study

330   of young Koreans means that the findings may not be generalizable to other populations of different

331   ages, ethnicities, or with different comorbidities.

332   Our study has several notable strengths. The longitudinal, prospective design enabled us to

333   observe the temporal associations between NAFLD, with the risk of incident hypertension. Furthermore,

334   the large sample size, the use of carefully standardized clinical, imaging, and laboratory procedures,

335   and the inclusion of lifestyle factors, and the repeated measurements allowed us to account for changes

336   in possible confounders over time, between baseline and follow up, as time-varying covariates. In

337   addition, the outcome was ascertained using multiple BP measurements, which reduced the potential

338   errors in diagnosis (e.g., white coat hypertension). Lastly, the inclusion of relatively healthy, younger

339   individuals i) reduced the potential for survivor bias caused by selecting subjects with severe diseases

340   as well as comorbidity-related bias, which is a common limitation of previous studies involving patients

341   with biopsy-proven advanced stage NAFLD and ii) eliminated the potential confounding effects of

342   menopause which is known to affect both NAFLD pathophysiology [29, 47] as well as BP [42],

343   allowing us to better determine sex-specific differences in the associations.

344

345   **Conclusion**

346   Our results demonstrate that NAFLD and its severity increase the risk of young-onset incident

347   hypertension, even in lean individuals, showing a stronger association in young women than in men.

348   As NAFLD is becoming an important public health concern, especially among young adults, the sex-

349   specific multisystem consequences of NAFLD in younger people deserves more attention.

350

351   **Author Contributions**

REFERENCES

[1] Hinton TC, Adams ZH, Baker RP, Hope KA, Paton JFR, Hart EC, et al. Investigation and Treatment of High Blood Pressure in Young People. Hypertension. 2020;75:16-22.doi:10.1161/HYPERTENSIONAHA.119.13820.
[2] Sundstrom J, Neovius M, Tynelius P, Rasmussen F. Association of blood pressure in late adolescence with subsequent mortality: cohort study of Swedish male conscripts. BMJ. 2011;342:d643.10.1136/bmj.d643.
[3] Yano Y, Reis JP, Colangelo LA, Shimbo D, Viera AJ, Allen NB, et al. Association of Blood Pressure Classification in Young Adults Using the 2017 American College of Cardiology/American Heart Association Blood Pressure Guideline With Cardiovascular Events Later in Life. JAMA. 2018;320:1774-82.10.1001/jama.2018.13551.
[4] Yano Y, Lloyd-Jones DM. Isolated Systolic Hypertension in Young and Middle-Aged Adults. Curr Hypertens Rep. 2016;18:78.10.1007/s11906-016-0686-x.
[5] Shang X, Hill E, Zhu Z, Liu J, Ge BZ, Wang W, et al. The Association of Age at Diagnosis of Hypertension With Brain Structure and Incident Dementia in the UK Biobank. Hypertension. 2021;78:1463-74.10.1161/HYPERTENSIONAHA.121.17608.
[6] Allen AM, Therneau TM, Larson JJ, Coward A, Somers VK, Kamath PS. Nonalcoholic fatty liver disease incidence and impact on metabolic burden and death: A 20 year-community study. Hepatology. 2018;67:1726-36.10.1002/hep.29546.
[7] Ryoo JH, Suh YJ, Shin HC, Cho YK, Choi JM, Park SK. Clinical association between non-alcoholic fatty liver disease and the development of hypertension. J Gastroenterol Hepatol. 2014;29:1926-31.10.1111/jgh.12643.
[8] Sung KC, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. J Hepatol. 2014;60:1040-5.10.1016/j.jhep.2014.01.009.
[9] Zhao Y-C, Zhao G-J, Chen Z, She Z-G, Cai J, Li H. Nonalcoholic Fatty Liver Disease. Hypertension. 2020;75:275-84.10.1161/HYPERTENSIONAHA.119.13419.
[10] Connelly PJ, Currie G, Delles C. Sex Differences in the Prevalence, Outcomes and Management of Hypertension. Current Hypertension Reports. 2022;24:185-92.10.1007/s11906-022-01183-8.
[11] Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, et al. Sex Differences in Nonalcoholic Fatty Liver Disease: State of the Art and Identification of Research Gaps. Hepatology. 2019;70:1457-69.10.1002/hep.30626.
[12] Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, Brinton RD, Carrero JJ, DeMeo DL, et al. Sex and gender: modifiers of health, disease, and medicine. Lancet. 2020;396:565-82.10.1016/s0140-6736(20)31561-0.
[13] Connelly PJ, Currie G, Delles C. Sex Differences in the Prevalence, Outcomes and Management of Hypertension. Current Hypertension Reports. 2022.10.1007/s11906-022-01183-8.
[14] Sabbatini AR, Kararigas G. Estrogen-related mechanisms in sex differences of hypertension and target organ damage. Biology of Sex Differences. 2020;11:31.10.1186/s13293-020-00306-7.
[15] Chang Y, Ryu S, Sung KC, Cho YK, Sung E, Kim HN, et al. Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification: evidence from the Kangbuk Samsung Health Study. Gut. 2019;68:1667-75.10.1136/gutjnl-2018-317666.
[16] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120:1640-5.10.1161/CIRCULATIONAHA.109.192644.

[17] Lee S PH, Kim SM, Kwon HS, Kim DY, Kim DJ, Cho GJ, Han JH, Kim SR, Park CY, Oh SJ, Lee CB, Kim KS, Oh SW, Kim YS, Choi WH, Yoo HJ. Cut-off points of waist circumference for defining abdominal obesity in the Korean population. Korean J Obes. 2006;15:9.

[18] Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Hypertension. 2018;71:e13-e115.10.1161/HYP.0000000000000065.

[19] Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). European heart journal. 2018;39:3021-104.

[20] World Health Organization, Regional Office for the Western Pacific. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney: Health Communications Australia; 2000.

[21] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23.10.1002/hep.25762.

[22] Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, et al. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis. 2002;34:516-22.10.1016/s1590-8658(02)80111-6.

[23] Cho IY, Chang Y, Kang J-H, Kim Y, Sung E, Shin H, et al. Long or Irregular Menstrual Cycles and Risk of Prevalent and Incident Nonalcoholic Fatty Liver Disease. The Journal of Clinical Endocrinology & Metabolism. 2022;107:e2309-e17.10.1210/clinem/dgac068.

[24] Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Official journal of the American College of Gastroenterology| ACG. 2007;102:2708-15.

[25] Lee SY, Park HS, Kim DJ, Han JH, Kim SM, Cho GJ, et al. Appropriate waist circumference cutoff points for central obesity in Korean adults. Diabetes Res Clin Pract. 2007;75:72-80.10.1016/j.diabres.2006.04.013.

[26] Zhu XP, Han GC, Chen Q, Zhang ZY, Wang LS, Zhang B. Fatty liver is a sensitive early warning for hypertension and its complication in the Chinese population. Clin Exp Hypertens. 2022:1-7.10.1080/10641963.2022.2029469.

[27] Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? J Hepatol. 2018;68:335-52.10.1016/j.jhep.2017.09.021.

[28] Ciardullo S, Grassi G, Mancia G, Perseghin G. Nonalcoholic fatty liver disease and risk of incident hypertension: a systematic review and meta-analysis. European Journal of Gastroenterology & Hepatology. 2022;34:365-71.10.1097/meg.0000000000002299.

[29] Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology. 2014;59:1406-14.10.1002/hep.26761.

[30] Ramirez LA, Sullivan JC. Sex Differences in Hypertension: Where We Have Been and Where We Are Going. American Journal of Hypertension. 2018;31:1247-54.10.1093/ajh/hpy148.

[31] Ueda K, Fukuma N, Adachi Y, Numata G, Tokiwa H, Toyoda M, et al. Sex Differences and Regulatory Actions of Estrogen in Cardiovascular System. Frontiers in Physiology. 2021;12.10.3389/fphys.2021.738218.

[32] Khalid YS, Dasu NR, Suga H, Dasu KN, Reja D, Shah A, et al. Increased cardiovascular events and mortality in females with NAFLD: a meta-analysis. Am J Cardiovasc Dis. 2020;10:258-71.

[33] Allen AM, Therneau TM, Mara KC, Larson JJ, Watt KD, Hayes SN, et al. Women With Nonalcoholic Fatty Liver Disease Lose Protection Against Cardiovascular Disease: A Longitudinal Cohort Study. Am J Gastroenterol. 2019;114:1764-71.10.14309/ajg.0000000000000401.

[34] Huebschmann AG, Huxley RR, Kohrt WM, Zeitler P, Regensteiner JG, Reusch JEB. Sex differences in the burden of type 2 diabetes and cardiovascular risk across the life course. Diabetologia. 2019;62:1761-72.10.1007/s00125-019-4939-5.

[35] Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, Caldwell SH, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: Focus on high-risk groups. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2015;47:997-1006.10.1016/j.dld.2015.08.004.

[36] Kim Y, Chang Y, Ryu S, Wild SH, Byrne CD. NAFLD improves risk prediction of type 2 diabetes: with effect modification by sex and menopausal status. Hepatology. 2022;76:1755-65.10.1002/hep.32560.

[37] Faulkner JL, Belin de Chantemele EJ. Sex Differences in Mechanisms of Hypertension Associated With Obesity. Hypertension. 2018;71:15-21.10.1161/HYPERTENSIONAHA.117.09980.

[38] Adolph TE, Grander C, Grabherr F, Tilg H. Adipokines and Non-Alcoholic Fatty Liver Disease: Multiple Interactions. Int J Mol Sci. 2017;18.10.3390/ijms18081649.

[39] Rojas E, Rodriguez-Molina D, Bolli P, Israili ZH, Faria J, Fidilio E, et al. The role of adiponectin in endothelial dysfunction and hypertension. Curr Hypertens Rep. 2014;16:463.10.1007/s11906-014-0463-7.

[40] Chidambaram M, Duncan JA, Lai VS, Cattran DC, Floras JS, Scholey JW, et al. Variation in the renin angiotensin system throughout the normal menstrual cycle. J Am Soc Nephrol. 2002;13:446-52.10.1681/ASN.V132446.

[41] Dogru T, Genc H, Tapan S, Ercin CN, Ors F, Aslan F, et al. Elevated asymmetric dimethylarginine in plasma: an early marker for endothelial dysfunction in non-alcoholic fatty liver disease? Diabetes Res Clin Pract. 2012;96:47-52.10.1016/j.diabres.2011.11.022.

[42] Tikhonoff V, Casiglia E, Gasparotti F, Spinella P. The uncertain effect of menopause on blood pressure. J Hum Hypertens. 2019;33:421-8.10.1038/s41371-019-0194-y.

[43] Lopez-Ruiz A, Sartori-Valinotti J, Yanes LL, Iliescu R, Reckelhoff JF. Sex differences in control of blood pressure: role of oxidative stress in hypertension in females. Am J Physiol Heart Circ Physiol. 2008;295:H466-74.10.1152/ajpheart.01232.2007.

[44] Ojeda NB, Hennington BS, Williamson DT, Hill ML, Betson NE, Sartori-Valinotti JC, et al. Oxidative stress contributes to sex differences in blood pressure in adult growth-restricted offspring. Hypertension. 2012;60:114-22.10.1161/HYPERTENSIONAHA.112.192955.

[45] Ballestri S, Mantovani A, Byrne CD, Lonardo A, Targher G. Diagnostic accuracy of ultrasonography for the detection of hepatic steatosis: an updated meta-analysis of observational studies. Metabolism and Target Organ Damage. 2021;1:7.10.20517/mtod.2021.05.

[46] Lee TT, Rausch ME. Polycystic Ovarian Syndrome: Role of Imaging in Diagnosis. RadioGraphics. 2012;32:1643-57.10.1148/rg.326125503.

[47] Wegermann K, Garrett ME, Zheng J, Coviello A, Moylan CA, Abdelmalek MF, et al. Sex and Menopause Modify the Effect of Single Nucleotide Polymorphism Genotypes on Fibrosis in NAFLD. Hepatology Communications. 2021;5:598-607.https://doi.org/10.1002/hep4.1668.

Table 1. Estimated mean values (95% CI) and adjusted[a] proportions (95% CI) of baseline characteristics for population strata defined by sex and NAFLD status among young adults under the age of 40 years (n = 153,342)

Characteristics	Women		P value	Men		p-value
	No NAFLD	NAFLD		No NAFLD	NAFLD	
Number of participants	80,593	5,196		45,102	22,451	
Age (years)	32.8 (32.8-32.8)	33.8 (33.7-33.9)	< 0.001	32.8 (32.8-32.9)	33.9 (33.9-34.0)	< 0.001
Alcohol intake (%) [b]	11.8 (11.6-12.1)	13.0 (12.1-13.9)	0.014	43.5 (43.0-43.9)	43.1 (42.5-43.8)	0.378
Current smoker (%)	1.7 (1.6-1.8)	2.7 (2.3-3.2)	< 0.001	28.6 (28.2-29.1)	30.7 (30.1-31.3)	< 0.001
Higher education (%) [d]	85.2 (84.9-85.4)	73.8 (72.5-75.1)	< 0.001	93.2 (93.0-93.4)	93.3 (92.9-93.6)	0.790
HEPA (%) [c]	11.0 (10.8-11.2)	11.4 (10.5-12.2)	0.449	17.8 (17.5-18.2)	14.0 (13.5-14.5)	< 0.001
Lipid-lowering medication use (%)	0.1 (0.1-0.1)	0.3 (0.2-0.5)	< 0.001	0.4 (0.4-0.5)	1.0 (0.9-1.1)	< 0.001
Obesity (%) [e]	5.4 (5.3-5.6)	52.5 (51.2-53.9)	< 0.001	19.2 (18.9-19.6)	59.9 (59.3-60.6)	< 0.001
Body mass index (kg/m^2)	20.7 (20.7-20.8)	25.7 (25.6-25.8)	< 0.001	23.1 (23.1-23.1)	25.9 (25.9-26.0)	< 0.001
Waist circumference (cm)	73.1 (73.1-73.2)	85.1 (84.9-85.3)	< 0.001	81.7 (81.7-81.8)	89.6 (89.5-89.6)	< 0.001
SBP (mmHg)	98.9 (98.8-98.9)	104.3 (104.0-104.5)	< 0.001	109.2 (109.2-109.3)	112 (111.9-112.1)	< 0.001
DBP (mmHg)	62.9 (62.9-63.0)	65.6 (65.4-65.7)	< 0.001	67.9 (67.8-67.9)	69.7 (69.7-69.8)	< 0.001
Glucose (mg/dl)	89.2 (89.2-89.3)	93.6 (93.4-93.8)	< 0.001	92.2 (92.2-92.3)	94.5 (94.4-94.6)	< 0.001
HbA1c (%)	5.4 (5.4-5.4)	5.6 (5.6-5.6)	< 0.001	5.4 (5.4-5.4)	5.5 (5.5-5.5)	< 0.001
Total cholesterol (mg/dl)	179.8 (179.6-180.0)	192.9 (192.1-193.7)	< 0.001	188.0 (187.7-188.3)	201.6 (201.1-202.0)	< 0.001
LDL-C(mg/dl)	103.9 (103.7-104.1)	123.1 (122.4-123.8)	< 0.001	120.2 (120.0-120.5)	135.1 (134.7-135.5)	< 0.001
HDL-C (mg/dl)	68.1 (68.0-68.2)	55.3 (54.9-55.6)	< 0.001	57.0 (56.9-57.2)	48.4 (48.2-48.5)	< 0.001
Triglycerides (mg/dl)	71.6 (71.4-71.9)	117.0 (116.0-118.0)	< 0.001	97.8 (97.2-98.5)	148.0 (147.1-148.9)	< 0.001
GGT (U/L)	14.0 (14.0-14.1)	22.7 (22.4-23.0)	< 0.001	26.6 (26.3-26.9)	42.8 (42.3-43.2)	< 0.001
ALT (U/L)	13.6 (13.5-13.7)	23.4 (23.1-23.7)	< 0.001	22.2 (22.0-22.3)	40.4 (40.1-40.6)	< 0.001
AST (U/L)	17.5 (17.5-17.6)	20.8 (20.5-21.0)	< 0.001	21.6 (21.4-21.7)	27.2 (27.0-27.4)	< 0.001
hs-CRP (mg/L)	0.76 (0.74-0.78)	1.77 (1.70-1.84)	< 0.001	0.96 (0.93-0.99)	1.36 (1.32-1.40)	< 0.001
HOMA-IR	1.19 (1.19-1.20)	2.43 (2.41-2.46)	< 0.001	1.18 (1.17-1.19)	1.94 (1.93-1.95)	< 0.001

[a]Adjusted for age; [b]≥10 g/day; [c]health-enhancing physical activity; [d]≥college graduate; [e]BMI ≥25 kg/m^2

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; GGT, gamma-glutamyl transpeptidase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HEPA, health-enhancing physical activity; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure

Table 2. Absolute and relative estimates of stage 1 hypertension incidence for population strata defined by sex and NAFLD status among young adults under the age of 40 years (n = 153,342)

	Person-years	Incident cases	Incidence density (/ 10^3 PY)	Age adjusted HR (95% CI)	Multivariable-adjusted HR[a] (95% CI)	HR (95% CI)[b] in a model with time-dependent variables
Women (n = 85,789)						
No NAFLD	408,783	5,701	13.9	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD	23,981	1,035	43.2	2.99 (2.79-3.19)	1.67 (1.56-1.80)	1.70 (1.59-1.81)
Men (n = 67,553)						
No NAFLD	208,605	10,727	51.4	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD	93,083	8,428	90.5	1.69 (1.64-1.74)	1.24 (1.20-1.28)	1.21 (1.17-1.25)

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).

[a] Estimated from Cox proportional hazards models; multivariable Model 1 was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.

[b] Estimated from Cox proportional hazard models with NAFLD status, smoking status, alcohol consumption, physical activity, BMI, and lipid-lowering medication, as time-dependent categorical variables, and baseline age, center, year of screening examination, and education level as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; PY, person-years

Table 3. Absolute and relative estimates of stage 1 hypertension incidence for population strata defined by sex and NAFLD severity status based on ultrasound among young adults under the age of 40 years (n = 153,342)

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Age adjusted HR (95% CI)	Multivariable-adjusted HR[a] (95% CI)	HR (95% CI)[b] in a model with time-dependent variables
Women (n = 85,789)						
No NAFLD	408,783	5,701	13.9	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD, mild	21,357	859	40.2	2.76 (2.57-2.97)	1.67 (1.54-1.80)	1.68 (1.56-1.80)
NAFLD, moderate/severe	2,624	176	67.1	4.91 (4.23-5.71)	1.78 (1.52-2.08)	1.83 (1.60-2.09)
p for trend				< 0.001	< 0.001	< 0.001
Men (n = 67,553)						
No NAFLD	208,605	10,727	51.4	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD, mild	76,071	6,495	85.4	1.58 (1.54-1.63)	1.23 (1.19-1.27)	1.21 (1.17-1.25)
NAFLD, moderate/severe	17,012	1,933	113.6	2.16 (2.06-2.27)	1.28 (1.22-1.35)	1.23 (1.17-1.30)
p for trend				< 0.001	< 0.001	< 0.001

The *p-value* for the interaction of sex and NAFLD categories for the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.
[b] Estimated from Cox proportional hazard models with NAFLD categories, smoking, alcohol consumption, physical activity, BMI, medication for lipid-lowering as time-dependent categorical variables and baseline age, center, year of screening examination, and education level as time-fixed variables.
Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; PY, person-years

Table 4. Absolute and relative estimates of stage 1 hypertension incidence for population strata defined by sex and adiposity status by NAFLD among young adults under the age of 40 years

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)
Strata by overweight	Non-overweight (n = 94,910)				Overweight[b] (n = 58,432)			
Women								
No NAFLD	348,462	4,061	11.7	1.00 (reference)	60,321	1,640	27.2	1.00 (reference)
NAFLD	6,949	170	24.5	1.95 (1.67-2.28)	17,032	865	50.8	1.83 (1.68-1.98)
Men								
No NAFLD	109,440	4,557	41.6	1.00 (reference)	99,165	6,170	62.2	1.00 (reference)
NAFLD	14,281	835	58.5	1.35 (1.25-1.45)	78,802	7,593	96.4	1.50 (1.45-1.55)
p for interaction by sex				<0.001				<0.001
Strata by abdominal obesity	No abdominal obesity (n = 131,314)				Abdominal obesity[c] (n = 22,028)			
Women								
No NAFLD	385,740	4,961	12.9	1.00 (reference)	23,042	740	32.1	1.00 (reference)
NAFLD	12,758	431	33.8	2.44 (2.21-2.69)	11,222	604	53.8	1.65 (1.48-1.84)
Men								
No NAFLD	188,134	9,105	48.4	1.00 (reference)	20,471	1,622	79.2	1.00 (reference)
NAFLD	54,273	4,111	75.7	1.49 (1.44-1.55)	38,811	4,317	111.2	1.38 (1.30-1.46)
p for interaction by sex				<0.001				<0.001

[a] Estimated from Cox proportional hazards models; multivariable Model 1 was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, and lipid-lowering medication.
[b] Overweight was defined as body mass index (BMI) of ≥23 kg/m².
[c] Abdominal obesity was defined as waist circumference ≥90 cm for men and ≥85 cm for women.
Abbreviations: CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; PY, person-years

FIGURE LEGENDS

Figure 1. Selection of study participants

Participants under age 40 years who underwent a comprehensive health examination between January 2011 and December 2019 and had at least one follow-up visit before 31 December 2020 (n=235,193)

Exclusions (n = 81,851): some individuals met more than one criterion for exclusion
- Excessive alcohol consumption (men ≥30g/day; women ≥20g/day) (n = 31,625)
- Liver steatogenic medication (n = 971)
- History of hepatitis and medication for hepatitis (n = 5,762)
- Serologic positivity for hepatitis B or hepatitis C virus (n = 6,376)
- Liver cirrhosis based on ultrasound (n = 25)
- History of cancer (n = 3,012)
- History of hysterectomy, bilateral oophorectomy, radiation or chemotherapy-related menopause (n = 2,307)
- History of cardiovascular disease (n = 933)
- Hypertension at baseline (n = 36,086)
- Diabetes at baseline (n = 2,606)
- Missing data on hypertension, alcohol consumption, or metabolic parameters (n = 13,225)

153,342 participants included in the analysis
   (85,789 women and 67,553 men)

Study design

Results

- ✓ **Design:** A retrospective cohort study

- ✓ **Study participants:** 245,054 adults aged <40 years without hypertension at baseline

- ✓ **Exposure and outcome measurement:** i) Hepatic steatosis was assessed by liver ultrasound; ii) Hypertension was defined based on two thresholds of blood pressure (BP) ≥130/80 mmHg or ≥140/90 mmHg; self-reported history of physician-diagnosed hypertension; or current use of BP-lowering medications.

Women (n=85,789)

No NAFLD NAFLD mild NAFLD moderate/severe

Median follow-up: 4.5 years

Adjusted HR

1.00 1.67 1.78

Risk of incident hypertension

Men (n=67,553)

No NAFLD NAFLD mild NAFLD moderate/severe

Median follow-up: 4.5 years

Adjusted HR

1.00 1.23 1.28

Risk of incident hypertension
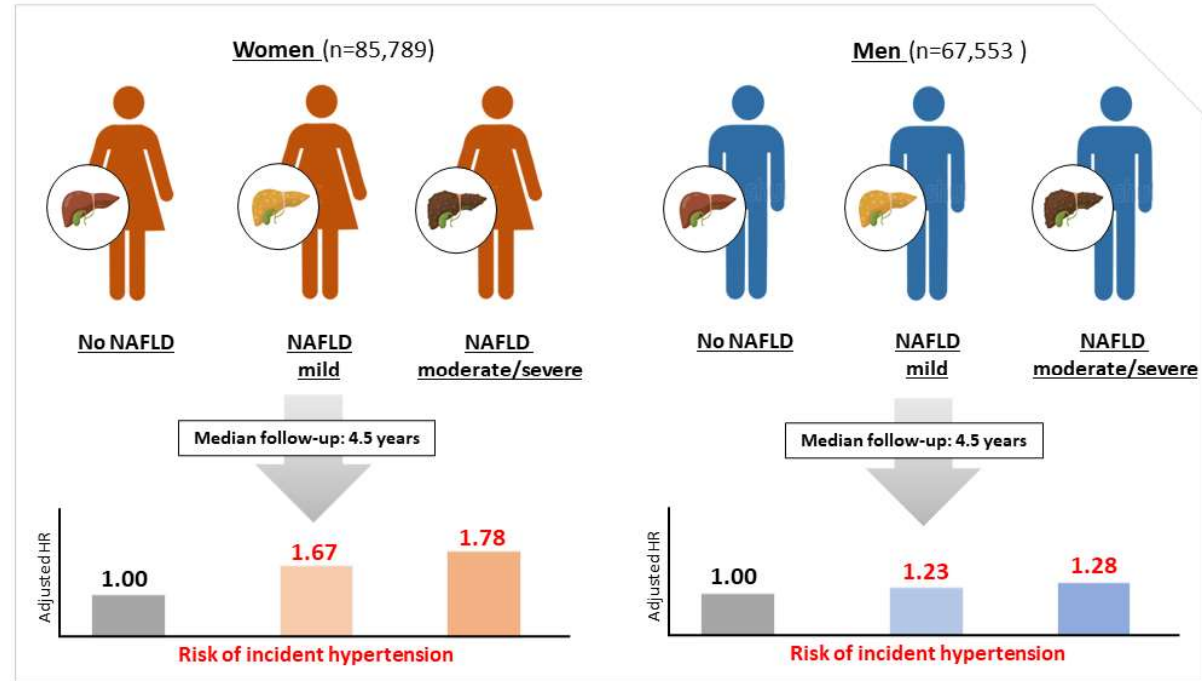
Conclusion: NAFLD is a potential risk factor for young-onset hypertension with a relatively greater impact in women and in those with more severe hepatic steatosis.

HIGHLIGHTS

- Non-alcoholic fatty liver disease (NAFLD) increases risks of young-onset hypertension.

- The association between NAFLD and young-onset hypertension was independent of obesity.

- The presence of NAFLD attenuates protection against hypertension in young women.

- Sex-specific multisystem consequences of NAFLD in younger people deserves more attention.

April 20, 2023

Maurizio Averna, MD
Editor-in-Chief
Nutrition, Metabolism & Cardiovascular Diseases

Dear Dr. Averna,

Thank you for your constructive suggestions regarding our manuscript #NMCD-D-23-00312 entitled, "**Nonalcoholic fatty liver disease and risk of incident young-onset hypertension: effect modification by sex**" We have revised the manuscript according to the reviewers' recommendations and comments. Furthermore, we have conducted additional analyses that considerably strengthened the validity of our study. These additional analyses do not alter our original conclusions. We are pleased to submit the revised version of the manuscript for publication in *Nutrition, Metabolism & Cardiovascular Diseases*.

In addition to the revised version of the manuscript, we have uploaded a version with tracked changes, reflecting the modifications to the manuscript, as well as our point-by-point responses to the reviewers' comments, which detail the changes made in response to the comments.

The authors declare no conflicts of interest related to this manuscript, including financial conflicts. The manuscript has not been submitted for publication elsewhere and is not under consideration by any other journal.

Thank you for your consideration of our manuscript. Please feel free to contact me if you have any questions related to our manuscript. We look forward to hearing from you.

Sincerely,

Seungho Ryu, MD, PhD
Professor of Occupational and Environmental Medicine
Kangbuk Samsung Hospital
Sungkyunkwan University

SUPPLEMENTARY MATERIALS

S1. Exclusion criteria

A total of 81,851 subjects were excluded on the basis of the following criteria (**Figure 1**): excessive alcohol consumption (men ≥30g/day; women ≥20g/day) (n = 31,625); liver steatogenic medication (n = 971); history of hepatitis and medication for hepatitis treatment (n = 5,762); serologic positivity for hepatitis B or hepatitis C virus (n = 6,376); liver cirrhosis based on ultrasound (n = 25); history of CVD (n = 933); history of cancer (n = 3,012); diabetes at baseline (n = 2,606), as some glucose-lowering medications have BP-lowering effects [1]; history of hysterectomy, bilateral oophorectomy, radiation or chemotherapy-related menopause (n = 2,307); hypertension at baseline (n = 36,086); and missing information on hypertension, alcohol consumption, or metabolic parameters (n = 13,225). Some participants met more than one exclusion criterion, and the final sample yielded 153,342 participants without hypertension at baseline, comprising 85,789 women and 67,553 men.

S2. Data collection and measurement

Current average alcohol consumption per day was assessed using the frequency of alcohol consumption per week and the amount of alcohol consumed per drinking day. Physical activity levels were assessed using the validated Korean version of the International Physical Activity Questionnaire short form [2]. Physical activity levels were categorized into three groups: inactive, minimally active, and health-enhancing physical activity (HEPA) [2]. HEPA was defined as follows: (1) vigorous activity ≥3 days/week, with ≥1,500 accumulated metabolic equivalent (MET)-min/week, or (2) a combination of walking and moderate- or vigorous-intensity activities over 7 days for a total of ≥3,000 MET-min/week.

The homeostatic model assessment of insulin resistance (HOMA-IR) index was determined using the following equation: fasting blood insulin (mU/mL) × fasting blood glucose (mmol/L)/22.5; the cutoff value of 2.5 was used to define insulin resistance [3]. Glycated hemoglobin (HbA1c) levels were measured using a Cobas Integra 800 (Roche Diagnostics, Rotkreuz, Switzerland) with a

turbidimetric inhibition immunoassay for hemolyzed whole blood. The intra- and inter-assay coefficients of variation were 2.3% and 2.4 %, respectively.

Being metabolically healthy was defined as having none of the following metabolic abnormalities [4]: 1) fasting glucose level ≥100 mg/dL or current use of glucose-lowering agents, 2) Blood pressure (BP) ≥130/80 mmHg or current use of BP-lowering agents, 3) elevated triglyceride level (≥150 mg/dL) or current use of lipid-lowering agents, 4) low high-density lipoprotein-cholesterol (HDL-C) (<40 mg/dL in men or <50 mg/dL in women), 5) abdominal obesity (waist circumference ≥90 cm for men and ≥85 cm for women), or 6) insulin resistance, defined as a HOMA-IR score ≥2.5.

Two noninvasive fibrosis indices were used to further assess NAFLD severity: the Fibrosis-4 Index for Liver Fibrosis (FIB-4) and NAFLD fibrosis score (NFS). The FIB-4 index was calculated using the following formula: FIB-4 = (age [years] × aspartate transaminase [AST; U/L]) / (platelet count [× 10^9/L] × alanine aminotransferase [ALT; U/L]$^{1/2}$). The cutoff values of the FIB-4 index were used to define low (FIB-4 <1·30), intermediate (FIB-4 1·30-2·67), and high (FIB-4 ≥2·67) probabilities of advanced fibrosis [5]. The NFS was calculated on the basis of the following published formula: NFS = -1·675 + 0·037 × age (years) + 0·094 × body mass index (BMI; kg/m^2) + 1·13 × impaired fasting glycemia or diabetes (yes = 1, no = 0) + 0·99 × AST/ALT ratio – 0·013 × platelet (×10^9/L) – 0·66 × albumin (g/dL).[6] The NFS scores were categorized into three groups: high (NFS >0·676), intermediate (NFS 0·676 to -1·455), and low (NFS <-1·455) [6].

S3. Supplementary tables

eTable 1. Absolute and relative estimates of stage 2 hypertension incidence for population strata defined by sex and NAFLD status among young adults under the age of 40 years (n = 153,342)

eTable 2. Development of stage 1 hypertension for population strata defined by sex and NAFLD status among young adults under the age of 40 years after further adjustment for HOMA-IR

eTable 3. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on ultrasound among young adults under the age of 40 years after further adjustment for HOMA-IR

eTable 4. Absolute and relative estimates of stage 2 hypertension incidence for population strata defined by sex and NAFLD severity status based on ultrasound among young adults under the age of 40 years (n = 153,342)

eTable 5. Development of stage 1 hypertension by NAFLD status or NAFLD severity based on ultrasound among metabolically healthy young adults under the age of 40 years (n = 91,628)

eTable 6. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on NFS among young adults under the age of 40 years

eTable 7 Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on FIB-4 among young adults under the age of 40 years

eTable 1. Absolute and relative estimates of stage 2 hypertension incidence for population strata defined by sex and NAFLD status among young adults under the age of 40 years (n = 153,342)

	Person-years (PY)	Incident cases	Incidence density (/ 10³ PY)	Age adjusted HR (95% CI)	Multivariable-adjusted HRᵃ (95% CI)	HR (95% CI)ᵇ in a model with time-dependent variables
Women (n = 85,789)						
No NAFLD	423,216	420	1.0	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD	26,737	135	5.0	4.79 (3.94-5.81)	2.17 (1.75-2.68)	2.06 (1.69-2.51)
Men (n = 67,553)						
No NAFLD	241,449	666	2.8	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD	121,177	765	6.3	2.09 (1.89-2.32)	1.34 (1.19-1.50)	1.47 (1.30-1.65)

The *P*-value for the interaction of sex, menopausal status, and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
ᵃ Estimated from Cox proportional hazards models; multivariable Model 1 was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, hyperlipidemia medication, and BMI
ᵇ Estimated from Cox proportional hazard models with NAFLD status, smoking status, alcohol consumption, physical activity, BMI, and hyperlipidemia medication, as time-dependent categorical variables, and baseline age, center, year of screening examination, and education level as time-fixed variables
Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 2. Development of stage 1 hypertension for population strata defined by sex and NAFLD status among young adults under the age of 40 years after further adjustment for HOMA-IR

	Multivariable-adjusted HR[a] (95% CI)
Women	
No NAFLD	1.00 (reference)
NAFLD	1.64 (1.53-1.76)
Men	
No NAFLD	1.00 (reference)
NAFLD	1.23 (1.19-1.27)

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).

[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, BMI and HOMA-IR.

Abbreviation: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model for insulin resistance; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 3. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on ultrasound among young adults under the age of 40 years after further adjustment for HOMA-IR

	Multivariable-adjusted HR[a] (95% CI)
Women	
No NAFLD	1.00 (reference)
NAFLD, mild	1.66 (1.55-1.78)
NAFLD, moderate/severe	0.96 (0.64-1.44)
p for trend	<0.001
Men	
No NAFLD	1.00 (reference)
NAFLD, mild	1.23 (1.19-1.27)
NAFLD, moderate/severe	1.17 (1.02-1.33)
p for trend	<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, BMI and HOMA-IR.
Abbreviation: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model for insulin resistance; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 4. Absolute and relative estimates of stage 2 hypertension incidence for population strata defined by sex and NAFLD severity status based on ultrasound among young adults under the age of 40 years (n = 153,342)

	Person-years (PY)	Incident cases	Incidence density (/ 10³ PY)	Age adjusted HR (95% CI)	Multivariable-adjusted HRᵃ (95% CI)	HR (95% CI)ᵇ in a model with time-dependent variables
Women (n = 85,789)						
No NAFLD	423,216	420	1.0	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD, mild	23,696	107	4.5	4.21 (3.41-5.21)	2.12 (1.69-2.66)	2.01 (1.63-2.48)
NAFLD, moderate/severe	3,041	28	9.2	9.90 (6.75-14.51)	2.62 (1.73-3.97)	2.45 (1.73-3.45)
P for trend				< 0.001	< 0.001	< 0.001
Men (n = 67,553)						
No NAFLD	241,449	666	2.8	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD, mild	97,828	568	5.8	1.90 (1.70-2.13)	1.32 (1.17-1.49)	1.45 (1.28-1.64)
NAFLD, moderate/severe	23,349	197	8.4	2.93 (2.50-3.44)	1.45 (1.21-1.72)	1.55 (1.31-1.82)
P for trend				< 0.001	< 0.001	< 0.001

The *P* value for the interaction of sex and NAFLD categories for the risk of hypertension was <0·001 (Multivariable-adjusted model).
ᵃ Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.
ᵇ Estimated from Cox proportional hazard models with NAFLD categories, smoking, alcohol consumption, physical activity, BMI, medication for lipid-lowering as time-dependent categorical variables and baseline age, center, year of screening examination, and education level as time-fixed variables.
Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 5. Development of stage 1 hypertension by NAFLD status or NAFLD severity based on ultrasound among metabolically healthy young adults under the age of 40 years (n = 91,628)

	Multivariable-adjusted HR[a] (95% CI)		
	Among metabolically healthy young women (n = 64,412)	Among metabolically healthy young men (n = 27,216)	P for interaction
By NAFLD			<0.001
No NAFLD	1.00 (reference)	1.00 (reference)	
NAFLD	1.64 (1.40-1.92)	1.22 (1.14-1.31)	
By NAFLD severity			<0.001
No NAFLD	1.00 (reference)	1.00 (reference)	
NAFLD, mild	1.62 (1.38-1.90)	1.22 (1.14-1.32)	
NAFLD, moderate/severe	2.26 (1.01-5.04)	1.21 (0.96-1.53)	
P for trend	< 0.001	< 0.001	

[a] Estimated from Cox proportional hazards models; multivariable Model 1 was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, hyperlipidemia medication, and BMI
Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 6. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on NFS among young adults under the age of 40 years

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)
Women				
No NAFLD	408,384.8	5,691	13.9	1.00 (reference)
NAFLD, low NFS	23,403.5	1,010	43.2	1.63 (1.51-1.76)
NAFLD, intermediate / high NFS	561.8	24	42.7	1.73 (1.48-2.03)
p for trend				<0.001
Men				
No NAFLD	208,576.0	10,727	51.4	1.00 (reference)
NAFLD, low NFS	91,121.4	8,194	89.9	1.46 (1.38-1.54)
NAFLD, intermediate / high NFS	1,944.8	233	119.8	1.16 (1.13-1.18)
p for trend				<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.
Abbreviation: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score

eTable 7 Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on FIB-4 score among young adults under the age of 40 years

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)
Women				
No NAFLD	408,384.8	5,691	13.9	1.00 (reference)
NAFLD, low FIB-4	23,815.9	1,027	43.1	1.64 (1.53-1.76)
NAFLD, intermediate / high FIB-4	149.4	7	46.9	1.59 (0.76-3.33)
p for trend				<0.001
Men				
No NAFLD	208,576.0	10,727	51.4	1.00 (reference)
NAFLD, low FIB-4	92,190.1	8,345	80.5	1.23 (1.19-1.27)
NAFLD, intermediate / high FIB-4	879.3	83	94.4	1.19 (0.96-1.47)
p for trend				<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.
Abbreviation: BMI, body mass index; CI, confidence interval; FIB-4, fibrosis-4 index; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

REFERENCES

[1] Kario K, Ferdinand KC, Vongpatanasin W. Are SGLT2 Inhibitors New Hypertension Drugs? Circulation. 2021;143:1750-3.doi:10.1161/CIRCULATIONAHA.121.053709.

[2] Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381-95.10.1249/01.MSS.0000078924.61453.FB.

[3] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9.10.1007/BF00280883.

[4] Chang Y, Kim BK, Yun KE, Cho J, Zhang Y, Rampal S, et al. Metabolically-healthy obesity and coronary artery calcification. J Am Coll Cardiol. 2014;63:2679-86.10.1016/j.jacc.2014.03.042.

[5] Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7:1104-12.10.1016/j.cgh.2009.05.033.

[6] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846-54.10.1002/hep.21496.

Reviewer #1

In the present manuscript the authors report the results of a cohort study investigating the association between NAFLD and incident hypertension in young men and women from the general Korean population. The topic is certainly of interest, as few studies focused specifically on this age group.

Response: We are grateful to the Reviewer for their constructive suggestions for our work. We have addressed the specific comments made by the Reviewer and revised the manuscript accordingly.

I have the following comments:
1)   Apart from identifying liver steatosis and excluding excessive alcohol intake, diagnosis of NAFLD also requires exclusion of other forms of liver disease or use of steatogenic medications. There is no mention of viral hepatitis screening in the present study, nor of the use of steatogenic medication. Please report whether data are available and, if so, please exclude these patients from the analysis. Otherwise, this should be listed as a major limitation of the current study.

<u>Response:</u> Thank you for your comment. In our study, NAFLD was defined as the presence of fatty liver in the absence of excessive alcohol use (<20 g/day and <30 g/day for women and men, respectively) or any other identifiable cause [1]. Therefore, individuals with potential secondary cause of hepatic steatosis were excluded from the study, including a history of hepatitis or medication for hepatitis treatment (n = 5,762), serologic positivity for viral hepatitis (HBV and HCV) (n = 6,376) as well as those using steatogenic medication (n = 971). We also excluded participants with liver cirrhosis based on ultrasound (n = 25), or history of any cancers (n = 3,012). This information was not described in detail in the original manuscript due to the word limit but was presented in **Figure 1**. We have now added these details also to the 'Exclusion criteria' section in the **Supplementary Materials**.

2)   Given that you have data on insulin resistance, which can be reliably estimated using HOMA-IR, it would be interesting to evaluate whether inclusion of HOMA-IR in the multivariable models modifies the association in a significant way, to understand whether NAFLD has a role independently from insulin resistance in the development of hypertension.

<u>Response:</u> Following the Reviewer's suggestion, we performed analyses that included HOMA-IR as a covariate. According to the results (**eTable 2** and **eTable 3**), the associations between the presence of NAFLD and risk of incident hypertension were comparable to the original analyses.

For women with moderate/severe NAFLD (n=176 or 17% of all women with NAFLD), the association was no longer significant. The loss of significance may be due to low statistical power in this stratum of women with the very small number of cases with moderate/severe NAFLD. Alternatively, a severe degree of liver steatosis likely manifests a state of systemic metabolic dysregulation [2], and impaired insulin resistance may in fact be one of the key mediators in the pathway leading to hypertension [3] and possibly to the loss of CV protection among women with severe NAFLD. That said, we still observed remarkably similar point estimates of the HRs for the associations with mild NAFLD, before and after the adjustment of HOMA-IR. Thus, these data suggest that NAFLD does contribute to the development of hypertension, independent of insulin resistance. The mechanistic role of insulin resistance in the relationship between NAFLD and risk of hypertension should be further examined.

eTable 2. Development of stage 1 hypertension for population strata defined by sex and NAFLD status among young adults under the age of 40 after further adjustment for HOMA-IR

	Multivariable-adjusted HR[a] (95% CI)
Women	
No NAFLD	1.00 (reference)
NAFLD	1.64 (1.53-1.76)
Men	
No NAFLD	1.00 (reference)
NAFLD	1.23 (1.19-1.27)

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, BMI and HOMA-IR.
Abbreviation: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model for insulin resistance; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

eTable 3. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity based on ultrasound among young adults under the age of 40 after further adjustment for HOMA-IR

	Multivariable-adjusted HR[a] (95% CI)
Women	
No NAFLD	1.00 (reference)
NAFLD, mild	1.66 (1.55-1.78)
NAFLD, moderate/severe	0.96 (0.64-1.44)
p for trend	<0.001
Men	
No NAFLD	1.00 (reference)
NAFLD, mild	1.23 (1.19-1.27)
NAFLD, moderate/severe	1.17 (1.02-1.33)
p for trend	<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, BMI and HOMA-IR.

3)   Another interesting point would be to have a clue on whether NAFLD severity is associated with higher risk. Apart from the degree of steatosis (which is not a good predictor of outcomes), I suggest looking at whether presence of probable at-risk NASH (e.g. using the Fibrotic NASH Index) or advanced liver fibrosis (e.g. using Fib-4 or NFS).

<u>Response:</u> We performed additional analyses evaluating the risks of hypertension by NAFLD severity based on NFS and FIB-4. Although significance was not detected for the group with intermediate/high FIB-4 possibly due to a low case number; overall, we obtained similar results, with stronger excess risks among women compared with men. The findings have been presented in **eTable 6** and **eTable 7**.

eTable 6. Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on NFS among young adults under the age of 40 years

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)
Women				
No NAFLD	408,384.8	5,691	13.9	1.00 (reference)
NAFLD, low NFS	23,403.5	1,010	43.2	1.63 (1.51-1.76)
NAFLD, intermediate / high NFS	561.8	24	42.7	1.73 (1.48-2.03)
p for trend				<0.001
Men				
No NAFLD	208,576.0	10,727	51.4	1.00 (reference)
NAFLD, low NFS	91,121.4	8,194	89.9	1.46 (1.38-1.54)
NAFLD, intermediate / high NFS	1,944.8	233	119.8	1.16 (1.13-1.18)
p for trend				<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).
[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI.
Abbreviation: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score

eTable 7 Development of stage 1 hypertension for population strata defined by sex and NAFLD severity status based on FIB-4 among young adults under the age of 40 years

	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Multivariable-adjusted HR[a] (95% CI)
Women				
No NAFLD	408,384.8	5,691	13.9	1.00 (reference)
NAFLD, low FIB-4	23,815.9	1,027	43.1	1.64 (1.53-1.76)
NAFLD, intermediate / high FIB-4	149.4	7	46.9	1.59 (0.76-3.33)
p for trend				<0.001
Men				
No NAFLD	208,576.0	10,727	51.4	1.00 (reference)
NAFLD, low FIB-4	92,190.1	8,345	80.5	1.23 (1.19-1.27)
NAFLD, intermediate / high FIB-4	879.3	83	94.4	1.19 (0.96-1.47)
p for trend				<0.001

The *P*-value for the interaction of sex and NAFLD status with the risk of hypertension was <0·001 (Multivariable-adjusted model).

[a] Estimated from Cox proportional hazards models; multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, lipid-lowering medication, and BMI. Abbreviation: BMI, body mass index; CI, confidence interval; FIB-4, fibrosis-4 index; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease

4)   As a summary of available evidence on the topic, I would mention the results of a recent meta-analysis evaluating the association between NAFLD and incident hypertension (doi: 10.1097/MEG.0000000000002299).

<u>Response:</u> Thank you for your suggestion. As recommended, we have cited the meta-analysis in the **Discussion** section.

"Previous epidemiological studies have shown that NAFLD and its severity are associated with prevalent and incident hypertension in general populations [4-6]. A recent meta-analysis of 11 cohort studies suggests that NAFLD is associated with approximately a ~1.6-fold increased risk of incident hypertension [7]. "

Reviewer #2

GENERAL COMMENT

Previously deemed to be "the hepatic manifestation of the metabolic syndrome (MetS)", NAFLD is rather mutually and bi-directionally associated with the MetS and its individual components. However, compared to arterial hypertension, obesity, diabetes and dyslipidemia have all more strongly been associated with NAFLD. With this backset, although the extent to which this association is mediated by obesity or diabetes remains incompletely defined, an ever-increasing body of evidence has also supported a strong association of NAFLD with arterial hypertension (DOI: 10.1111/jgh.12643; DOI: 10.1016/j.jhep.2014.01.009; PMID: 31865799).
Additionally, a strong line of research has disclosed the sexually dimorphic nature not only of cardiometabolic risk factors, but also of NAFLD/NASH (DOI: 10.1016/S0140-6736(20)31561-0; DOI: 10.1038/s41574-020-00431-8; doi: 10.1016/j.cgh.2020.04.067; doi: 10.3390/jcm9051278).
In the setting of the above background information, submission NMCD-D-00312 followed for a median 4.5-year time frame a large adult Korean population (85,789 women and 67,553 men aged <40 years) who were normo-tensive at the baseline and who were submitted to comprehensive annual or biennial examinations at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea. Data have shown that the presence of mild or moderate/severe NAFLD at the baseline was a significant risk factor for incident hypertension, particularly among women regardless of obesity/central obesity.

Response: We are grateful to the Reviewer for constructive suggestions for our work. We have addressed the specific comments made by the Reviewer and revised the manuscript accordingly.

SPECIFIC COMMENTS

MAJOR

I enjoyed reading this innovative study, which has a strong scientific rationale and fully plausible findings. Among its definite points of strength I highlight, a) the very large study population; b) sex specific analysis of data; c) the use of semi-quantitative ultrasonographic indices to assess steatosis; and d) choice of fully appropriate bibliographic references.

Regarding point d) I would like to highlight that, at variance with other researchers, these authors have omitted citing the old study by Saadeh (doi: 10.1053/gast.2002.35354) which I believe to be both outdated and misleading as to the true diagnostic accuracy of modern ultrasonographic equipment.

Probably, authors may be willing to discuss that one of the advantages of their study design is that young individuals allow better comparison among sexes by eliminating the confounding factor of menopause which might affect findings, presumably through its pro-fibrogenic action on the liver (doi: 10.1002/hep.26761; doi: 10.1002/hep4.1668).

Response: Thank you for the suggestion. We have cited the references in the **Discussion** section as follows:

"Lastly, the inclusion of relatively healthy, younger individuals i) reduced the potential for survivor bias caused by selecting subjects with severe diseases as well as comorbidity-related bias, which is a common limitation of previous studies involving patients with biopsy-proven advanced stage NAFLD

and ii) eliminated the potential confounding effects of menopause which is known to affect both NAFLD pathophysiology [8, 9] as well as BP [10], allowing us to better determine sex-specific differences in the associations."

Adding a graphical abstract could facilitate readers and Scholars utilizing this submission for dissemination (in Academic teaching, publications, and medical symposia) as well as for planning future studies.

Response: We have added a graphical abstract along with the revised manuscript.

MINOR

1. Abstract - Please, make sure that all initialisms (for example NAFLD) are explained when used for the first time.

Response: We have ensured that all abbreviations are fully written in the first use.

2. Throughout the manuscript - rephrase "non-alcoholic" to "nonalcoholic". Although both spellings are commonly encountered, only the latter is consistent with pioneer disease definitions by Ludwig, Schaffner & Thaler (DOI: 10.3390/ijms21165888).

Response: As suggested by the Reviewer, we have made changes accordingly.

Editorial comments: The text requires English editing.

Response: Thank you, we have done as you suggest.

References

[1] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23.10.1002/hep.25762.

[2] Gehrke N, Schattenberg JM. Metabolic Inflammation—A Role for Hepatic Inflammatory Pathways as Drivers of Comorbidities in Nonalcoholic Fatty Liver Disease? Gastroenterology. 2020;158:1929-47.e6.10.1053/j.gastro.2020.02.020.

[3] Nakagami H. Mechanisms underlying the bidirectional association between nonalcoholic fatty liver disease and hypertension. Hypertension Research. 2023;46:539-41.10.1038/s41440-022-01117-6.

[4] Sung KC, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. J Hepatol. 2014;60:1040-5.10.1016/j.jhep.2014.01.009.

[5] Zhu XP, Han GC, Chen Q, Zhang ZY, Wang LS, Zhang B. Fatty liver is a sensitive early warning for hypertension and its complication in the Chinese population. Clin Exp Hypertens. 2022:1-7.10.1080/10641963.2022.2029469.

[6] Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? J Hepatol. 2018;68:335-52.10.1016/j.jhep.2017.09.021.

[7] Ciardullo S, Grassi G, Mancia G, Perseghin G. Nonalcoholic fatty liver disease and risk of incident hypertension: a systematic review and meta-analysis. European Journal of Gastroenterology & Hepatology. 2022;34:365-71.10.1097/meg.0000000000002299.

[8] Wegermann K, Garrett ME, Zheng J, Coviello A, Moylan CA, Abdelmalek MF, et al. Sex and Menopause Modify the Effect of Single Nucleotide Polymorphism Genotypes on Fibrosis in NAFLD. Hepatology Communications. 2021;5:598-607.https://doi.org/10.1002/hep4.1668.

[9] Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology. 2014;59:1406-14.10.1002/hep.26761.

[10] Tikhonoff V, Casiglia E, Gasparotti F, Spinella P. The uncertain effect of menopause on blood pressure. J Hum Hypertens. 2019;33:421-8.10.1038/s41371-019-0194-y.