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NAFLD and Cardiometabolic Risk Factors: The Liver Fibrosis Trajectory Through the Lens of Biological Interactions

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NAFLD often co-occurs with one or more cardiometabolic risk factors[1] that are shared with NAFLD-associated fibrosis, suggesting that multiple etiologic pathways are involved in the disease biology.[2] As hepatic steatosis and cardiometabolic traits shape one another,[1] this interrelationship drives multiple burdens of predisposing conditions, making it difficult to discriminate the extent and determinants of progression to severe liver disease. As a result, the role of steatosis in predisposing an individual to liver fibrosis in the context of multiple and concomitant risk factors has not been adequately studied.

In this issue, Long et al. reported on a cross-sectional analysis involving 3,276 middle-aged and older adults who formed a subsample of a much larger Framingham Heart Study (FHS) community-based cohort.[3] The investigators showed that fibrosis—as measured by vibration-controlled transient elastography—is positively associated with multiple cardiovascular (CV) risk factors, including obesity, type 2 diabetes (T2D), and hypertension, and is negatively associated with high-density lipoprotein and total cholesterol. Based on these findings, the authors concluded that the association between fibrosis and cardiometabolic traits is independent of hepatic steatosis measured by the controlled attenuation parameter.[3] However, as Long et al. pointed out, lack of longitudinal data was one of the limitations of their study. This point is undoubtedly relevant because, since its launch in 1948, the FHS has become a multigenerational and paradigmatic study as a part of which CV and metabolic disease patterns are analyzed.

The elucidation of liver fibrosis risk factors via population-based epidemiological studies has deepened the knowledge of NAFLD and NASH-fibrosis pathogenesis. Thus, robust epidemiological studies are essential for obtaining quantitative data physicians can use when formulating best-practice recommendations that ultimately control the disease's progression. The research dilemma with NAFLD/NASH cross-sectional data is that they only provide a snapshot of NASH fibrosis. As a result, a "cause and effect" relationship cannot be established, and the natural history of fibrosis cannot be appraised.

Abbreviations: FHS, Framingham Heart Study; HbA1c, glycated hemoglobin; NHANES, National Health and Nutrition Examination Survey; T2D, type 2 diabetes.

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Making Sense of the Complex Association Among Steatosis, Cardiometabolic Risk Factors, and Fibrosis

We can make an abstraction from the "cause and effect" dilemma and consider the road less traveled in cross-sectional epidemiological studies on NAFLD. Specifically, rather than striving to demonstrate "causality," we can analyze biological interactions. We will contribute to this line of research by analyzing the data of 3,359 participants from the National Health and Nutrition Examination Survey (NHANES) study conducted in the United States during the 2017-2018 period. This cohort is presumably comparable with the FHS.

Long et al. observed moderate correlations between cardiometabolic variables and fibrosis in both women and men. In this context, rather than focusing on correlations, we can examine the joint involvement of two factors in causing a phenotype with emphasis on sex. We could, for example, hypothesize that liver fibrosis arises not only from independent effects but also the interaction of cardiometabolic risk factors and steatosis, whereby the effect of this putative biological interaction is sex-dependent.

Figure 1 shows sex differences in the interaction effects of steatosis and cardiometabolic risk factors on the probability of having hepatic fibrosis based on the NHANES 2017-2018 data. It is evident that glycated hemoglobin (HbA1c) (Fig. 1A), waist circumference (Fig. 1B), systolic blood pressure (Fig. 1C), and total cholesterol (Fig. 1D) may interact with steatosis, thereby affecting the odds of having fibrosis. The interaction effects, whether synergistic or antagonistic, seem to differ between women and men. Most importantly, the presumed independence of cardiometabolic risk factors from the probability of having fibrosis is not supported by the analysis of interactions, particularly those among HbA1c levels, total cholesterol, and central obesity (Fig. 1). For example, even in the presence of steatosis, participants unaffected by abdominal obesity have low chances of developing fibrosis, especially if they are men.

Furthermore, linear logistic regression analysis on NHANES 2017-2018 data without terms for the interaction between factors (corresponding to the unconditional analysis performed by Long et al.) suggests a modest protective effect of total cholesterol on the probability of having fibrosis (OR, 0.996; 95% CI, 0.994-0.999; P = 0.001), which is in agreement with the findings reported by Long et al.[3] Nevertheless, the interaction model suggests that this presumably protective effect of total cholesterol on fibrosis disappears in the presence of steatosis (Fig. 1D). On the other hand, we concur with Long et al. that statin treatment is a putative confounder.

Although it is tempting to draw inferences about the independence of variables and/or associations between phenotypes, it appears that statistical tests should be used judiciously, as different statistical models may lead to different biological interpretations.

Limited Ethnic Diversity in Studies Concerning NAFLD and NASH Represents a Gap in the Liver Research Field

Most epidemiological studies on NAFLD and NASH, including genetic association studies,[4] are based on relatively homogenous samples in which certain ethnicities are underrepresented. For example, White populations (91.6%) predominated in the sample examined by Long et al,[3] making it difficult to generalize their findings to other populations. In the NHANES 2017-2018 cycle, ethnic information was self-reported, allowing its inclusion in the analyses, the findings of which suggest that the role of ethnicity on liver fibrosis should be investigated. For instance, Hispanic participants with steatosis present a ~54% higher risk of fibrosis after accounting for all cardiometabolic risk factors (OR, 1.54; 95% CI, 0.977-2.436; P = 0.06). On the other hand, Mexican Americans have the greatest chances of having fibrosis in the presence of steatosis (OR, 1.958; 95% CI, 1.094-3.504; P = 0.02). It appears that non-Hispanic

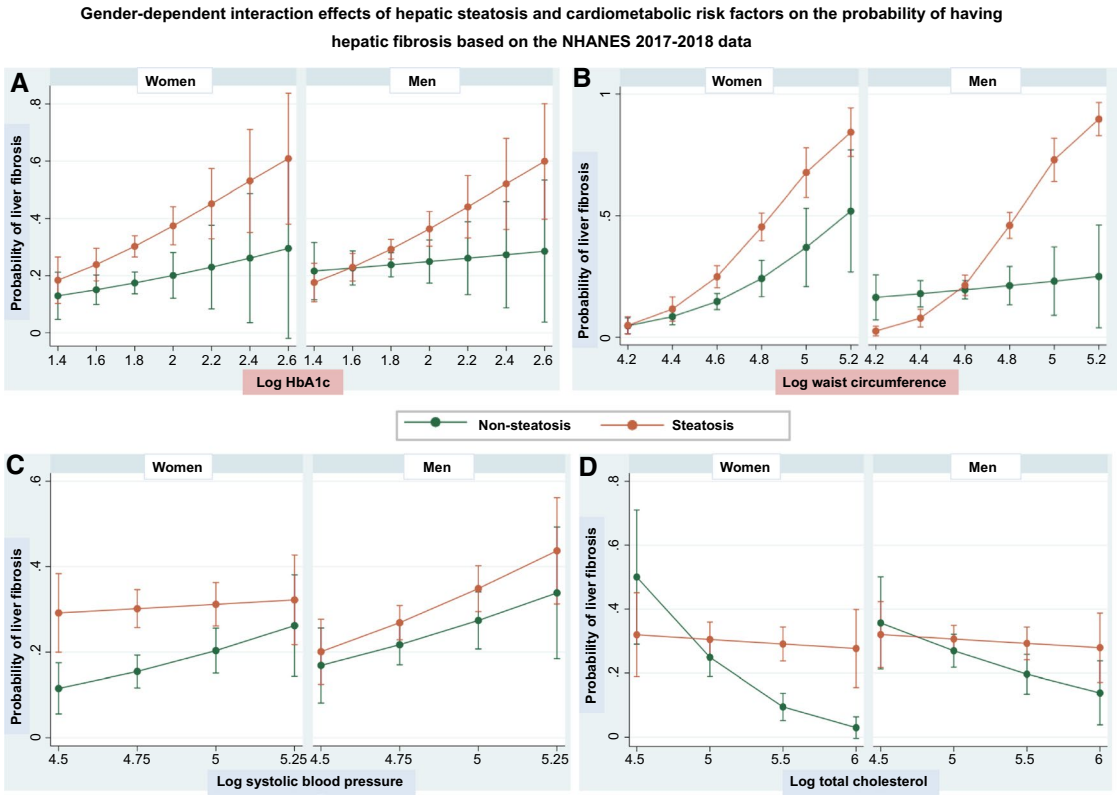

FIG. 1. Steatosis and cardiometabolic traits: A snapshot on the interaction effects on liver fibrosis. Charts show the interaction effects between hepatic steatosis and cardiometabolic risk factors—(A) log HbA1c, (B) Log waist circumference, (C) Log systolic blood pressure, (D) Log total cholesterol—on the probability of having hepatic fibrosis according to sex. To avoid skewed distribution, variables were log-transformed (Log). Interaction analyses were done by linear logistic regression adding all possible interaction terms among the presence of steatosis (no = 0, yes = 1), sex (women = 0, men = 1) and the specific continuous variable. Except when the specific variable was included in the interaction terms, age, waist, diabetes, HbA1c, total cholesterol, systolic blood pressure, and triglycerides were also included as cofactors. Probability of liver fibrosis was estimated by margins as implemented in Stata software. Data analysis was based on NHANES 2017-2018, which is, to the best of our knowledge, the only available survey with liver fibrosis assessment via transient elastography (FibroScan) examination. The National Center for Health Statistics Research Ethics Review Board approved the NHANES protocol, and participants gave informed consent. Data sets and further information are available online (https://www.cdc.gov/nchs/nhanes/index.htm). Liver steatosis and liver fibrosis were defined by the controlled attenuation parameter and liver stiffness measurements, respectively. Only participants who consumed less than 30 and 20 g alcohol for men and women, respectively, were included in the present analysis. Those participants with positive tests of viral hepatitis were excluded.

Black participants are less susceptible (~38% less risk) to developing fibrosis in the presence of steatosis (OR, 0.618; 95% CI, 0.401-0.952; P = 0.03) even after adjustment for all cardiometabolic covariates. When interpreting these results, it should be noted that the stratification by ethnicity has low statistical power for the other groups distinct of non-Hispanic White groups. Nevertheless, extrapolating findings from U.S. White participants to the rest of the population is challenging.

What Is the Next Step in the Effort to Decipher the Mechanisms of Disease From Interactions?

A variety of approaches and assumptions can be formulated to infer mechanisms of disease from the analysis of biological interactions, including

the investigation of gene–environment interactions and network biology approaches.[1] Behavioral risk factors can be incorporated into the analysis. For example, alcohol consumption can be considered to understand whether genetically mediated susceptibility to the effects of drinking influences the risk of fibrosis in the presence of cardiometabolic risk factors. Mendelian randomization studies may also be used to examine the combined effect of genetic determinants of steatosis and cardiometabolic factors as instrumental variables on fibrosis risk. For example, Parisinos et al. performed a Mendelian randomization study using data from the UK Biobank to test the causal effects of several metabolic traits on fibrosis as assessed by imaging studies.[5] The authors found that fibrosis and steatosis share some but not all etiopathogenic mechanisms. A similar approach was used by Liu et al., who found that genetically driven NAFLD causally promotes T2D and central obesity, whereas genetically driven T2D, obesity, and central obesity causally increase the risk of NAFLD.[6] Much less is known about this causal relationship for liver fibrosis.

To conclude, cross-sectional population and/or community-based epidemiological studies suggest that cardiometabolic risk factors increase the chances of having hepatic fibrosis. Although possible, the inverse relationship is more difficult to biologically explain. However, the analysis of interactions between phenotypes suggests that cardiometabolic traits and steatosis exert a moderating influence on each other's effect on fibrosis. Moreover, the observed quantitative differences in the magnitude of the impact between men and women suggest sexual dimorphism.

Assessments of biological interactions provide a robust conceptual framework that facilitates the translation of complex statistical equations and formulae into readable clinical guidelines. Ultimately, understanding the biological effects of cardiometabolic risk factors on fibrosis occurrence or *vice versa* will facilitate development of effective therapeutic approaches. In this regard, it appears that any antifibrotic therapy is unlikely to be effective if glucose and body weight are not controlled.

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