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Clinical Research Article

Association of Hepatic Steatosis and Fibrosis Indices With Insulin Sensitivity and Inflammation in the POP-ABC Study

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Abstract

Context: The cardiometabolic significance of subclinical liver fat in otherwise healthy individuals is unclear.

Objective: This work aimed to evaluate the association of hepatic steatosis/fibrosis with cardiometabolic risk markers and incident prediabetes among healthy adults.

Methods: This is a post hoc analysis of data from the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study. The participants underwent assessments, including clinical examination, oral glucose tolerance test, insulin sensitivity, insulin secretion, plasma high-sensitivity C-reactive protein (hsCRP), and adiponectin levels, with the primary outcome of incident prediabetes during 5-year follow-up. Liver steatosis and fibrosis were assessed using the hepatic steatosis index (HSI) and the Fibrosis-4 (Fib-4) index, and participants were stratified by baseline quartiles (Q) of each index.

Results: Among 343 (193 African American, 150 European American) participants (mean age 44.2 ± 10.6 years, body mass index 30.2 ± 7.28, fasting glucose 91.8 ± 6.80 mg/dL, and 2-hour glucose 125 ± 26.5 mg/dL), the mean baseline HSI was 39.7 ± 8.21 and Fib-4 index was 0.80 ± 0.41. Baseline HSI correlated with insulin sensitivity ($r = -0.44$; $P < .0001$), hsCRP ($r = 0.37$; $P < .0001$), and adiponectin ($r = -0.24$; $P < .0001$), as did Fib-4 index: insulin sensitivity ($r = 0.14$; $P = .046$), hsCRP ($r = -0.17$; $P = .0021$), adiponectin ($r = -0.22$; $P < .0001$). During 5 years of follow-up, prediabetes occurred in 16.2%, 21.6%, 31.5%, and 30.6% among participants in Q1 to Q4 of baseline HSI, respectively (log-rank $P = .02$). The prediabetes hazard ratio was 1.138 (95% CI, 1.027-1.261) for baseline HSI.

Conclusion: Among initially normoglycemic individuals, hepatic steatosis predicted progression to prediabetes, probably via mechanisms that involve insulin resistance and inflammation.

Key Words: impaired fasting glucose, impaired glucose tolerance, fatty liver, insulin resistance, inflammatory markers, race/Ethnicity

Abbreviations:: 2hrPG, 2-hour plasma glucose; AIRg, acute insulin response to intravenous glucose; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DPP, Diabetes Prevention Program; Fib-4, Fibrosis-4; FPG, fasting plasma glucose; GCRC, University of Tennessee General Clinical Research Center; HbA_{1c}, glycated hemoglobin A_{1c}; hsCRP, high-sensitivity C-reactive protein; HSI, hepatic steatosis index; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; POP-ABC, Pathobiology of Prediabetes in a Biracial Cohort; Q, quartile; Si-clamp, insulin sensitivity measured with hyperinsulinemic euglycemic clamp; T2DM, type 2 diabetes mellitus.

Nonalcoholic fatty liver disease (NAFLD), recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD), has an estimated prevalence of 32.4% worldwide [1-3]. The sequelae of MASLD include inflammation leading to steatohepatitis, liver cell damage, and increased risks of cirrhosis and hepatocellular carcinoma [2, 3]. High-risk groups for MASLD include people with cardiometabolic disorders, such as obesity, insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia [4-9]. Approximately 60% to 80% of individuals with MASLD concurrently have obesity, T2DM, and insulin resistance [10]. Thus, the recent change of nomenclature from NAFLD to MASLD correctly emphasizes the metabolic ramifications of the condition [1]. Insulin resistance promotes hepatic de novo lipogenesis and activates inflammatory pathways, thereby contributing to the development and progression of MASLD [10-13].

The spectrum of MASLD includes hepatic steatosis, nonalcoholic steatohepatitis, and liver fibrosis [1-6, 11]. Although MASLD is clinically silent, diagnostic approaches include demonstration of elevated liver enzymes, imaging studies, and liver biopsy. Early in its course, liver enzymes and even imaging studies often are within the normal reference ranges in people with MASLD [5, 6]. Liver biopsy remains the gold standard to determine severity of disease and stage of liver fibrosis. However, it is an invasive procedure with increased risks, complications, sampling error, and limited cost-effectiveness [6]. Noninvasive techniques such as ultrasonography, computed tomography, magnetic resonance imaging, and elastography are often deployed in the evaluation of patients with MASLD. These techniques have variable sensitivity and specificity for detecting the different stages of MASLD [13].

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Given the increasing prevalence of MASLD, there is a need for inexpensive and noninvasive diagnostic approaches. Thus, approaches based on calculated indices from readily available clinical and laboratory data have been validated as surrogate diagnostic measures of hepatic steatosis or fibrosis. Examples of such validated indices include the hepatic steatosis index (HSI) and the Fibrosis-4 index (Fib-4) [5, 6, 10, 14-23]. The HSI and Fib-4 index have yielded valuable information on the burden of MASLD in people with T2DM [14-23] and the association between MASLD and cardiorenal disease in cross-sectional and prospective studies [4, 14, 24].

In the present report we determined the association between MASLD and incident prediabetes in a prospective cohort. The Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study assessed progression from normoglycemia to prediabetes during 5 years of follow-up of African American and European American adults with parental history of T2DM [25-27]. The POP-ABC study identified baseline adiposity, insulin sensitivity, insulin secretion, and inflammatory markers as significant predictors of incident prediabetes [28]. The present post hoc analysis evaluated the association between baseline surrogate MASLD measures (HSI and Fib-4) and incident prediabetes among initially normoglycemic participants in the POP-ABC study. We further assessed the association of HSI and Fib-4 index with cardiometabolic risk markers, such as glycemia, lipid profile, insulin sensitivity, insulin secretion, and inflammatory cytokines.

Materials and Methods

Participants

This is a post hoc analysis of data from participants in the POP-ABC study. The design, methods, and baseline characteristics of the POP-ABC study have been published previously [25-27]. In brief, eligible for inclusion were non-Hispanic White (European American) or non-Hispanic Black (African American) men and women, aged 18 to 65 years, who were biological offspring of one or both parents with T2DM, had no personal history of diabetes, and were in good general health [25-27]. Sex and race/ethnicity were self-reported by participants. Potential POP-ABC study participants underwent a screening 75-g oral glucose tolerance test (OGTT) and were enrolled if they had normal fasting plasma glucose (FPG) (<100 mg/dL [5.6 mmol/L]) and/or normal glucose tolerance (2 hours plasma glucose [2hrPG] < 140 mg/dL [7.8 mmol/L]) [25-27]. By design, the POP-ABC study enrolled approximately 75% of participants with normal fasting glucose and normal glucose tolerance and approximately 25% with normal fasting glucose or normal glucose tolerance along with one marker of prediabetes (impaired fasting glucose [IFG] or impaired glucose tolerance [IGT]) [27, 28]. The latter subgroup provided the opportunity to study the natural history of progression to dual markers of prediabetes. The confirmation of normal fasting glucose and normal glucose tolerance status was based on the American Diabetes Association criteria [29, 30]. Individuals enrolled in active weight loss programs and those taking medications known to alter blood glucose or body weight were excluded from participation. Other exclusion criteria were current pregnancy or being within 12 months post partum, and hospitalization within 6 weeks of the screening visit [25-27].

The institutional review board at the University of Tennessee approved the protocol of the POP-ABC study. Written informed consent was obtained from all participants before initiation of the study, which was conducted at the University of Tennessee General Clinical Research Center (GCRC) in accordance with the principles of the Declaration of Helsinki.

Assessments

Study participants were seen at quarterly intervals for 5 years at the GCRC, with instructions to fast overnight before study visits. Assessments included physical examination; measurement of weight, height, waist circumference, and blood pressure; and a standard 75-g OGTT. Body mass index (BMI) was calculated as weight (in kilograms) divided by the height (in meters) squared. Biochemical measurements included plasma high-sensitivity C-reactive protein (hsCRP) and adiponectin levels at enrollment; quarterly FPG; and annual OGTT, glycated hemoglobin A_1c (HbA_{1c}), lipid profile, liver function tests, and body composition (dual-energy x-ray absorptiometry). Insulin secretion was measured annually and insulin sensitivity in years 1, 3, and 5.

Assessment of insulin sensitivity

Whole-body insulin sensitivity was determined with the hyperinsulinemic euglycemic clamp, as previously described [25, 27] In brief, a primed, continuous infusion of regular insulin (2 $mU/kg^{-1}min^{-1}$; 12 $pmol/kg^{-1}min^{-1}$) was administered intravenously for 180 minutes along with a separate adjustable-rate dextrose (20%) infusion to maintain blood glucose level at approximately 100 mg/dL (5.6 mmol/L). Arterialized blood samples for measurement of plasma glucose and insulin levels were drawn every 10 minutes. The total insulin-stimulated glucose disposal rate (M) was determined during steady state (final 60 minutes) and corrected for steady-state plasma insulin level to obtain values for insulin sensitivity (Si-clamp) [25, 27].

Assessment of insulin secretion

Acute insulin secretory response was assessed using frequently sampled intravenous glucose tolerance test after a bolus infusion of dextrose (25 g), as previously described [25, 27]. The acute insulin response to intravenous glucose (AIRg) was calculated as the mean incremental plasma insulin concentration at 3 to 5 minutes after the dextrose bolus [25, 27].

Biochemical analysis

A glucose oxidase technique using the YSI glucose analyzer (Yellow Spring Instruments Co Inc) was used to determine plasma glucose levels. Plasma levels of insulin, hsCRP, and adiponectin were measured in our Endocrine Research Laboratory using commercial kits. Plasma hsCRP levels were measured with a chemiluminescent assay (Immulite, Siemens Ltd, catalog No. LKCRP1, RRID:AB_2750938 http://antibodyregistry.org/AB_2750938). The limit of detection of the hsCRP assay was 0.1 mg/L and the within-run and between-run coefficients of variation were less than 5%. Plasma total adiponectin level (including all the multimeric forms of circulating adiponectin) was measured with enzyme-linked immunosorbent assay (ELISA, Millipore, catalog No. EZHADP-61 K, RRID:AB_2801457 https://www.antibodyregistry.org/AB_2801457); the assay sensitivity was 0.78 ng/mL and within-batch, and between-batch coefficients of variation were 1.8% and 6.2%, respectively. Hepatic alanine transaminase (ALT) and aspartate

Table 1. Baseline clinical characteristics of POP-ABC study participants

Characteristic	All participants	African American	European American	*P*
No. (women/men)	343 (245/98)	193 (141/52)	150 (104/46)	
Age, y	44.2 ± 10.6	42.5 ± 10.3	46.5 ± 10.5	.0003
Weight, kg	85.0 ± 21.2	87.8 ± 21.1	81.8 ± 20.9	.004
BMI	30.2 ± 7.20	31.2 ± 7.40	28.8 ± 6.80	.001
Waist, cm				
Women	92.7 ± 15.6	94.4 ± 15.1	90.4 ± 15.8	.03
Men	97.6 ± 15.6	97 ± 18.1	98.3 ± 11.0	.97
SBP, mm Hg	121 ± 16.0	122 ± 16.0	120 ± 16.0	.1469
DBP, mm Hg	73.0 ± 9.00	73.0 ± 9.00	72.0 ± 9.00	.2136
Fasting plasma glucose, mg/dL	91.8 ± 6.80	90.8 ± 6.80	93.1 ± 6.50	.008
2-h plasma glucose, mg/dL	124 ± 25.8	123 ± 27.4	125 ± 23.3	.45
HbA_{1c}, %	5.56 ± 0.45	5.63 ± 0.47	5.44 ± 0.32	<.0001
Platelet count, $\times 10^9$/L	277.5 ± 71.6	288 ± 77.6	262 ± 59.7	.0004
Total fat mass, kg	30.5 ± 13.5	31.8 ± 13.7	29.4 ± 13.0	.11
Trunk fat mass, kg	15.1 ± 7.20	15.3 ± 7.50	14.8 ± 7.00	.48

Values are shown as the mean ± SD.
To convert the values for glucose to millimoles per liter, multiply by 0.0555.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; POP-ABC, Pathobiology of Prediabetes in a Biracial Cohort; SBP, systolic blood pressure.

transaminase (AST) levels, complete blood count, HbA_{1c}, and fasting plasma lipid profiles were measured in a contract clinical laboratory.

Definition of Outcomes

Determination of liver indices

The HSI was calculated from baseline data in our normoglycemia POP-ABC enrollees with the following formula:

$$8 \times \frac{ALT}{AST} + \text{BMI} + 2(\text{if female}) + 2(\text{if diabetes mellitus}) \quad (31)$$

As none of our POP-ABC study participants had diabetes at baseline, the diabetes extension of the formula was not applicable.

The HSI is a validated tool for assessing the presence of hepatic steatosis [31]. HSI values of 36 or greater are suggestive of the presence of hepatic steatosis [31].

We also calculated the baseline Fib-4 index, a noninvasive grading tool for the likelihood of liver fibrosis [32]. The Fib-4 index was determined according to the equation:

$$[\text{Age (yr)} \times \text{AST (IU/L)}] \div \left[\text{platelet count } (10^9/\text{L}) \times \sqrt{\text{ALT}}(\text{IU/L})\right] \quad (32)$$

The presence of advanced fibrosis is generally associated with Fib-4 scores that are greater than 2.67 [32].

Participants were divided into quartiles (Q1-Q4) for assessments of prediabetes risk, using baseline HSI and Fib-4 data. For HSI: Q1 ($0 \le$ HSI $\le$34.0), Q2 (34.1 $\le$ HSI $\le$ 39.0), Q3 (39.1 $\le$ HSI score $\le$ 44.0), and Q4 (HSI score $\ge$ 44.1). For Fib-4: Q1 ($0 \le$ Fib-4 index $\le$0.50), Q2 (0.51 $\le$ Fib-4 index $\le$0.70), Q3 (0.71 $\le$ Fib-4 index $\le$ 0.99), and Q4 (Fib-4 index $\ge$ 1.0).

Table 2. Baseline liver indices and inflammatory markers in POP-ABC study participants

Characteristic	All participants	African American	European American	*P*
Aspartate transaminase, U/L	19.4 ± 7.80	19.7 ± 8.70	19.0 ± 6.40	.3958
Alanine transaminase, U/L	19.9 ± 14.6	19.2 ± 14.6	20.9 ± 14.6	.2588
Hepatic steatosis index	39.7 ± 8.20	40.6 ± 8.40	38.6 ± 7.90	.0377
Fibrosis-4 index	0.80 ± 0.40	0.84 ± 0.50	0.79 ± 0.50	.1839
hsCRP, mg/L	3.80 ± 5.84	4.69 ± 6.63	2.77 ± 4.55	.0027
Adiponectin, µg/mL	9.40 ± 5.30	4.40 ± 4.90	10.7 ± 5.44	<.0001

Values are shown as the mean ± SD.
Abbreviations: hsCRP, high-sensitivity C-reactive protein; POP-ABC, Pathobiology of Prediabetes in a Biracial Cohort.

Prediabetes outcome

The primary outcome of the POP-ABC study was progression from normoglycemia to prediabetes during 5 years of follow-up [25-28]. Participants meeting the primary outcome were those who developed IFG (FPG 100-125 mg/dL [5.6-6.9 mmol/L]) or IGT (2hrPG 140-199 mg/dL [7.8-11.0 mmol/L]) during a 75-g OGTT, based on the American Diabetes Association criteria [29, 30]. By design, the POP-ABC study enrolled a majority of participants with normal fasting glucose and normal glucose tolerance and approximately 25% with either normal fasting glucose and normal glucose tolerance along with one marker of prediabetes (IFG or IGT) [27, 28]. For those participants enrolled with normal FPG (<100 mg/dL [5.5 mmol/L]) who had isolated IGT at baseline (ie, 2hrPG levels of 140-199 mg/dL [7.8-11.0 mmol/L]), progression

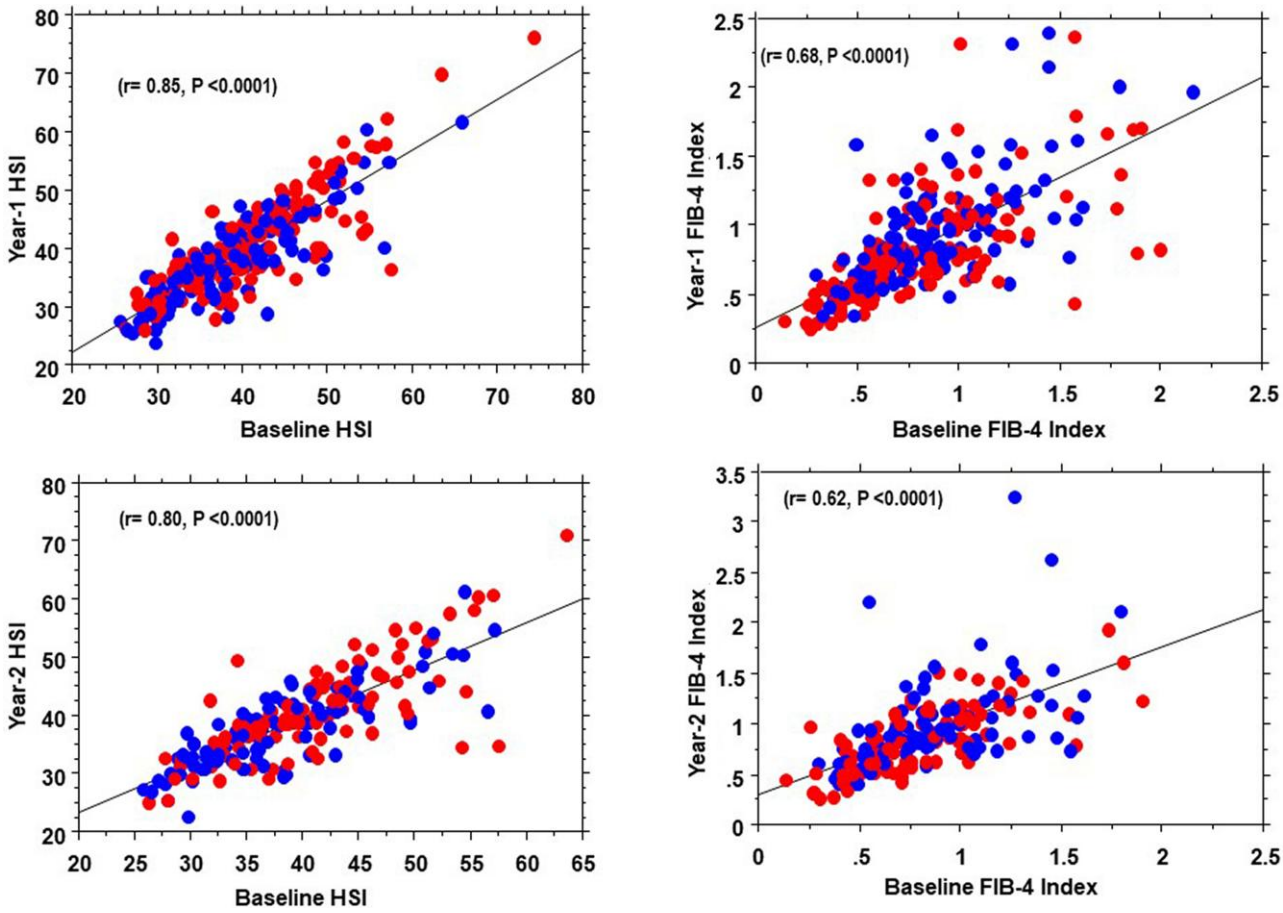


Figure 1. Reproducibility of hepatic steatosis index (HSI) and fibrosis-4 (FIB-4) index in African American (red) and European American (blue) participants.

to IFG (ie, FPG levels of 100-125 mg/dL [5.5-6.9 mmol/L]) constituted an end point occurrence. For those enrolled with normal glucose tolerance (ie, 2hrPG less than 140 mg/dL (7.8 mmol/L) and isolated IFG at baseline, progression to IGT constituted an end point occurrence. For all participants, any occurrence of diabetes (FPG ≥126 mg/dL [7.0 mmol/L], 2hrPG ≥ 200 mg/dL [11.1 mmol/L]) or prescription of an antidiabetes medication constituted an end point [27, 28]. In the present post hoc report from the main POP-ABC study, participants (N = 10) who developed diabetes during follow-up were excluded from analysis.

Participants reaching a study end point underwent confirmatory OGTT, usually within 6 weeks. All outcomes were adjudicated by the institutional data and safety officer (Murray Heimberg, MD, PhD).

Statistical Analysis

Data were reported as means ± SD. Statistical significance level was set as *P* less than .05.

Using the percentile distributions of HSI and Fib-4 scores, our study population was divided into quartiles as follows: less than or equal to 25th percentile, greater than 25th to 50th percentile, greater than 50th to 75th percentile, and greater than 75th percentile. The corresponding values for HSI quartiles were as follows: Q1: less than 34 (N = 86); Q2: 34.1 to 39 (N = 88); Q3: 39.1 to 44 (N = 79); Q4: greater than 44 (N = 90). The corresponding values for Fib-4 quartiles were as follows: Q1: less than 0.5 (N = 89); Q2: 0.5 to 0.7 (N = 88); Q3: 0.71 to 0.99 (N = 69); Q4: greater than 0.99 (N = 97).

Differences in continuous or discrete variables between defined groups were analyzed using descriptive statistics or chi-square test, respectively. Linear and multivariable regression models were used to analyze the association of HSI and Fib-4 index with metabolic variables and inflammatory markers. The association between quartiles of HSI and Fib-4 index at baseline and incident prediabetes during 5 years of follow-up in the POP-ABC study was analyzed using Kaplan-Meier survival plots and Cox proportional-hazards regression. All analyses were completed using StatView statistical software (SAS Institute Inc).

Results

Cohort Characteristics

The baseline characteristics of POP-ABC study participants have been previously reported [27]. The present report focuses on previously unpublished data based on liver indices. Of the 376 participants (217 African American, 159 European American) enrolled in the main POP-ABC study, 343 participants (193 African American, 150 European American) had evaluable baseline and follow-up data that were analyzed for the present report. Table 1 shows the characteristics of study participants by ethnicity. Overall, the cohort had normal mean values for FPG (91.8 ± 6.80 mg/dL), 2hrPG (125 ± 26.5) mg/dL, and HbA_{1c} (5.56 ± 0.45%) at enrollment. Ethnic

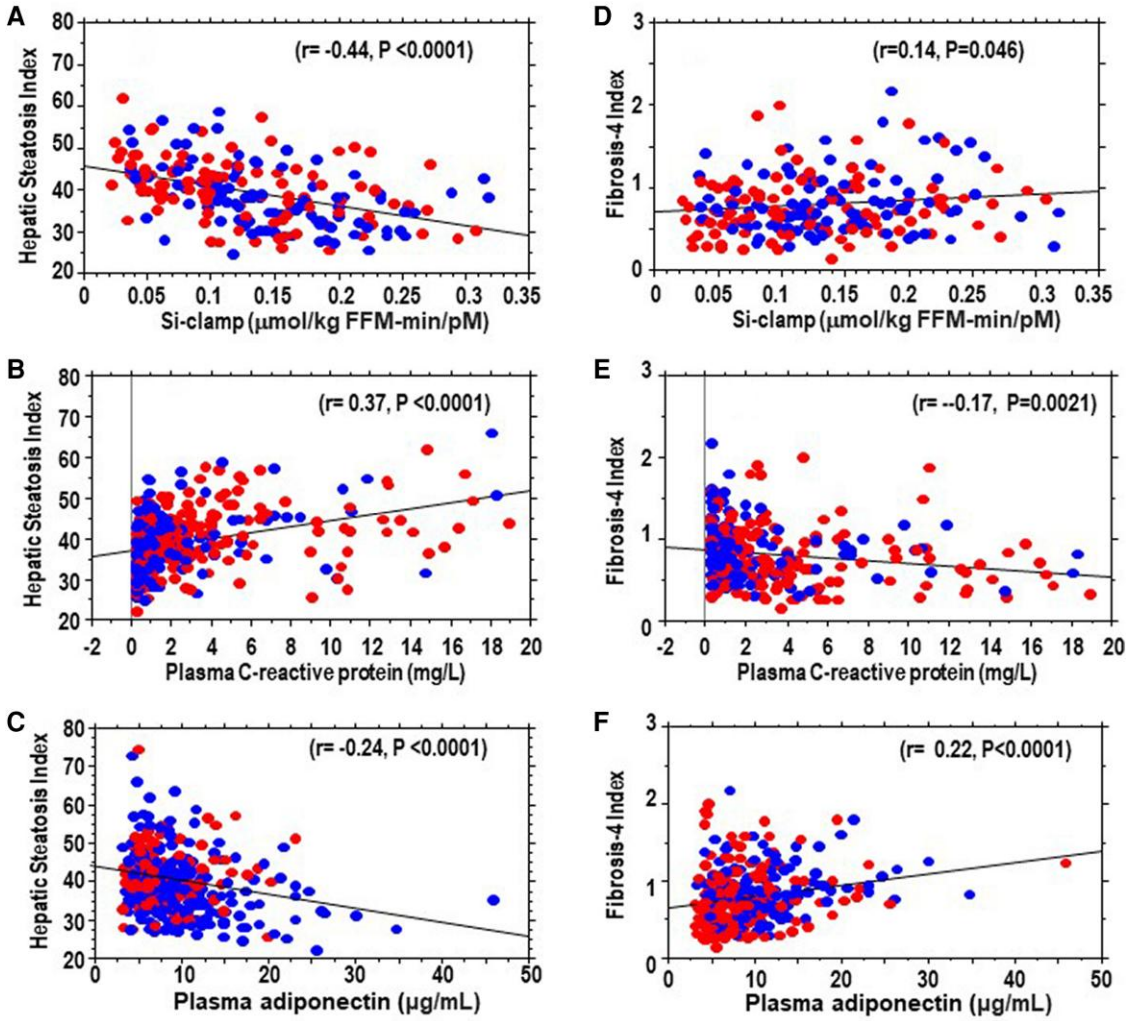


Figure 2. Association of A to C, hepatic steatosis index, and D to F, fibrosis-4 index with whole-body insulin sensitivity (Si-clamp), high sensitivity C-reactive protein, and adiponectin levels in African American (red) and European American (blue) participants. FFM, fat-free mass.

disparities were noted in some baseline measures such as adiposity, FPG, triglycerides, and platelet count (see Table 1).

Baseline Liver Indices and Biochemical Characteristics

Table 2 shows baseline values for hepatic transaminases, liver indices, and markers of inflammation among POP-ABC study participants. The mean baseline HSI was 39.7 ± 8.21, and the mean Fib-4 index was 0.80 ± 0.41. There were no ethnic differences in mean baseline levels of AST and ALT, or Fib-4 index. However, European American participants had significantly lower values for HSI and hsCRP, but higher values for plasma adiponectin, compared with African American participants.

Reproducibility of Liver Indices

Values for HSI and Fib-4 index were reproducible over 3 consecutive years (Fig. 1). The Pearson correlation coefficients between baseline vs year 1 or year 2 values for HSI were 0.85 ($P < .0001$) and 0.80 ($P < .0001$), respectively. The corresponding correlation coefficients for Fib-4 index were 0.68 ($P < .0001$) for baseline vs year 1 and 0.62 ($P < .0001$) for baseline vs year 2 values, respectively (see Fig. 1).

Liver Indices and Cardiometabolic Risk Markers

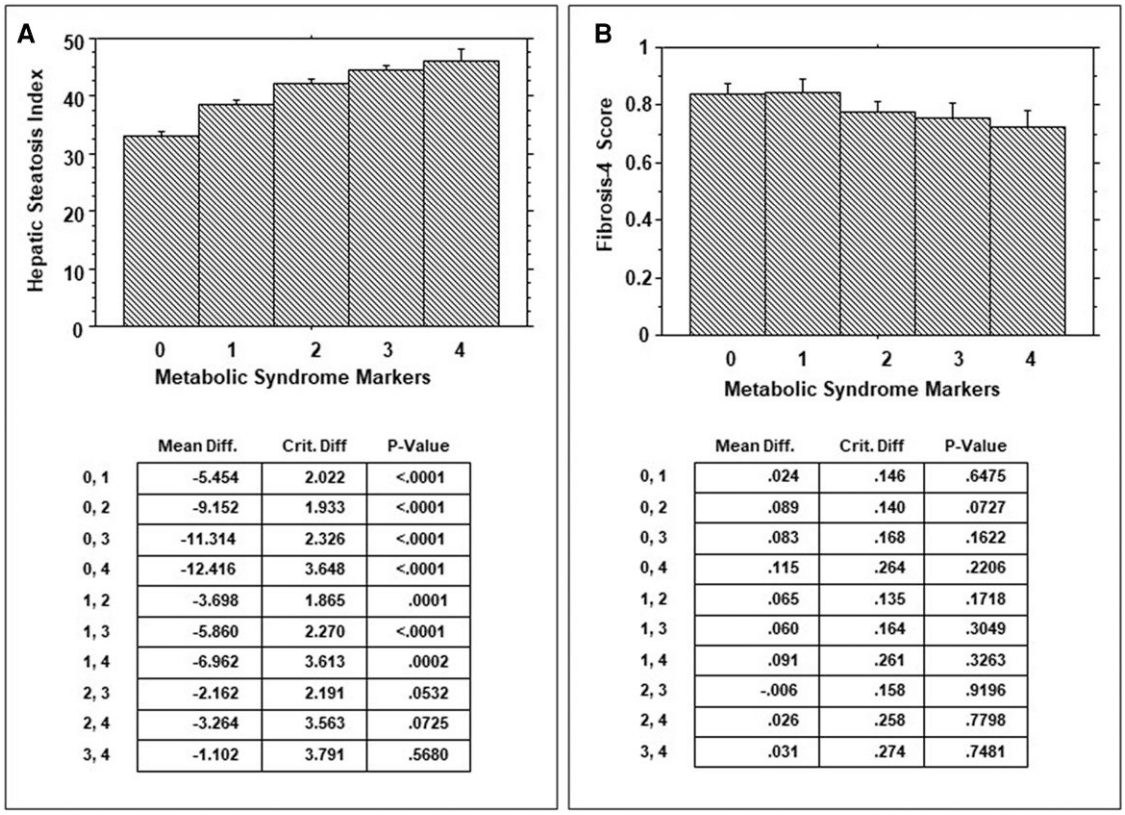
In linear regression models, baseline HSI and Fib-4 index were significantly associated with Si-clamp, hsCRP, and adiponectin levels (Fig. 2). The baseline liver indexes also correlated with homeostasis model assessment of insulin resistance (HOMA-IR) (HSI: $r = 0.43$, $P < .0001$; Fib-4: $r = -0.17$; $P = .004$). (The Si-clamp estimates whole-body [predominantly skeletal muscle] insulin-mediated glucose disposal, whereas HOMA-IR assesses peripheral and hepatic insulin resistance [33]). In a multivariate model that included FPG, 2hrPG, HbA_{1c}, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, insulin sensitivity, insulin secretion, hsCRP, and adiponectin levels, HSI (but not Fib-4) showed significant associations with plasma glucose, insulin sensitivity, and hsCRP (Table 3).

Next, we stratified study participants by presence of metabolic syndrome components. As POP-ABC study participants were all normoglycemic at enrollment, we used modified criteria for metabolic syndrome based on waist circumference greater than or equal to 100 cm in men or 88 cm in women, blood pressure greater than 120/80 mm Hg, triglycerides greater than or equal to 150 mg/dL, and HDL cholesterol

Table 3 Partial correlations of baseline hepatic steatosis index and Fibrosis-4 index and glucoregulatory, cardiometabolic, and inflammatory variables

	HSI	Fib-4	FPG	2hrPG	HbA_{1c}	LDL	HDL	Trig.	Tchol	HOMA-IR	Si-clamp	AIRg	DI	hsCRP	Adipo.
HSI	1	−.245	.173	−.182	.047	−.080	082	−.071	.084	.131	−.219	−.004	−.010	.193	.018
Fib-4	−.245	1	.057	−.004	.068	−.056	−.043	−.045	.052	−.094	−.060	−.045	−.041	−.013	.132
FPG	.173	.057	1	−.121	.120	−.056	−.053	.017	.056	.202	.104	−.078	−.039	−.061	−.157
2hrPG	−.182	−.004	−.121	1	.050	−.106	−.056	−.016	.095	.145	.009	.052	−.131	.169	−.090
HbA_{1c}	.047	.068	.120	.050	1	−.005	−.028	−.082	.019	−.013	−.022	.043	.001	.116	.004
LDL-c	−.080	−.056	−.056	−.106	−.005	1	−.962	−.928	.995	−.051	−.073	−.014	.003	−.010	.077
HDL-c	−.082	−.043	−.053	−.056	−.028	−.962	1	−.934	.967	−.028	−.027	−.006	−.011	.002	.165
Trig.	−.071	−.045	.017	−.016	−.082	−.928	−.934	1	.938	−.010	−.082	.004	−.002	−.008	.107
Tchol	.084	.052	.056	.095	.019	.995	.967	.938	1	.048	.073	.009	−.002	.015	−.070
HOMA-IR	.131	−.094	.202	.145	−.013	−.051	−.028	−.010	.048	1	−.229	.103	−.041	−.018	.084
Si-clamp	−.219	−.060	.104	.009	−.022	−.073	−.027	−.082	.073	−.229	1	−.635	.652	−.108	.141
AIRg	−.004	−.045	−.078	.052	.043	−.014	−.006	.004	.009	.103	−.635	1	.828	−.060	−.020
DI	−.010	−.041	−.039	−.131	.001	.003	−.011	−.002	−.002	−.041	.652	.828	1	.082	−.005
hsCRP	.193	−.013	−.061	.169	.116	−.010	.002	−.008	.015	−.018	−.108	−.060	.082	1	−.062
Adipo.	.018	.132	−.157	−.090	.004	.077	.165	.107	−.070	.084	.141	−.020	−.005	−.062	1

Correlations with an absolute value greater than 0.14 are significant ($P = .04 - <.0001$).
Abbreviations: 2hrPG, 2-hour plasma glucose; Adipo., adiponectin; AIRg, acute insulin response to intravenous glucose; DI, disposition index (insulin secretion corrected for insulin sensitivity); Fib-4, Fibrosis-4; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; HIS, hepatic steatosis index; LDL-c, low-density lipoprotein cholesterol; Si-clamp, insulin sensitivity measured with hyperinsulinemic euglycemic clamp; Tchol, total cholesterol.



	Mean Diff.	Crit. Diff	P-Value
0, 1	-5.454	2.022	<.0001
0, 2	-9.152	1.933	<.0001
0, 3	-11.314	2.326	<.0001
0, 4	-12.416	3.648	<.0001
1, 2	-3.698	1.865	.0001
1, 3	-5.860	2.270	<.0001
1, 4	-6.962	3.613	.0002
2, 3	-2.162	2.191	.0532
2, 4	-3.264	3.563	.0725
3, 4	-1.102	3.791	.5680

	Mean Diff.	Crit. Diff	P-Value
0, 1	.024	.146	.6475
0, 2	.089	.140	.0727
0, 3	.083	.168	.1622
0, 4	.115	.264	.2206
1, 2	.065	.135	.1718
1, 3	.060	.164	.3049
1, 4	.091	.261	.3263
2, 3	-.006	.158	.9196
2, 4	.026	.258	.7798
3, 4	.031	.274	.7481

Figure 3. Association of A, hepatic steatosis index, and B, fibrosis-4 index with accumulation of metabolic syndrome components. A significant interaction was observed between metabolic syndrome markers and hepatic steatosis index (ANOVA $P < .0001$) but not fibrosis-4 index (ANOVA $P > .05$).

Table 4. Comparison of baseline liver indices and clinical characteristics of participants who progressed to prediabetes during 5 years of follow-up vs nonprogressors

Characteristic	All participants	Progressors	Nonprogressors	*P*
No.	343	111	232	
Women/Men	245/98	111 (65/46)	232 (180/52)	.0003
Age, y	44.2 ± 10.6	47.3 ± 8.9	43.8 ± 10.8	.0017
BMI	30.2 ± 7.20	31.4 ± 6.90	29.6 ± 7.40	.0013
FPG, mg/dL	91.8 ± 6.80	94.0 ± 7.00	91.0 ± 6.00	.0027
HbA_{1c}, %	5.56 ± 0.50	5.70 ± 0.50	5.50 ± 0.40	.0190
2hPG, mg/dL	124 ± 26.0	130 ± 27.0	121 ± 25.0	.0036
AST, U/L	19.4 ± 7.80	19.6 ± 8.10	19.3 ± 7.70	.7675
ALT, U/L	19.9 ± 14.6	21.7 ± 15.3	19.1 ± 14.3	.115
Hepatic steatosis index	39.7 ± 8.20	41.33 ± 7.49	39.01 ± 8.47	.0143
Fibrosis-4 index	0.8 ± 0.40	0.83 ± 0.36	0.79 ± 0.42	.4039
hsCRP, mg/L	3.80 ± 5.80	4.40 ± 6.70	3.50 ± 5.40	.215
Adiponectin, µg/mL	9.40 ± 5.30	8.50 ± 4.40	9.90 ± 5.70	.031

Values are shown as mean ± SD.
Abbreviations: 2hrPG, 2-hour plasma glucose; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin A_{1c}; hsCRP, high-sensitivity C-reactive protein.

less than 40 mg/dL in men and less than 50 mg/dL in women. We then examined the interaction between accumulation of metabolic syndrome components with HSI and Fib-4 index. Compared with participants who did not harbor any metabolic syndrome component, there was a progressive increase in HSI values among participants with increasing number of metabolic syndrome components (analysis of variance [ANOVA] $P < .0001$) (Fig. 3). The Fib-4 index did not show any such interaction with metabolic syndrome components (Fig. 3).

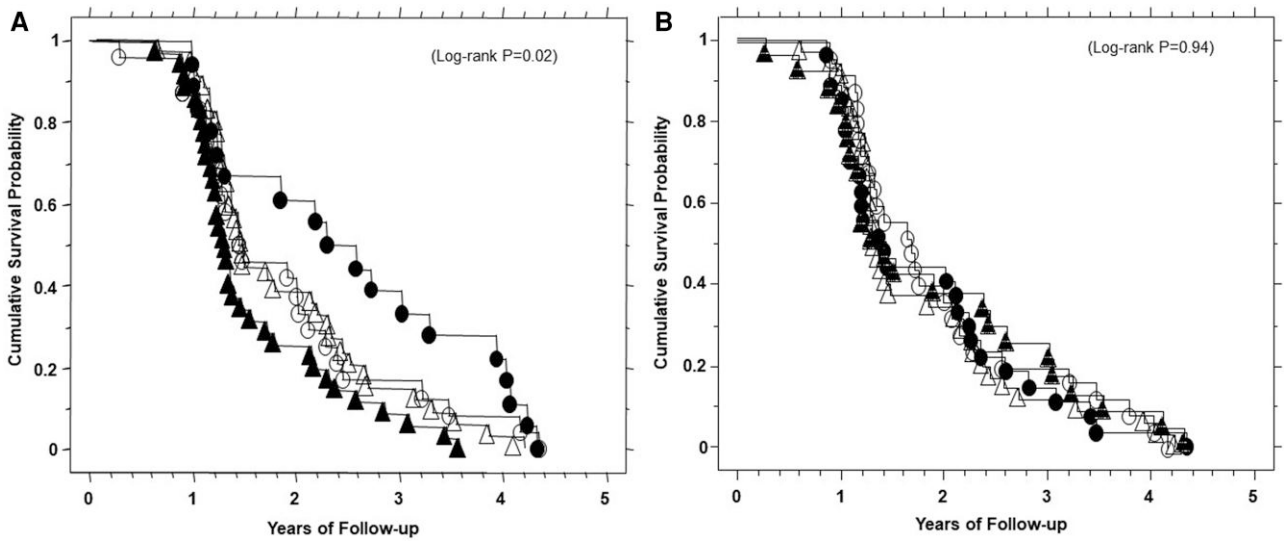


Figure 4. Kaplan-Meier analysis showing lower probability of prediabetes survival with increasing quartiles of baseline A, hepatic steatosis index, but not B, fibrosis-4 index. Quartiles: Q1, closed circles; Q2, open circles; Q3, closed triangles; Q4, open triangles.

Liver Indices and Incident Prediabetes

During 5 years of follow-up of our initially normoglycemic cohort, 111 progressed to prediabetes and 232 remained normoglycemic (nonprogressors). Table 4 summarizes the baseline characteristics of progressors to prediabetes and nonprogressors. Compared with nonprogressors, the progressors to prediabetes had higher values for HSI ($P = .0143$) but similar Fib-4 scores ($P = .40$), ALT ($P = .80$), and AST ($P = .11$) values (see Table 4). As previously reported in the primary results of the POP-ABC study [28], progressors to prediabetes had significantly higher mean age, FPG, 2hrPG, HbA_{1c}, BMI, and HOMA-IR, and lower values for insulin sensitivity, disposition index, and plasma adiponectin, compared with nonprogressors (see Table 4).

Next, we examined the association between the baseline distribution (Qs) of HSI and Fib-4 index and progression from normoglycemia to prediabetes among POP-ABC participants. During 5 years of follow-up, the cumulative incidence of prediabetes was 16.2%, 21.6%, 31.5% and 30.6% across quartiles 1, 2, 3, and 4 of baseline HSI distributions, respectively. Kaplan-Meier analysis showed decreased prediabetes survival probability with increasing Qs of baseline HSI ($P = .02$) but not Fib-4 index (Fig. 4). In minimally adjusted Cox proportional-hazards models, the hazard ratio (HR) for prediction of incident prediabetes was 1.138 (95% CI, 1.027-1.261) for baseline HSI and 1.041 (95% CI, 0.940-1.154] for baseline Fib-4 index (Table 5). After full adjustment for race, sex, FPG, 2hrPG, insulin sensitivity, insulin secretion, and total body fat mass, baseline HSI (HR 1.005 [95% CI, 0.836-1.208]) and baseline Fib-4 index (HR 0.997 [95% CI, 0.865-1.149]) were not independent predictors of prediabetes (see Table 5).

Table 5. Cox regression of baseline hepatic steatosis index and Fibrosis-4 index as predictors of incident prediabetes

Model 1	Hazard ratio	95% CI
Hepatic steatosis index	1.138	1.027-1.261
Fibrosis-4 index	1.041	0.940-1.154
Model 2	**Hazard ratio**	**95% CI**
Hepatic steatosis index	1.005	0.836-1.208
Fibrosis-4 index	0.997	0.865-1.149

Model 1: adjusted for race and sex; model 2:adjusted for race, sex, fasting plasma glucose, 2-hour plasma glucose, insulin sensitivity, insulin secretion, and total body fat mass.

Discussion

Cross-sectional studies have observed a significant association between T2DM and hepatic steatosis [14-16, 19]. However, those studies do not permit inferences regarding causality, nor do they reveal the direction of the relationship between hepatic steatosis and dysglycemia. The prospective Diabetes Prevention Program (DPP) followed individuals with prediabetes for the primary outcome of progression to T2DM. During year 14 of postrandomization follow-up, liver fat was measured as liver attenuation in Hounsfield units in 1876 DPP participants and compared in progressors to T2DM vs nonprogressors [20]. The investigators reported that progressors to diabetes had a greater prevalence of fatty liver than nonprogressors [20]. Furthermore, the DPP investigators observed significant associations between fatty liver and fasting insulin (an index of insulin resistance), waist circumference, and serum triglycerides [20]. The report from the prospective DPP suggests that the well-known cross-sectional association between hepatic steatosis and T2DM probably has an earlier origin, perhaps during the prediabetes stage. However, the lack of baseline data on hepatic steatosis, when all DPP participants were at the prediabetes stage, makes it difficult to interpret the longitudinal findings observed after 14 years of follow-up [20]. Although the DPP participants who developed diabetes during 14 years of follow-up had greater amounts of liver fat than nonprogressors, the direction of the association cannot be ascertained without baseline liver fat data.

In the present report from the prospective POB-ABC study, we explored the association of baseline indices of hepatic steatosis and fibrosis with the risk of progression from

normoglycemia to prediabetes during 5 years of follow-up. Our findings showed that increasing Qs of baseline HSI were significantly associated with higher risk of incident prediabetes. These findings extend previous observations linking hepatic steatosis to diabetes by demonstrating a significant association with incident prediabetes. Like the report from the DPP [20], we found congruent direct associations between hepatic steatosis and cardiometabolic risk markers, including glycemia, insulin resistance, and proinflammatory hsCRP. Indeed, after adjustments for baseline insulin sensitivity, insulin secretion, and adiposity in our POP-ABC study participants, the prediabetes HR associated with baseline HSI decreased and the CI widened.

Thus, the association between HSI and increased prediabetes risk might be mediated by mechanisms involving adiposity, insulin sensitivity, insulin secretion, and inflammation, among other possible factors.

The strengths of the present report include the prospective design of the POP-ABC study, large sample size, diverse cohort, extensive follow-up duration, rigorous ascertainment of end points, and the use of robust methodology for measuring insulin sensitivity and secretion. However, there are limitations that should be considered when interpreting the results presented here. First, the distribution of baseline HSI and Fib-4 index showed that most of our study participants had values that were lower than levels seen in people with overt fatty liver disease. Thus, our findings are best interpreted as reflecting mostly the relationship between subclinical steatosis and early cardiometabolic dysregulation. Furthermore, all POP-ABC participants were offspring of parents with T2DM; as such, the present findings may not be generalizable to people without a parental history of diabetes. Furthermore, although we measured fatty liver indices over 3 consecutive years, to assess reproducibility, only baseline values were analyzed as predictors of incident prediabetes. Analysis of temporal change in liver indices could provide potentially valuable additional information, but that was outside the scope of the present report.

Despite these limitations, our study demonstrates significant associations between baseline noninvasive measures of hepatic steatosis/fibrosis and several cardiometabolic risk markers. Importantly, our findings show a significant association between subclinical values of HSI and the risk of incident prediabetes during 5-year follow-up of initially normoglycemic African American and European American adults with parental history of T2DM.

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Author Contributions

S.D.-J. was the principal investigator, developed the study concept and design, analyzed data, and drafted, reviewed, and revised the manuscript; B.C.-B. collected data, and drafted, reviewed, and revised the manuscript; P.A. analyzed data, and reviewed and revised the manuscript; and S.E. collected data, and reviewed and revised the manuscript.

Disclosures

The authors have no conflicts of interest regarding the content of the present manuscript.

Data Availability

The data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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