Arteriosclerosis, Thrombosis, and Vascular Biology

ORIGINAL RESEARCH

High-Mobility Group Box-1 Is Associated With Obesity, Inflammation, and Subclinical Cardiovascular Risk Among Young Adults

A Longitudinal Cohort Study

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OBJECTIVE: We aimed to characterize circulating HMGB1 (high-mobility group box-1) levels, one of the better-characterized damage-associated molecular patterns, with respect to age, sex, and race in the general population, and investigate the longitudinal associations of HMGB1 with inflammatory markers, obesity, and preclinical markers of cardiovascular disease.

APPROACH AND RESULTS: The analyses included 489 participants (50% Blacks, aged 24.6±3.3 years at the first visit) with up to 4 follow-up visits (1149 samples) over a maximum of 8.5 years. Systolic blood pressure, diastolic blood pressure, carotid-femoral pulse wave velocity, and carotid intima-media thickness together with plasma HMGB1, hs-CRP (high-sensitivity C-reactive protein), IFN-γ (interferon-γ), IL-6 (interleukin-6), IL-10 (interleukin-10), and TNF-α (tumor necrosis factor-α) were measured at each visit. At baseline, plasma HMGB1 concentrations were higher in Blacks compared with Whites (3.86 versus 3.20 ng/mL, $P<0.001$), and in females compared with males (3.75 versus 3.30 ng/mL, $P=0.005$). HMGB1 concentrations increased with age ($P=0.007$), and higher levels of obesity measures ($P<0.001$). Without adjustment for age, sex, race, and body mass index, HMGB1 concentrations were positively associated with hs-CRP, IL-6, TNF-α, systolic blood pressure, diastolic blood pressure, and carotid-femoral pulse wave velocity ($P<0.05$) but not IL-10, IFN-γ or carotid intima-media thickness. After covariate adjustments, the associations of HMGB1 with hs-CRP, and carotid-femoral pulse wave velocity remained statistically significant ($P<0.05$).

CONCLUSIONS: This is the first study to demonstrate the age, sex, and race differences in circulating HMGB1. The increasing circulating concentrations of HMGB1 with age suggest a potential role of HMGB1 in the pathogenesis of chronic low-grade inflammation, obesity, and subclinical cardiovascular disease risk.

Key Words: blood pressure ■ C-reactive protein ■ inflammation ■ obesity ■ tumor necrosis factor

Damage-associated molecular patterns are endogenous danger signals released upon cellular stress or tissue injury, which alert the body about danger and induce potent inflammatory responses by activating the innate immune system during noninfectious inflammation.[1] HMGB1 (high-mobility group box-1), a 30-kDa nuclear and cytosolic ubiquitous protein, is one of the best-characterized damage-associated molecular patterns.[2–6] HMGB1 is actively secreted by innate immune cells and released into the extracellular space, stimulating other cytokines and playing an important role in inflammatory and immune responses.[7–11] Indeed, elevated circulating levels of HMGB1 have been found in acute inflammatory conditions, yet, HMGB1 has been understudied in low-grade chronic inflammatory conditions with systemic

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Nonstandard Abbreviations and Acronyms

BMI	body mass index
BP	blood pressure
cf-PWV	carotid-femoral pulse wave velocity
cIMT	carotid intima-media thickness
CVD	cardiovascular disease
DBP	diastolic blood pressure
GSH	Georgia Stress and Heart
HMGB1	high-mobility group box-1
hs-CRP	high-sensitivity C-reactive protein
IFN-γ	interferon-γ
IL-6	interleukin-6
IL-10	interleukin-10
SBP	systolic blood pressure
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α
WHR	waist-to-hip ratio

Highlights

- To our knowledge, this is the first longitudinal study to comprehensively examine the associations of human HMGB1 (high-mobility group box-1) with several inflammatory markers, obesity, and subclinical cardiovascular risk.
- We observe race and sex differences in plasma HMGB1.
- Circulating HMGB1 increases with age.
- Circulating HMGB1 is associated with obesity, inflammation, and subclinical cardiovascular disease risk.

and long-term effects, which are involved in the development of multiple diseases.

Although the cause of obesity is complex, obesity is associated with low-grade chronic inflammation, where its status is conditioned by the innate immune system activation in adipose tissue that promotes an increase in proinflammatory cytokines.[12] Studies have established a key role for low-grade systemic inflammation in the early stage of blood pressure (BP) elevation, arterial stiffness, atherosclerosis, and cardiovascular disease (CVD).[13–16] Further, inflammatory mechanisms linking obesity to its metabolic and cardiovascular complications are already activated in childhood obesity.[17] In a cross-sectional study, HMGB1 levels were higher in obese children (n=60) than their healthy controls (n=40), and circulating HMGB1 levels were associated with body mass index (BMI) and IL-6 (interleukin-6).[18] In another cross-sectional study of Chinese adults, circulating HMGB1 levels were higher in participants with type II diabetes mellitus (n=76) versus healthy participants (n=79), and HMGB1 levels were also higher in the obese group than in the normal-weight group.[19] Moreover, HMGB1 levels positively correlated with waist-to-hip ratio (WHR), BP, and IL-6.[19] Japanese participants with peripheral artery disease (n=24) showed higher HMGB1 levels compared with their healthy controls (n=10), and there was a significant difference in HMGB1 levels between the mild peripheral artery disease (n=12) and severe peripheral artery disease (n=12), although hs-CRP (high-sensitivity C-reactive protein) demonstrated no significant correlation with HMGB1.[5] A recent cross-sectional study from Turkey demonstrated that serum HMGB1 concentrations were significantly increased in participants with coronary artery disease (n=55) compared with healthy participants (n=50).[20] Despite numerous small studies suggesting increases in HMGB1 correlate with the low-grade inflammation found in metabolic and CVD, longitudinal studies with larger sample sizes are urgently needed.

The GSH (Georgia Stress and Heart) Study, a prospective multiethnic cohort study (≈50% Blacks) with repeated physiological and subclinical CVD risk measures over time, provided an excellent opportunity to examine the longitudinal relationships of HMGB1 with obesity and related CVD risk factors. For the first time, the present study evaluated the longitudinal associations of HMGB1 with inflammatory markers, obesity, and preclinical markers of CVD among young adults. Furthermore, to our knowledge, this is the first study to describe the variations of circulating HMGB1 levels with respect to age, sex, and race.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

The participants were from the longitudinal GSH study.[21] The inclusion criteria were (1) aged 5 to 16 years in 1989, (2) Blacks or Whites, (3) normotensive for age- and sex-based on BP screening, and (4) apparently healthy. Participants were classified as Black if (1) both parents reported being of African heritage, (2) the parents and the child were born and raised in the United States, and (3) parents considered themselves and their child to be Black, or Afro-American. Participants were classified as White if (1) both parents reported that they were of European ancestry, (2) the parents and the child were born and raised in the United States, and (3) they considered themselves and their child to be White, European American, or White. All participants were screened for eligibility and recruited using family health history questionnaires obtained from a county-wide (Richmond County, Georgia) public school screening of children in kindergarten through eighth grade whose families volunteered for health prevention research. The original GSH cohort consisting of 740 participants was established in 1989. Since visit 9, blood samples have been collected and properly stored. A total of 489 participants (66% of the original 740

participants) with up to 4 visits, including 1149 blood samples with valid HMGB1 measurements, one from each person-visit were analyzed for the current study. The annualized attrition rate was <4% because of geographic relocation, official discontinuation of death, and loss of interest. There have been no significant differences in age, ethnicity, and sex distributions between dropouts and the participants who remained in the study. The Institutional Review Board at the Medical College of Georgia gave approval for the study. Informed consent was provided by all participants or by parents if participants were <18 years.

Anthropometry Measurements

Height was measured to the nearest 0.1 cm by a wall-mounted stadiometer (Tanita Corporation of American, Arlington Heights, IL); weight was measured to the nearest 0.1 kg by a calibrated electronic scale with the participants not wearing shoes and in light clothing (model CN2OL; Cardinal Detecto, Webb City, MO). BMI was computed as weight (in kilogram) per square of height (in square meters). Overweight was defined as BMI ≥25 kg/m^2, and obese was defined as ≥30 kg/m^2. Waist circumference was measured at the center of the umbilicus. Hip circumference was measured at the widest part of the hips. WHR was computed as waist circumference divided by hip circumference.

Preclinical CVD Phenotypic Measurements

BP was recorded by Dinamap (model 1864 SX). After attachment of an appropriately sized BP cuff to the right arm, the participant was placed in a supine position on a medical table with head propped on a pillow and then given instructions to relax as completely as possible for 15 minutes. BP measurements were taken from the Dinamap at the end of the 11th, 13th, and 15th minutes. As recommended by the National Health and Nutrition Examination Survey procedures for BP measurement, the average of the last 2 readings was used to represent resting systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Arterial stiffness was measured among 253 participants on visit 15 using the SphygmoCor Pulse Wave Analysis System (SphygmoCor, Sydney, Australia). Carotid-femoral pulse wave velocity (cf-PWV) was automatically calculated from measurements of pulse transit time and the distance traveled by the pulse between the distal and proximal measurement sites. Carotid artery IMT was available among 299 participants on visit 15 and 271 participants on visit 16. A total of 348 participants were measured for IMT for both visits. Hewlett-Packard Sonos 5500 (Andover, MA) equipped with a 7.5 MHz linear array probe was used to measure the common carotid artery IMT. There were 570 valid observations for IMT measurement. Left and right common carotid, carotid bulb, internal carotid, and external carotid were first visualized in transverse then in longitudinal planes. Measurements were made at a point 2 cm proximal to the bifurcation on both near and far wall that showed the intima-media boundaries most clearly. IMT was derived from a computer program Vascular Tool (Medical Imaging Application, Iowa City, IA). Common carotid's IMT was measured as the distance from leading edge of first echogenic line to that of the second echogenic line. Ten frames of common carotid artery were analyzed by one experienced sonographer. The mean carotid IMT for far wall was used in this analysis.

Plasma Inflammatory Markers Measurement

hs-CRP, IFN-γ (interferon-γ), IL-10 (interleukin-10), IL-6, and TNF-α (tumor necrosis factor-α) were measured using Simple Plex assay, which was based on microfluidics and glass nanoreactor technology (Simple Plex, Protein Simple corp, San Jose, California).[22] The intra-assay and inter-assay CVs were <7.0%.

Table 1. General Characteristics in Each Visit

Characteristics	First Visit	Second Visit	Third Visit	Fourth Visit
N	489	372	223	65
Age, y	24.6± 3.3	27.1±3.5	29.4±3.3	30.4±3.4
Female, N (%)	249 (51)	193 (52)	115 (52)	31 (48)
Black, N (%)	246 (50)	194 (52)	123 (55)	36 (55)
BMI, kg/m^2	29.1±7.9	29.7±8.1	30.0±8.3	31.2±9.5
Waist circumference, inch	36.4±6.8	37.2±7.0	37.7±6.7	39.0±7.3
WHR	0.84±0.07	0.85±0.08	0.86±0.07	0.87±0.07
HMGB1, ng/mL	3.5±1.8	3.8±1.8	4.5±1.8	4.7±1.5
hs-CRP, mg/L	3.3±6.2	3.4±5.5	3.4±6.5	3.7±4.9
IFN-γ, pg/mL	0.8±1.0	0.8±0.8	0.8±0.9	0.9±1.4
IL-10, pg/mL	2.4±1.2	2.4±1.0	2.3±0.9	2.4±1.1
IL-6, pg/mL	3.4±2.7	3.2±2.3	3.4±3.1	4.6±3.8
TNF-α, pg/mL	6.2±1.7	6.1±1.8	6.2±1.8	6.2±2.4
SBP, mm Hg	114.5±13.0	115.1±13.3	115.1±13.3	119.1±13.7
DBP, mm Hg	64.6±8.1	66.9±8.5	69.0±9.4	70.1±9.6
cf-PWV, m/s	5.9±1.2	6.2±1.4	6.7±1.4	6.9±1.1
cIMT, mm	0.5±0.1	0.5±0.1	0.5±0.1	N/A

The observations included in this analysis were reordered according the order of the appearance of each participants with valid HMGB1 measurement. BMI indicates body mass index; cf-PWV, carotid-femoral pulse wave velocity; cIMT, carotid intima-media thickness; DBP, diastolic blood pressure; HMGB1, high-mobility group box-1; hs-CRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; IL-6, interleukin-6; IL-10, interleukin-10; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α; and WHR, waist-to-hip ratio.

HMGB1 Measurement

Plasma HMGB1 concentrations were quantified in 1149 samples by ELISA using the kit from IBL International according to the manufacturer's instruction. High-sensitive range of standard curve is from 0.2 to 10 ng/mL, the intra-assay and inter-assay CVs are 5.8% and 7.6%, respectively. Because of the skewed and tailed distribution, HMGB1 was log-transformed in regression models. Residuals of log-transformed HMGB1 from linear regressions against test plates were taken as adjusted values of HMGB1 to account for the plate bias.

Statistical Analysis

Descriptive statistics of participant characteristics were summarized by visit and presented in Table 1. Bar charts in Figure 1 illustrated the distributions of baseline HMGB1 by sex and race. Longitudinal associations of HMGB1 with measures of obesity (BMI, waist circumference, and WHR) were evaluated using mixed-effects models adjusting for age, sex, and race. The longitudinal associations of HMGB1 with inflammatory markers (hs-CRP, IL-6, IFN-γ, IL-10, and TNF-α) and CVD phenotypes (SBP, DBP, cf-PWV, and carotid intima-media thickness) were also investigated using mixed-effects models, with and without adjustments for age, race, sex, and BMI. Autoregressive structure of order 1 of the within-group errors was used to account for serial correlations among observations on the same participant. cf-PWV was analyzed cross-sectionally using multiple linear regression model as it was measured only on visit 15. Similar statistical analyses were carried out to test the interactions between HMGB1 and age, sex, or race with regard to the inflammatory markers and CVD phenotypes, and subgroup analyses were performed by age, sex, and race groups. HMGB1, cf-PWV, and all 5 inflammatory markers were log-transformed in the regression models. No potential nonlinear associations were

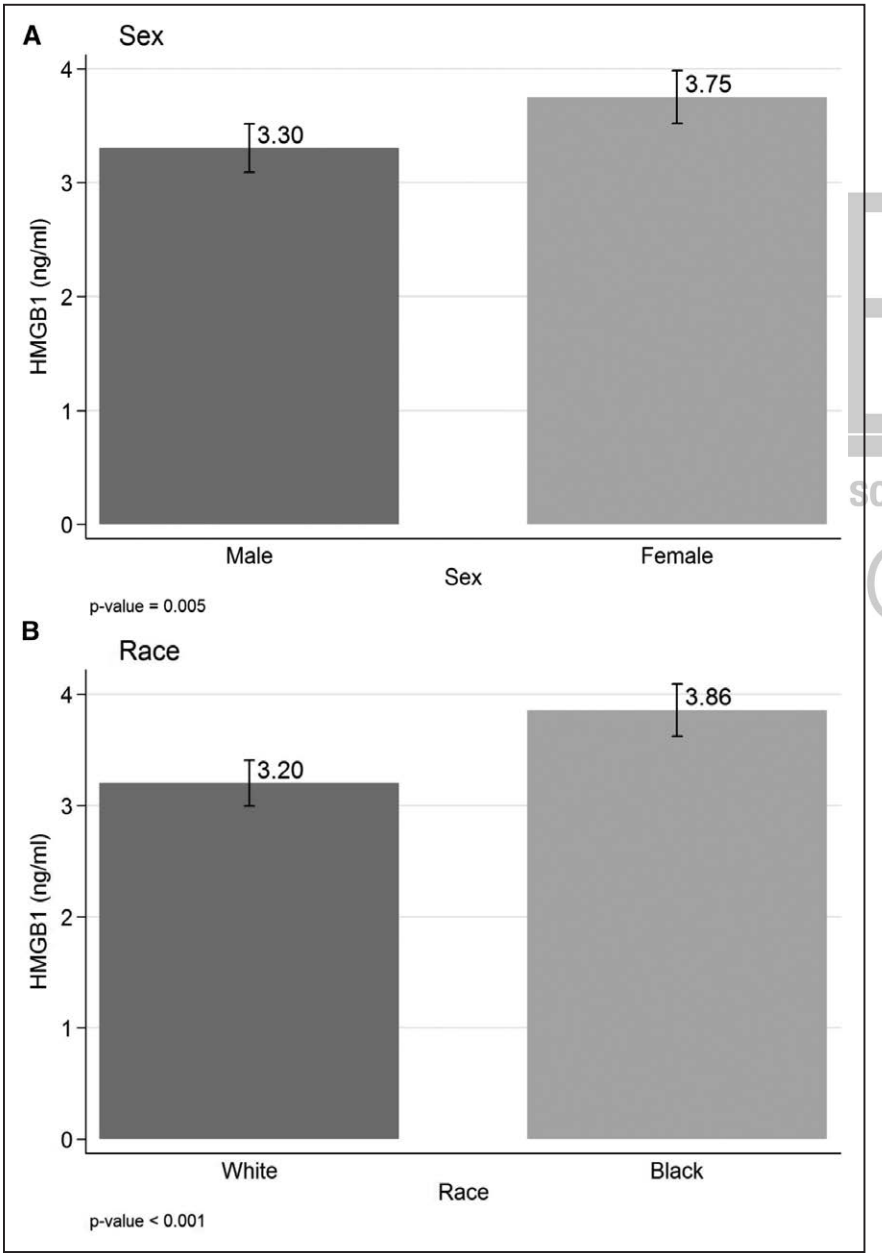

Figure 1. The baseline differences in HMGB1 (high-mobility group box-1) concentrations between sex and race.
The bar charts present the baseline differences in HMGB1 regarding sex (**A**), race (**B**).

identified using scatter plot. A *P* value <0.05 was considered statistically significant. All statistical analyses were performed using Stata version 12.0 (College Station, TX 77845).

RESULTS

General Characteristics of the Participants

The observations included in this analysis were reordered according to the order of the appearance of each participant with valid HMGB1 measurement. The first visit was the first observation of each participant who was included in this analysis. The 489 participants averaged 24.6±3.3 years in age at the first visit that was included in this study (Table 1). Among those, 249 (51%) were female, and 246 (50%) were Black.

Variations in Plasma HMGB1 by Participant Characteristics

Using the first visit as the baseline, higher plasma HMGB1 concentrations were associated with older age (*P*=0.007), and higher BMI, waist circumference, and WHR (*P*<0.001). Figure 1 shows the differences in baseline HMGB1 concentrations by sex and race. Plasma HMGB1 concentrations were higher among females than males, and among Blacks than Whites (*P*<0.05).

Longitudinal Associations of HMGB1 With Obesity Measures

Longitudinally, plasma concentrations of HMGB1 were positively associated with BMI, WHR, and waist circumference without (*P*<0.001) or with adjustment for age, sex, and race (*P*<0.001). The participants were categorized into groups according to the baseline measurements of BMI, waist circumference, and WHR. Among the 489 participants, 172 (35%) were lean, 156 (32%) were overweight, and 161 (33%) were obese. Compared with the lean participants (BMI<25 kg/m^2), higher HMGB1 concentrations were observed among the obese participants (BMI>30 kg/m^2; *P*<0.001), but not among the overweight participants (BMI between 25 and 30 kg/m^2; *P*=0.078). Compared with those in the lower tertile, participants in the middle and upper tertiles of WHR had higher HMGB1 (*P*=0.010 and *P*<0.001, respectively), and participants in the middle and upper tertiles of waist circumference had higher HMGB1 (*P*<0.001 for both) (Figure 2). We further tested the associations between HMGB1 and obesity with additional adjustment of the inflammatory markers. The associations of HMGB1 with BMI and waist circumference remained statistically significant after further adjustment of IFN-γ, IL-10, IL-6, and TNF-α (*P*<0.05). HMGB1 was no longer associated with WHR with adjustment of any of the inflammatory markers (*P*>0.05). HMGB1 was no longer associated with any of the obesity measures when adjusted for hs-CRP (*P*>0.05).

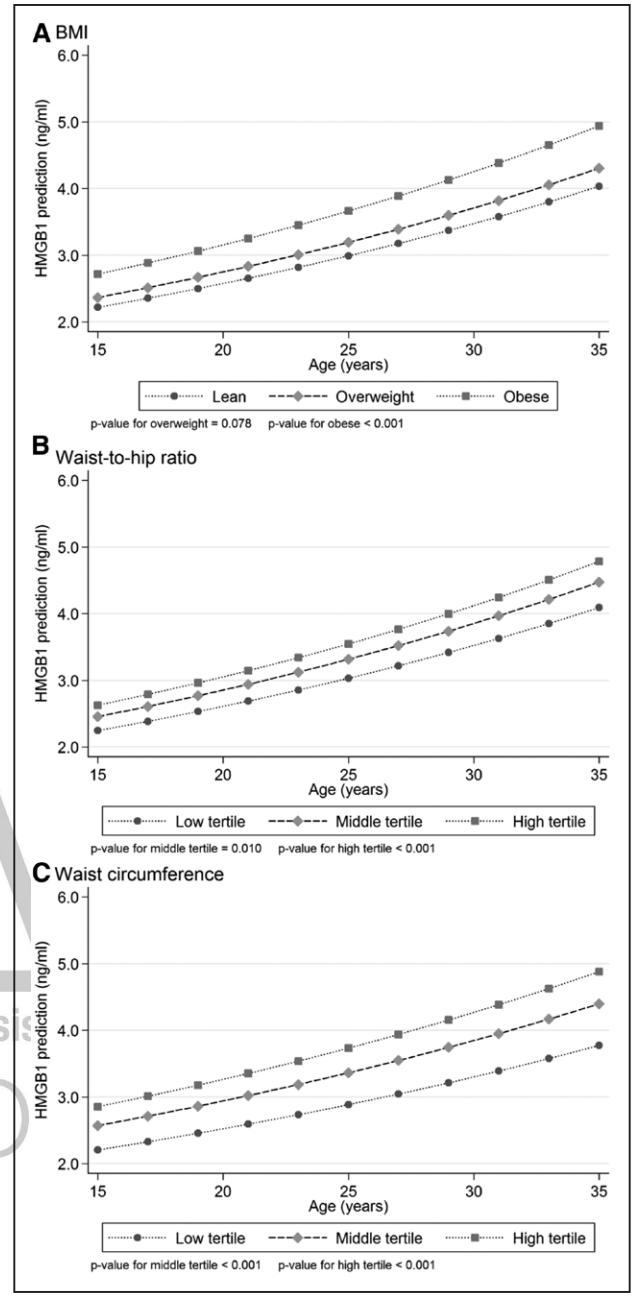

Figure 2. Longitudinal associations between obesity measures and HMGB1 (high-mobility group box-1). Marginal plots between obesity measures and HMGB1 are based on mixed-effects models adjusting for age, sex, and race. **A**, Obesity; (**B**) tertiles of waist circumference; (**C**) tertiles of waist-to-hip ratio. *P* values labeled in each plot are the significant levels of the corresponding groups compared with the lean group (**A**) or to the low tertile (**B** and **C**). BMI indicates body mass index.

Longitudinal Associations of HMGB1 With Inflammatory Markers

As shown in Table 2, plasma concentrations of HMGB1 were positively associated with hs-CRP, IL-6, and TNF-α without (*P*<0.01) or with adjustment for age, sex, and race (*P*<0.01). HMGB1 remained significantly associated with hs-CRP with further adjustment of BMI (*P*<0.001). In

Table 2. The Longitudinal Associations of HMGB1 With Inflammatory Markers

	hs-CRP (N=1129)		IFN-γ (N=888)		IL-10 (N=1122)		IL-6 (N=643)		TNF-α (N=1134)	
	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Model 1	0.67 (0.07)	<0.001	0.04 (0.04)	0.308	0.02 (0.02)	0.348	0.15 (0.04)	<0.001	0.03 (0.01)	0.006
Model 2	0.57 (0.07)	<0.001	0.05 (0.04)	0.178	0.03 (0.02)	0.205	0.12 (0.04)	0.006	0.03 (0.01)	0.009
Model 3	0.42 (0.06)	<0.001	0.04 (0.04)	0.331	0.01 (0.02)	0.658	0.06 (0.04)	0.139	0.02 (0.01)	0.147

The numbers of observations were smaller than total sample size (1149 observations of 489 participants) because of missing values. Two-level mixed-effects models were used. Autoregressive structure of order 1 of the within-group errors was assumed to account for successive observations with the groups. Model 1 was unadjusted; model 2 was adjusted for age, race, and sex; model 3 was adjusted for body mass index in addition to model 2. There were significant interaction of age and HMGB1 on hs-CRP (β=−0.04, *P*=0.009), of sex and HMGB1 on IFN-γ (β=−0.16, *P*=0.038), and of sex and HMGB1 on TNF-α (β=−0.07, *P*=0.005). No other significant interaction of age, sex, or race on the association between HMGB1 and inflammatory markers was detected. HMGB1 and all 5 inflammatory markers were log-transformed. HMGB1 indicates high-mobility group box-1; hs-CRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; IL-6, interleukin-6; IL-10, interleukin-10; and TNF-α, tumor necrosis factor-α.

the fully adjusted model, a 10% increase in HMGB1 was associated with ≈4% increase in hs-CRP. The associations of HMGB1 with IL-6 and TNF-α were not significant with further adjustment of BMI (*P*>0.05). The associations of HMGB1 with IFN-γ and IL-10 were not significant (*P*>0.05). In addition, we tested whether the associations of HMGB1 with the inflammatory markers were modified by age, sex, or race. There were significant modifications of HMGB1 effects on hs-CRP by age (β=−0.04, *P*=0.009), on IFN-γ (β=−0.16, *P*=0.038) and TNF-α by sex (β=−0.07, *P*=0.005). No other significant interactions of HMGB1 by age, sex, or race was detected. Figure 3 shows age, sex, and race differences in the associations of HMGB1 with hs-CRP, IFN-γ, and TNF-α. The increase of hs-CRP associated with HMGB1 was significant in each of the subgroups (*P*<0.05). HMGB1 was associated with increased IFN-γ among males but not statistically significant (β=0.10, *P*=0.104). HMGB1 was significantly associated with increased TNF-α among the males (β=0.05, *P*=0.008), not the females (β=−0.00, *P*=0.993).

Longitudinal Associations of HMGB1 With Preclinical CVD Phenotypes

Table 3 presents the results of 2-level mixed-effects models associating circulating HMGB1 levels with cardiovascular phenotypes. Without covariate adjustment, HMGB1 was associated with elevated SBP, DBP, and cf-PWV (*P*<0.05) but not carotid intima-media thickness. After adjustment for age, sex, race, and BMI, the association between HMGB1 and cf-PWV remained statistically significant (*P*=0.005). In the fully adjusted model, a 100% increase in HMGB1 was associated with ≈7% increase in cf-PWV. However, the associations of HMGB1 with SBP or DBP were no longer significant (*P*>0.05), after the adjustment of the above confounding factors. No significant age, sex, or race effect modification on the association between HMGB1 and cardiovascular phenotypes was detected. In addition, the associations of inflammatory markers with preclinical CVD phenotypes were studied with the same regression models that were done for HMGB1. None of the inflammatory markers was significantly associated with preclinical CVD phenotypes (*P*>0.05). Moreover, the positive association between HMGB1 and cf-PWV remained significant with further adjustment of each of the inflammatory makers (*P*<0.05).

DISCUSSION

To our knowledge, this is the first study to characterize circulating HMGB1 levels in a bi-ethnic population by age, sex, and race. Our study demonstrates that circulating HMGB1 concentrations are higher in Blacks than Whites, and in females than males, and HMGB1 increases with age. This is the first report of the longitudinal associations of the circulating HMGB1 concentrations with obesity status, inflammation, and preclinical markers of CVD. The associations of HMGB1 with hs-CRP and cf-PWV are consistent across different age, sex, and race subgroups.

The plasma concentrations of HMGB1 increased with age in our cohort. Similar longitudinal patterns were seen in animal models. HMGB1 protein and mRNA were elevated in the hippocampus and cerebrospinal fluid of aged rats, and blocking the actions of HMGB1 might reduce age-associated inflammatory priming.[11] In a cross-sectional analysis, human serum concentrations of HMGB1 were found to be significantly higher in older (≥70 years old, n=18) than young adults (18–30 years old, n=19).[23] However, in another cross-sectional analysis, the serum levels of HMGB1 were lower in the aged group (>65 years, n=30) compared with those in the young group (≈40 years, n=30).[24] The discrepancies could be attributed to various factors such as the sample sizes, study designs (cross-sectional versus longitudinal), age range, race, lifestyles, and geographic regions. Nonetheless, given the plausible underlying mechanisms, the relationship between HMGB1 and aging warrants further investigations.

Notable differences in HMGB1 concentrations were observed between Black and White races and between sexes in our cohort. Blacks had higher concentrations than Whites. This is consistent with previous findings that Blacks tend to have greater levels of plasma inflammatory

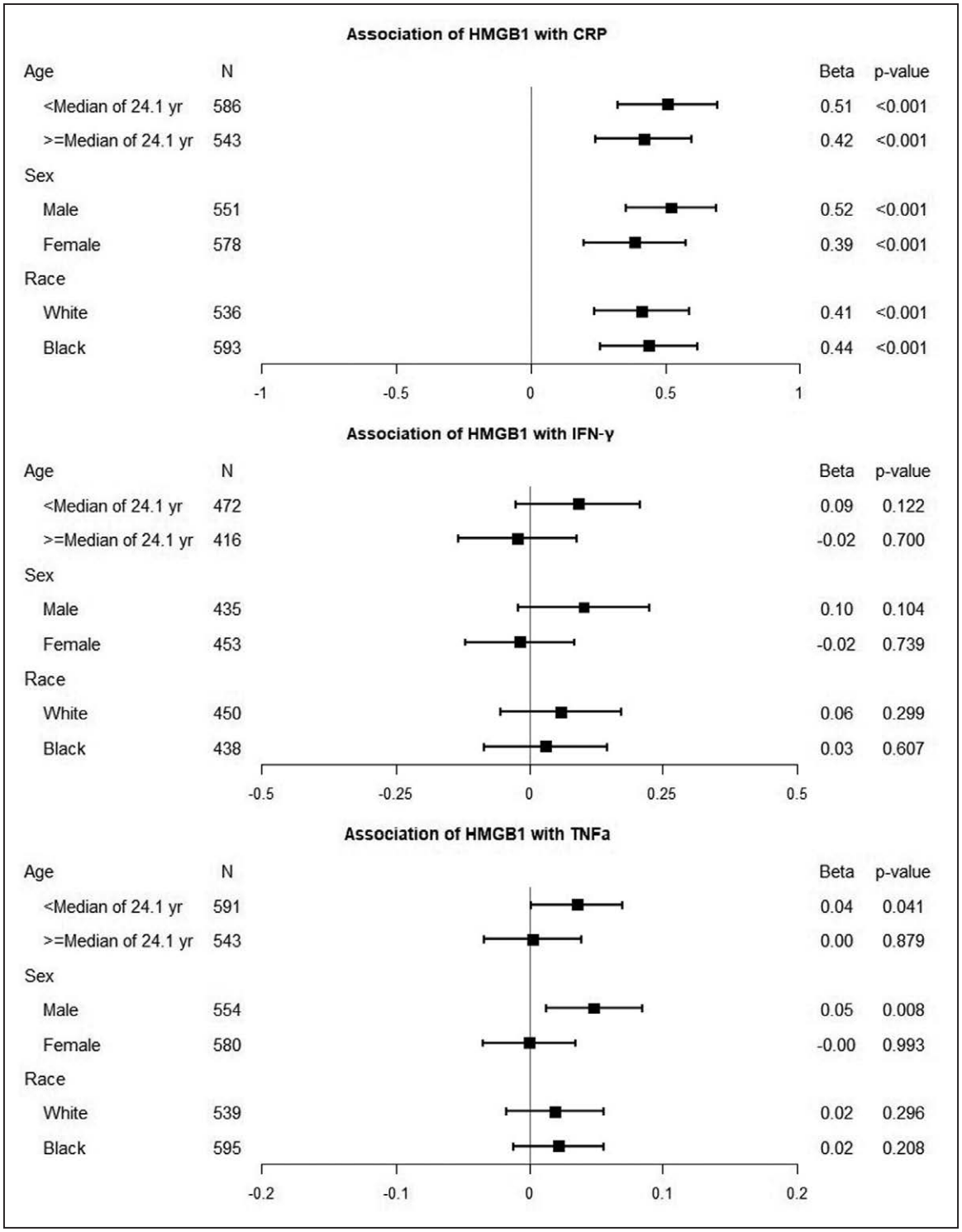

Figure 3. Subgroup analyses of the associations of HMGB1 (high-mobility group box-1) with hs-CRP (high-sensitivity C-reactive protein), IFN-γ (interferon-γ), and TNF-α (tumor necrosis factor-α).
Two-level mixed-effects models were used. Autoregressive structure of order 1 of the within-group errors was assumed to account for successive observations with the groups. Adjusted covariates included age, race, sex, and body mass index when appropriate. HMGB1, hs-CRP, IFN-γ, and TNF-α were log-transformed.

markers such as hs-CRP and IL-6.[25–27] Females had significantly higher HMGB1 than males, which could be related to females' higher percent body fat. However, the role of sex in HMGB1 biology deserves more attention.

In the present study, the plasma concentration of HMGB1 was significantly correlated with obesity over time. This is biologically plausible and supported by various animal studies, including our own.[11] In animal studies,

Table 3. The Longitudinal Associations of HMGB1 With Cardiovascular Phenotypes

	SBP (N=1145)		DBP (N=1145)		cf-PWV (N=253)		cIMT (N=570)	
	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Model 1	1.64 (0.66)	0.012	2.28 (0.46)	<0.001	0.12 (0.03)	<0.001	0.00 (0.01)	0.807
Model 2	0.60 (0.67)	0.371	0.34 (0.44)	0.438	0.08 (0.02)	0.001	−0.00 (0.01)	0.554
Model 3	−0.24 (0.65)	0.710	0.49 (0.44)	0.261	0.07 (0.02)	0.005	−0.01 (0.01)	0.101

The numbers of observations were smaller than total sample size (1149 observations of 489 participants) because of missing values. Two-level mixed-effects models were used except for cf-PWV. Autoregressive structure of order 1 of the within-group errors was assumed to account for successive observations with the groups. Multiple linear regression model was used for cf-PWV. Model 1 was unadjusted; model 2 was adjusted for age, race, and sex; model 3 was adjusted for body mass index in addition to model 2. No significant interaction of age, sex, or race on the association between HMGB1 and cardiovascular phenotypes was detected. HMGB1 and cf-PWV were log-transformed. cf-PWV indicates carotid-femoral pulse wave velocity; cIMT, carotid intima-media thickness; DBP, diastolic blood pressure; HMGB1, high-mobility group box-1; and SBP, systolic blood pressure.

it has been reported that serum levels of HMGB1 were increased in rats fed with a high-fat diet for 12 weeks.[28] Adipose tissue secrets HMGB1, which was expressed 2-fold more in adipose tissue from obese compared with normal-weight individuals.[29] Moreover, obesity-associated adipocyte death leads to the release of the intracellular contents such as HMGB1 into the extracellular milieu.[30] HMGB1 activates resident immune cells in the adipose tissue once released from necrotic adipocytes. Activated resident immune cells then secrete additional HMGB1, which then activates and recruits additional immune cells.[30] Therefore, HMGB1 plays a critical role in the initiation and maintenance of chronic inflammatory state in adipose tissue of obese participants.[30] HMGB1 binding to TLR (toll-like receptor), specifically TLR2 and TLR4, leads to the activation of nuclear factor κB, mitogen-activated protein kinases, and Jun N-terminal kinase, thereafter leading to immunoinflammatory response with the production of cytokines and other inflammatory molecules.[31] HMGB1 is also able to activate receptor for advanced glycation end products pathway, whose products lead to the activation of several intracellular oxidative stress-depending signals including nuclear factor κB, PI3K/Akt, mitogen-activated protein kinases, and then upregulate several proinflammatory genes.[32–34] In this study, we observed longitudinal associations of HMGB1 with hs-CRP, which supports these mechanistic findings in vitro and animal models.

The demonstrated associations of HMGB1 with the above inflammatory markers are of clinical importance because chronic inflammation plays a key role in the early stage of hypertension and CVD.[35] In our cohort, higher HMGB1 was associated with elevated SBP, DBP, and cf-PWV, although the correlations between HMGB1 and BP were no longer statistically significant after the adjustments of age, sex, race, and BMI. Moreover, our data show that HMGB1 could be a superior biomarker of cf-PWV to the inflammatory factors in the young adult population. In our study, HMGB1 seems to outperform hs-CRP in predicting arterial stiffness (pulse wave velocity), or that although HMGB1 and hs-CRP are closely correlated, HMGB1 may still comprise independent effect on CVD development. Given our sample size, our data had the power of 97.80% to detect a correlation between HMGB1 and cf-PWV. There are several potential mechanisms linking HMGB1 to cardiovascular phenotypes. HMGB1 may affect cardiovascular health through TLR-mediated inflammation. An animal study showed that hypertensive rats exhibited higher TLR4 in the paraventricular nucleus, which is a major receptor of HMGB1.[36] TLR4 inhibition decreased mean arterial pressure in rats.[36] In a human study, TLR polymorphism was associated with SBP and pulse pressure among patients with coronary artery disease.[37] Another study in vitro proposed that extracellular HMGB1 could enhance mechanical stress-induced cardiac hypertrophy via the RAGE/ERK1/2 signaling pathway.[38] HMGB1 may also play a role in Ang II-induced vascular smooth muscle cell phenotypic transformation.[39] Sustained release of HMGB1 in response to persistent hypertensive stimuli promotes vascular remodeling, endothelial dysfunction, cardiac hypertrophy, and increases in BP in models of pulmonary hypertension.[40,41] Our longitudinal data highlight that HMGB1 might play a critical role in the inflammatory response in the pathogenesis of CVD in humans. The finding that HMGB1 seemed to be associated with IFN-γ and TNF-α among the male warrants further investigation.

A major strength in the present study is our longitudinal cohort with the multiple HMGB1 measurements over time; with an equal distribution of Blacks and Whites, males and females; and with repeated detailed obesity and preclinical CVD measures. However, the study also has several limitations, including: (1) limited age range and relatively young age of our sample; (2) cf-PWV and carotid intima-media thickness were measured in a subset of the participants; (3) its observational nature that may not support causal inferences; and (4) we did not collect dietary intake information, which could be a confounding factor given that diets may differentially affect intestinal permeability and thus, low-grade chronic inflammation. (5) We only collected blood samples from the GSH cohort, and measured circulating HMGB1 in this study, which was not able to identify the potential source of HMGB1. Future studies examining the HMGB1 from peripheral blood mononuclear cells or adipose tissue are

warranted. Future interventional studies are also needed to validate our results.

In conclusion, for the first time, we report the longitudinal associations of circulating HMGB1 levels with obesity, proinflammatory cytokines, and preclinical CVD phenotypes in a cohort of White and Black races. Race and sex differences in some of these associations are evident. Further investigations of the variations in and biological mechanisms of HMGB1 in free-living human population may lead to better understanding of the role of HMGB1 and may confirm the value of circulating HMGB1 as a clinical biomarker and the development of anti-HMGB1 antibodies or antagonists in therapeutics for the targeted prevention, management, and treatment of chronic inflammation, obesity, and CVD.

ARTICLE INFORMATION

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Disclosures

None.

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