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The relationship between glucose metabolism, metabolic syndrome, and bone-specific alkaline phosphatase: A structural equation modeling approach

Running title: Insulin resistance and alkaline phosphatase

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Abstract

Context: Serum alkaline phosphatase plays a role in vascular calcification. It is found in various tissues, whereas bone-specific alkaline phosphatase (BAP) more specifically reflects mineral metabolism. The relationship of serum alkaline phosphatase (total and bone-specific) with diabetes and metabolic syndrome, two major risk factors of vascular calcification, is largely unknown.

Objective: We aimed to investigate the relationships between glucose metabolism, components of MetS, and alkaline phosphatase.

Design: This was a cross-sectional study.

Setting: Nationally representative sample of the US population in 1999-2004.

Participants: 3,773 non-diabetic participants of the National Health and Nutrition Examination Survey 1999-2004.

Main Outcome Measures: Serum BAP and total alkaline phosphatase.

Results: In multivariable linear regression, HOMA2-IR (β=0.068), HOMA2-B (β=0.081), insulin (β=0.065), mean arterial pressure (β=0.15), and HDL-cholesterol (β=0.209) were positively associated with BAP, whereas HOMA2-IS (β=-0.065) was negatively associated with BAP. On the other hand, only mean arterial pressure and HDL-cholesterol were significantly associated with total alkaline phosphatase. Moreover, structural equation model revealed that hypertension, low HDL, and

insulin resistance had significant direct effects on serum BAP levels, whereas obesity and inflammation might have indirect effects on serum BAP levels. The overall model showed very good fit to the data (comparative fit index = 0.995, root mean square error of approximation = 0.037, and standardized root mean square residual = 0.006).

Conclusion: Glucose metabolism and MetS are significantly related to serum BAP levels. How BAP mediates vascular calcification in diabetes and MetS warrants further studies.

Keywords: bone alkaline phosphatase; insulin resistance; vascular calcification; metabolic syndrome

Introduction

Diabetes mellitus and metabolic syndrome (MetS) are major risk factors of vascular calcification (1). Patients with diabetes have more extensive coronary artery disease, and atherosclerotic plaque morphology in diabetic patients may be predisposed to rupture and thrombosis (2). Among patients with acute coronary syndromes, patients with co-morbidity diabetes or MetS have a greater lesion length, plaque burden, necrotic core, and calcium content among nonculprit lesions (3). Moreover, higher incidence and progression of coronary artery calcium have also been demonstrated in subjects with MetS or diabetes (4). However, the underlying mechanism is poorly understood.

Extracellular pyrophosphate and alkaline phosphatase have been identified as key molecules in vascular calcification (1). Pyrophosphate is a potent inhibitor of tissue mineralization and vascular calcification (5). Pyrophosphate is produced by an enzyme, ectonucleotide pyrophosphatasephosphodiesterease 1 (NPP1), which is located in the cell membrane. It is then transported across the cell membrane to the extracellular matrix by the progressive ankylosis protein (ANK). Bone-specific alkaline phosphatase (BAP) hydrolyzes pyrophosphate and so promotes tissue mineralization (6-7). Knockout mice deficient in NPP1 and ANK have increased

aortic calcification (5) and medial calcification (8), respectively. On the other hand, blocking the hydrolyzing action of BAP ameliorated the calcification in vascular smooth muscle cells from NPP1- and ANK-deficient mice (7). Thus, alkaline phosphatase has a critical role in calcification, and it has been extensively used as a marker of calcification (6-7) and exploited as therapeutic target of vascular calcification (7, 9). Previous studies also showed that serum alkaline phosphatase is a predictor of mortality (10-11) and cardiovascular events (11).

A number of studies have investigated the association between serum alkaline phosphatase and diabetes/MetS owing to its role as a hepatobiliary marker. However, serum alkaline phosphatase consists of several alkaline phosphatase isoforms from various tissues, such as liver, bone, and kidney, with a large proportion are derived from liver and bone. Previous studies showed that BAP reflects mineral metabolism with a higher sensitivity and specificity than total alkaline phosphatase (12). Therefore in the current study, we aimed to evaluate the relationship between glucose metabolism, metabolic syndrome, alkaline phosphatase, and BAP in a large nationally-representative population.

Materials and Methods

The current study utilized data from National Health and Nutrition Examination Survey (NHANES) 1999–2004 (13). The NHANES included a stratified multistage probability sample which represented the civilian non-institutionalized U.S. population. Selection was based on counties, blocks, households, and individuals within households. It also included non-Hispanic blacks and Mexican-Americans to provide an adequate estimate of these ethic groups. Participants were required to sign a consent form before their participation, and ethical approval was obtained from the Human Subjects Committee of the U.S. Department of Health and Human Services.

Our analysis included non-pregnant participants aged 20 or above whose BAP and fasting glucose levels were available (n=5,057). We excluded participants with missing data in the multivariable models or without weight in the complex sampling model (n=757) and participants with diabetes (n=527, defined as a plasma glucose level ≥126 mg/dl [7.0 mmol/L] after fasting for a minimum of 8 hours, a glycohemoglobin (HbA1C) value ≥6.5%, self-reported diabetes or self-reported current use of oral hypoglycemic medication or insulin). After exclusion, 3,773 participants were included in the analysis.

BAP and total alkaline phosphatase

Serum BAP was measured using the Hybritech Tandem-MP ostase immunoenzymetric assay (1999-2001, 2003-2004) and the Beckman access ostase assay (2002). Since there were two methods in measuring BAP, therefore NHANES recommends the following regression equation to convert the Hybritech assay values to the Beckman assay values: Beckman assay serum BAP = exp(-0.5326*1.1139*x - 0.7963*(max(0,x -4.5151)) + 0.9660*(max(0, x-4.9030)), where x = log(Hybritech assay serum BAP).This conversion was used in the present analysis.

Serum total AP was measured as a routine laboratory blood parameter using Hitachi Model 704 multichannel analyzer in NHANES 1999–2000 and Beckman Synchron LX20 in NHANES 2003–2004. In NHANES 2001–2002, they were measured by either Hitachi Model 704 multichannel analyzer or Beckman Synchron LX20, and the reported values had been adjusted by regression equations to allow comparison across the two methods.

Indices of glucose metabolism

Serum insulin, plasma glucose, and HbA1C were measured at the Diabetes Diagnostic Laboratory, University of Missouri–Columbia. Plasma glucose and serum insulin were measured from fasting blood samples using hexokinase enzymatic method and

radioimmunoassay, respectively. HbA1C was measured using Primus CLC330 and Primus CLC 385 analyzers (Primus Corporation, Kansas City, MO). The updated homeostasis model assessment (HOMA2) is more accurate than the original HOMA1, therefore Homeostasis model assessment calculator (14) was used to calculate the HOMA2 beta cell function (%B), insulin sensitivity (%S), and hepatic insulin resistance (IR).

Components of MetS

Lipids (total cholesterol, HDL-cholesterol, triglycerides) were measured enzymatically using Hitachi 704 Analyzer at the lipoprotein analytical laboratory, Johns Hopkins University School of Medicine. Seated systolic and diastolic blood pressures (mm/Hg) were measured using a mercury sphygmomanometer according to the American Heart Association and Seventh Joint National Committee recommendations. Mean arterial pressure (MAP, mm/Hg) was calculated as 2/3 diastolic blood pressure + 1/3 systolic blood pressure.

Independent variables

Age (years), gender (male/female), race/ethnicity (Hispanics, non-Hispanic white, non-Hispanic Black, others), smoking status (current/ former/ non-smokers), alcohol

use (drinkers/ non-drinkers), level of education (< high-school, high-school, > high-school), and physical activity (active/ sedentary) were assessed using a questionnaire. We used same definition for never, current and former smokers that is used in the National Health Interview Surveys (NHIS) conducted by Center for Disease Control. Individuals who had not smoked more than 100 cigarettes in their lifetime were considered non-smokers; those who had smoked more than 100 cigarettes in their lifetime were considered former smokers if they answered negatively to the question "Do you smoke now?" and current smokers if they answered affirmatively. Individuals who had 12 alcohol drinks or more per year were considered as drinkers. Participants who reported doing moderate or vigorous activity for at least 10 minutes over the past 30 days on the Physical Activity Questionnaire were considered to be physically active. Body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by height in meters squared. However, as BMI is highly correlated with waist circumference ($r>0.8$), we only included waist circumference (a component of MetS) as the independent variable to avoid collinearity. C-reactive protein (CRP, mg/dL), gamma-glutamyl transpeptidase (GGT, U/L), aspartate aminotransferase (AST, U/L), alanine transaminase (ALT, U/L), N-terminal telopeptides, and fatty liver index (15) were also included as independent variables. Fatty liver index was derived using the equation: $FLI = (e^{0.953*\log e (triglycerides)}$

$^{+ 0.139*BMI + 0.718*loge (ggt) + 0.053*waist circumference - 15.745}) / (1 + e^{0.953*loge (triglycerides) + 0.139*BMI +}$ $^{0.718*loge (ggt) + 0.053*waist circumference - 15.745}) * 100.$ Details regarding the measurement of the independent variables are available at the NHANES website (13).

Statistical analyses

Glycemic traits and components of MetS were analyzed as continuous and quartile variables. Multivariable linear regression was used to evaluate the association when glycemic traits and components of MetS were modeled as continuous variables. Estimated mean and 95% CI of BAP or tissue non-specific alkaline phosphatase for each quartile were calculated using ANCOVA in a general linear model, and linear trend P was obtained using contrast coefficients for linear trend analysis. Structural equation modeling (SEM) was used to estimate the model goodness-of-fit and directional estimates between variables. The predefined model used in the current study was modified from an existing model of MetS from literature.(16) The global Goodness of Fit Index for the SEM included the comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR). These indices are less sensitive to sample size and complexity than χ^2 test for evaluating model fit (17). Values for the CFI of ≥0.90 and ≥0.95 indicate acceptable and good fit, respectively (18). Values for the RMSEA values of ≤0.06

represent good fit while values >0.10 represent unacceptable fit (18). A SRMR ≤0.08 and <0.10 represent good and acceptable fit, respectively (18). SEM was carried out using R package "lavaan.survey" that performs SEM while taking account of the complex sampling of NHANES data. Variables with skewed distributions were log-transformed before analysis. Sample weights that account for the unequal probabilities of selection, oversampling, and non-response were applied for all analyses using complex sampling module in SPSS version 18.0 software (SPSS Inc, Chicago, IL). All values presented are weighted to represent the U.S. civilian population.

Results

Table 1 shows the sex-stratified characteristics of the 3,773 NHANES 1999-2004 participants who were free from diabetes. Women were older, more likely to be non-Hispanic white, non-smokers., non-active drinkers and had lower physical activity. They had higher HDL-cholesterol levels, CRP, HOMA2-IS, and HOMA2-B. Conversely they had lower waist circumference, BAP, total alkaline phosphatase, triglyceride levels, MAP, ALT, AST, GGT, HbA1C, fasting glucose levels, insulin concentrations, and HOMA2-IR.

Table 2 shows the association between glycemic traits and alkaline phosphatase. After adjustment of age, race/ethnicity, education, smoking, drinking, physical activity , and waist circumference in model 1, HOMA2-IR, HOMA2-B, fasting glucose, and insulin were positively associated with BAP, while HOMA2-IS was negatively associated with BAP ($P<0.05$). After further adjustment of metabolic risk factors (CRP, total cholesterol, AST, ALT, GGT, triglycerides, HDL-cholesterol, and MAP) in model 2, the association between BAP and fasting glucose became insignificant. Similar results were obtained when urinary N-terminal telopeptides and fatty liver index were adjusted in model 3. On the other hand, three indices (HOMA2-IR, HOMA2-B, and insulin) and HOMA2-IS were positively and negatively associated with tissue non-specific alkaline phosphatase in model 1, respectively. All associations became insignificant after further adjustment in models 2 and 3. Similar results were obtained when glycemic traits were categorized as quartiles (Supplementary Table 1).

Table 3 shows the association between components of MetS and alkaline phosphatase. MAP and HDL-cholesterol showed significant association with BAP, while waist circumference and triglycerides showed a trend toward significant association with BAP ($0.05 < P < 0.1$). On the other hand, MAP and HDL-cholesterol showed significant association with tissue non-specific alkaline phosphatase, while no

association was observed with waist circumference and triglycerides. Similar results were obtained when components of MetS were categorized as quartiles (Supplementary Table 2).

Figure 1 illustrates our hypothesized relationships between BAP, inflammation, and components of MetS. All latent variables (denoted by oval circle) were adjusted for age, gender, race/ethnicity, drinking, smoking, and physical activity. As expected, obesity has direct association with hypertension, inflammation, and insulin resistance; inflammation has direct association with insulin resistance; insulin resistance has direct association with vascular calcification and dyslipidemia; and dyslipidemia and hypertension have direct association with vascular calcification. All of the standardized regression coefficients shown in the figure were statistically significant ($P \leq 0.001$). The CFI was 0.995 (value >0.95 indicates good fit), RMSEA was 0.037 (value <0.06 indicates good fit), and SRMR was 0.006 (value <0.08 indicates good fit), these fit indices consistently indicate that our hypothesized model is a good fitting model.

Discussions

The associations and inter-relationships between insulin resistance, components of

MetS, and BAP were unraveled in the current study of the US-population using structural equation modeling in addition to multivariable linear regression. Although tissue non-specific alkaline phosphatase is known to correlate with insulin resistance and metabolic syndrome owing to its role as a hepatobiliary marker, our study clearly shows that both hyperinsulinemia, insulin resistance, and MetS are in fact more strongly associated with BAP.

Our study excluded participants with diabetes allowing us to study the relationship of hyperinsulinemia or insulin resistance with BAP, without the confounding effect of significant hyperglycaemia. Note that the direct effect of insulin on osteoblasts is a stimulation of osteoblast proliferation & differentiation, and an increase in BAP activity *in vitro* (19). On the other hand, streptozotocin-induced insulin deficiency (type 1 diabetes) in rats is associated with reduced BAP which is reversed by insulin therapy (20). In humans, osteopenia is observed in type 1 diabetic patients even with good control. On the other hand, high glucose *per se* has deleterious effect on osteoblast function *in vitro* (rat primary osteoblast cell culture) and decreases BAP secreted (21). Only subjects with poorly controlled type 2 diabetes are at risk of osteoporosis.

Our study sheds light on the mechanism of elevated BAP. While insulin resistance is known to be a risk factor for vascular calcification, the mechanism is not completely understood. In the current study, we showed that multiple HOMA2 indices and insulin levels are robustly associated with BAP rather than total alkaline phosphatase. Although total alkaline phosphatase could be elevated due to obesity, insulin resistance, fatty liver, and hepatosteatosis, no significant association was observed between HOMA2-IR and total alkaline phosphatase in the fully adjusted model. This may be explained by the fact that other liver markers included in the model were more specific to liver dysfunction; ALT, AST, and GGT also showed significant associations ($P<0.05$) with HOMA2-IR in the fully adjusted model. When ALT, AST, and GGT were removed from the fully adjusted model, the association between alkaline phosphatase and HOMA2-IR became significant ($P = 0.015$). On the other hand, elevated BAP could be due to the presence of fatty liver. However, further adjustment for fatty liver index (15), a validated marker of fatty liver, did not change the association of HOMA2-IR, HOMA2-IS, HOMA2-B, and insulin with BAP (Table 2), suggesting that the association was independent of fatty liver. In addition, our study also showed that BAP is associated with multiple components of MetS, which suggested that hyperinsulinemia, insulin resistance, and MetS could lead to serum BAP elevation. In line with our previous studies (22-23), serum CRP was significantly

associated with alkaline phosphatase in the current study, while no association was observed with BAP ($P > 0.5$; data not shown). These observations further suggested that although total alkaline phosphatase and BAP are strongly correlated, the causes of elevation of total alkaline phosphatase and BAP could be different.

There have only been a few studies of BAP with insulin resistance and MetS. In agreement with a cross-sectional study carried out in 328 type 2 diabetes patients, we also observed no significant association between BAP and fasting plasma glucose and HbA1c (24). In a study of 54 healthy postmenopausal women, there was no correlation between BAP and any components of MetS (25), which could be due to the small sample size.

Serum alkaline phosphatase is routinely measured clinically, while BAP is not. Therefore, most epidemiological studies examined the association of serum alkaline phosphatase with mortality and cardiovascular diseases. Among 4,115 participants of the Cholesterol And Recurrent Events study, alkaline phosphatase was associated with all-cause mortality. This finding was subsequently replicated using NHANES III data, with significant association being observed between alkaline phosphatase and all-cause and cardiovascular mortality (10). Similar results are observed in patients

with maintenance hemodialysis (26) and acute stroke (27). In a retrospective study including 10,743 outpatients, serum alkaline phosphatase was robustly associated with mortality (including cardiovascular mortality) and cardiovascular related hospitalization (28). In addition to mortality, serum alkaline phosphatase has been reported to be associated with multiple cardiovascular diseases, such as stroke incidence (29), coronary artery calcification score (30), myocardial infarction, stent thrombosis, major adverse cardiac event, and coronary calcification (11). Our earlier study also showed that elevated alkaline phosphatase is associated with peripheral arterial disease (31). A few studies examined the role of BAP in mortality and cardiovascular diseases. In 130 osteoporotic patients, serum BAP was significantly higher in those with vascular calcification than those without (32). In a prospective study carried out in 800 dialysis patients, serum BAP was robustly associated with cardiovascular and non-cardiovascular mortality, and the effect size was much stronger than that of serum total alkaline phosphatase (33). Altogether, these studies suggested that alkaline phosphatase is an important predictor of mortality and cardiovascular events, while our current study provided evidence that BAP may be the link between insulin resistance and vascular calcification, cardiovascular diseases, or even mortality. Future studies on clinical outcomes should measure BAP in addition to total alkaline phosphatase (11).

BAP is known as a bone formation marker. Our previous study showed that genetic variation in *ALPL* gene was associated with hip geometry and bone mineral density in 1,513 Framingham Offspring Cohort participants (34). Therefore the associations observed in the current study could reflect difference in bone turnover. We then further adjusted for another bone turnover marker, urinary N-terminal telopeptides, in the logistic regression model (Table 2). Further adjustment for urinary N-terminal telopeptides attenuated the estimates but the associations remained statistically significant. These findings suggest that at least part of the association between glycemic traits and BAP is independent of bone turnover. Similarly, BAP may also be associated with growth. We then performed sensitivity analysis by excluding participants aged <25, and the results were essentially similar to the results before the exclusion (Supplementary Table 3). Future study is required to confirm our findings. The association of MetS with osteoporosis is controversial, with MetS was reported to be associated with higher BMD (35) and lower BMD (36). In previous study, blood pressure was shown to be negatively associated with bone mineral density (36). However, blood pressure was found to be positively associated with BAP in the current study. It should also be noted that, although BAP is mainly expressed in osteoblast, it is also expressed in other cells, such as B-cell (37) and vascular smooth muscle cells (38). Therefore, serum BAP could also be contributed by other cells,

although it is expected to be contributed predominantly by osteoblasts.

Population studies have shown that diabetes is preventable or even reversible. Therefore, identification of biomarkers may have the potential for early diagnosis of insulin resistance and hence prevention of diabetes (39-40). In this study, we showed that serum BAP concentration is associated with several metabolic traits, therefore BAP could potential be used as a biomarker. Future studies are required to examine whether addition of BAP as a predictive biomarker could enhance the performance of existing prevention strategies such as increased physical activity, healthy diet, and early treatment of hyperglycemia (39-40).

Our study has several strengths. The study population is large, multiethnic, nationally-representative, and well-characterized with data on multiple risk factors and confounders. The findings were shown to be robust in multiple statistical tests. Nevertheless, there are limitations. First, cross-sectional data cannot prove causality. Second, although the model in the current study showed a good fit in SEM, this may not be the only plausible model since the mechanisms underlying metabolic risk factors is complex. Future studies incorporating evidence from laboratory and human studies are required to investigate the mechanism in detail. Third, HOMA2 indices are

only surrogate markers, for example, HOMA2-IR is a surrogate marker of hepatic insulin resistance; and these indices are strongly correlated with insulin levels. It is unclear whether insulin resistance, insulin levels, or both leads to increased BAP. Future studies using euglycemic hyperinsulinemic and hyperglycemic clamp techniques are required to confirm our findings. Fourth, as aforementioned, the regulation of BAP is complex, therefore the results should be interpreted with caution.

In conclusion, our study provides a possible mechanism for the increased vascular calcification in subjects with increased insulin resistance such as MetS or type 2 diabetes. Future studies are required to examine whether improving insulin resistance and MetS could reduce the expression of BAP and the risk of vascular calcification and cardiovascular disease.

Conflict of interest

The authors reported no conflict of interest.

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Table 1. Characteristics of the study population (NHANES 1999-2004).

	Total (n=3,773)		Men (n=1,932)		Women (n=1,841)		
	%	(95% CI)	%	(95% CI)	%	(95% CI)	P-value
%			49.6	(48.4-50.8)	50.4	(49.2-51.6)	NA
Smoking (%)							
never	50.7	(47.9-53.6)	44.6	(41.3-48)	56.7	(53.2-60.1)	<0.001
former	23.3	(21.3-25.4)	26.3	(24.1-28.6)	20.3	(17.7-23.2)	<0.001
current	26	(23.8-28.4)	29	(26.2-32.1)	23	(20.7-25.6)	<0.001
Active drinker (%)	75	(71.2-78.4)	84.3	(80.5-87.6)	65.8	(61-70.3)	<0.001
Race/ethnicity (%)							
Mexican American	12.7	(9.8-16.4)	13.6	(10.5-17.4)	11.8	(8.8-15.7)	0.101
Non-Hispanic White	73.6	(69.9-77)	72.5	(68.5-76.2)	74.7	(71-78)	0.04
Non-Hispanic Black	10	(8.1-12.3)	9.7	(7.8-12.1)	10.2	(8.1-12.8)	0.516
Others	3.7	(2.8-5)	4.2	(3-5.9)	3.3	(2.1-5)	0.324
Education (%)							
< High school	18.5	(16.9-20.2)	18.8	(16.5-21.4)	18.2	(16.3-20.2)	0.667
High school	26.3	(23.9-28.9)	27.6	(24.8-30.5)	25.1	(22.2-28.3)	0.147
> High school	55.1	(52.2-58)	53.6	(50.2-56.9)	56.7	(53-60.3)	0.12
Had moderate or vigorous activity (%)	65.9	(63.5-68.2)	67.5	(64.6-70.3)	64.2	(61.4-66.9)	0.038
Age (yr)	39.94	(39.03-40.88)	38.84	(37.93-39.78)	41.05	(39.95-42.18)	<0.001
BMI (m/kg^2)	26.99	(26.75-27.23)	27.09	(26.82-27.36)	26.90	(26.53-27.26)	0.357
Waist circumference (cm)	93.35	(92.73-93.99)	96.92	(96.12-97.75)	89.97	(89.1-90.87)	<0.001
Bone-specific Alkaline phosphatase(ug/L)	13.76	(13.45-14.08)	14.96	(14.57-15.35)	12.68	(12.3-13.07)	<0.001
ALP (U/L)	67.05	(65.89-68.22)	70.36	(68.8-71.94)	63.94	(62.75-65.16)	<0.001
ALT (U/L)	22.50	(22.15-22.86)	27.25	(26.52-27.98)	18.65	(18.25-19.05)	<0.001
AST (U/L)	22.86	(22.51-23.2)	25.10	(24.58-25.63)	20.84	(20.46-21.23)	<0.001
GGT (U/L)	21.67	(21.09-22.26)	26.95	(25.98-27.96)	17.48	(16.93-18.06)	<0.001
C-reactive protein(mg/dL)	0.18	(0.17-0.19)	0.15	(0.14-0.16)	0.22	(0.21-0.24)	<0.001
Total cholesterol (mg/dL)	196.47	(194.4-198.52)	196.34	(193.55-199.11)	196.61	(194.4-198.79)	0.847
Triglycerides (mg/dL)	115.50	(112.05-119.07)	124.17	(118.77-129.81)	107.60	(104.35-110.92)	<0.001
HDL-cholesterol (mg/dL)	49.75	(49-50.5)	44.99	(44.1-45.89)	54.92	(53.91-55.92)	<0.001
Mean arterial pressure (mm/Hg)	87.76	(87.22-88.31)	89.23	(88.63-89.85)	86.34	(85.59-87.08)	<0.001
Glycohemoglobin (%)	5.23	(5.21-5.26)	5.25	(5.22-5.28)	5.22	(5.19-5.24)	0.006
Glucose, plasma (mg/dL)	94.54	(93.99-95.1)	96.38	(95.76-97.03)	92.77	(92.15-93.39)	<0.001
Insulin (uU/mL)	8.87	(8.59-9.17)	9.21	(8.84-9.59)	8.55	(8.24-8.88)	0.001

HOMA2-IR	1.00	(0.97-1.04)	1.05	(1.01-1.09)	0.96	(0.93-1)	<0.001
HOMA2-IS (%)	99.52	(96.32-102.83)	95.50	(91.69-99.47)	103.63	(99.79-107.65)	<0.001
HOMA2-B (%)	86.28	(84.63-87.98)	85.15	(83.2-87.16)	87.42	(85.39-89.47)	0.049

Data are geometric means (95% CI). All values presented are weighted to represent the U.S. civilian population, 1999-2004. P-value represents differences in means or proportions, using ANOVA or χ^2 test.

Table 2. Association between glycemic traits and alkaline phosphatase (bone-specific or total) in NHANES 1999-2004

	Glycemic traits	Model 1 (N=3,773)			Model 2 (N=3,773)			Model 3 (N=3,009)*		
		Estimate	95% CI	P-value	Estimate	95% CI	P-value	Estimate	95% CI	P-value
Bone-specific alkaline phosphatase	HOMA-IR	0.109	(0.079-0.138)	<0.001	0.068	(0.038-0.097)	<0.001	0.044	(0.008-0.08)	0.018
	HOMA-IS	-0.107	(-0.137--0.077)	<0.001	-0.065	(-0.095--0.035)	<0.001	-0.041	(-0.079--0.004)	0.031
	HOMA-b	0.134	(0.088-0.18)	<0.001	0.081	(0.038-0.125)	<0.001	0.054	(0.009-0.1)	0.02
	Fasting glucose (mg/dL)	0.202	(0.038-0.366)	0.017	0.072	(-0.082-0.227)	0.351	0.021	(-0.167-0.208)	0.823
	Insulin (uU/mL)	0.107	(0.077-0.136)	<0.001	0.065	(0.035-0.095)	<0.001	0.042	(0.005-0.078)	0.026
	HbA1C (%)	0.22	(-0.033-0.473)	0.087	0.13	(0.085-0.346)	0.229	0.109	(-0.142-0.36)	0.382
Total alkaline phosphatase	HOMA-IR	0.072	(0.048-0.096)	<0.001	0.022	(-0.002-0.045)	0.071	-0.017	(-0.047-0.013)	0.259
	HOMA-IS	-0.07	(-0.095--0.046)	<0.001	-0.02	(-0.044-0.004)	0.098	0.02	(-0.011-0.051)	0.2
	HOMA-b	0.092	(0.055-0.13)	<0.001	0.032	(-0.002-0.065)	0.065	-0.014	(-0.05-0.023)	0.457
	Fasting glucose (mg/dL)	0.089	(-0.05-0.228)	0.202	-0.031	(-0.166-0.103)	0.643	-0.077	(-0.242-0.089)	0.35
	Insulin (uU/mL)	0.07	(0.046-0.094)	<0.001	0.02	(-0.004-0.043)	0.095	-0.019	(-0.049-0.011)	0.199
	HbA1C (%)	0.122	(-0.058-0.302)	0.179	0.004	(-0.157-0.164)	0.965	0.008	(-0.169-0.185)	0.93

Model 1: Adjusted for age, race/ethnicity, education, smoking, drinking, physical activity, and waist circumference; Model 2: Further adjusted for AST, ALT, GGT, total cholesterol, triglycerides, HDL-cholesterol, mean arterial pressure, and CRP; Model 3: Further adjusted for fatty liver index and urinary N-terminal telopeptides, while waist circumference was removed from the model due to high correlation with fatty liver index.

* Urinary N-telopeptides data was only available in NHANES 1999-2002.

Table 3. Association between components of MetS and alkaline phosphatase (bone-specific or total) in NHANES 1999-2004

Components of MetS	Bone-specific alkaline phosphatase				Total alkaline phosphatase			
	Estimate	95% CI		P-value	Estimate	95% CI		P-value
		Lower limit	Upper limit			Lower limit	Upper limit	
Waist Circumferences (cm)	-0.122	-0.244	0.001	0.052	-0.049	-0.153	0.056	0.352
Mean Arterial Pressure (mm/Hg)	0.15	0.041	0.259	0.008	0.119	0.045	0.193	0.002
HDL-cholesterol (mg/dL)	-0.209	-0.272	-0.147	<0.001	-0.167	-0.217	-0.117	<0.001
Triglyceride (mg/dL)	-0.041	-0.086	0.003	0.069	-0.025	-0.062	0.013	0.191

Components of MetS simultaneously included in the model with further adjustment of age, race/ethnicity, education, smoking, drinking, physical activity, AST, ALT, GGT, total cholesterol, HOMA-IR, and CRP.

Figure 1. Pre-defined structural equation model outlining relationships between insulin resistance, components of MetS, and BAP.

Oval circle indicates latent variable that is not measured directly. Standardized regression coefficients are at the base of each arrow. *** $P \leq 0.001$. HDL level, inflammation, obesity, IR, hypertension, and BAP level were adjusted for age, gender, race/ethnicity, drinking, smoking, and physical activity in the structural equation model.

Figure
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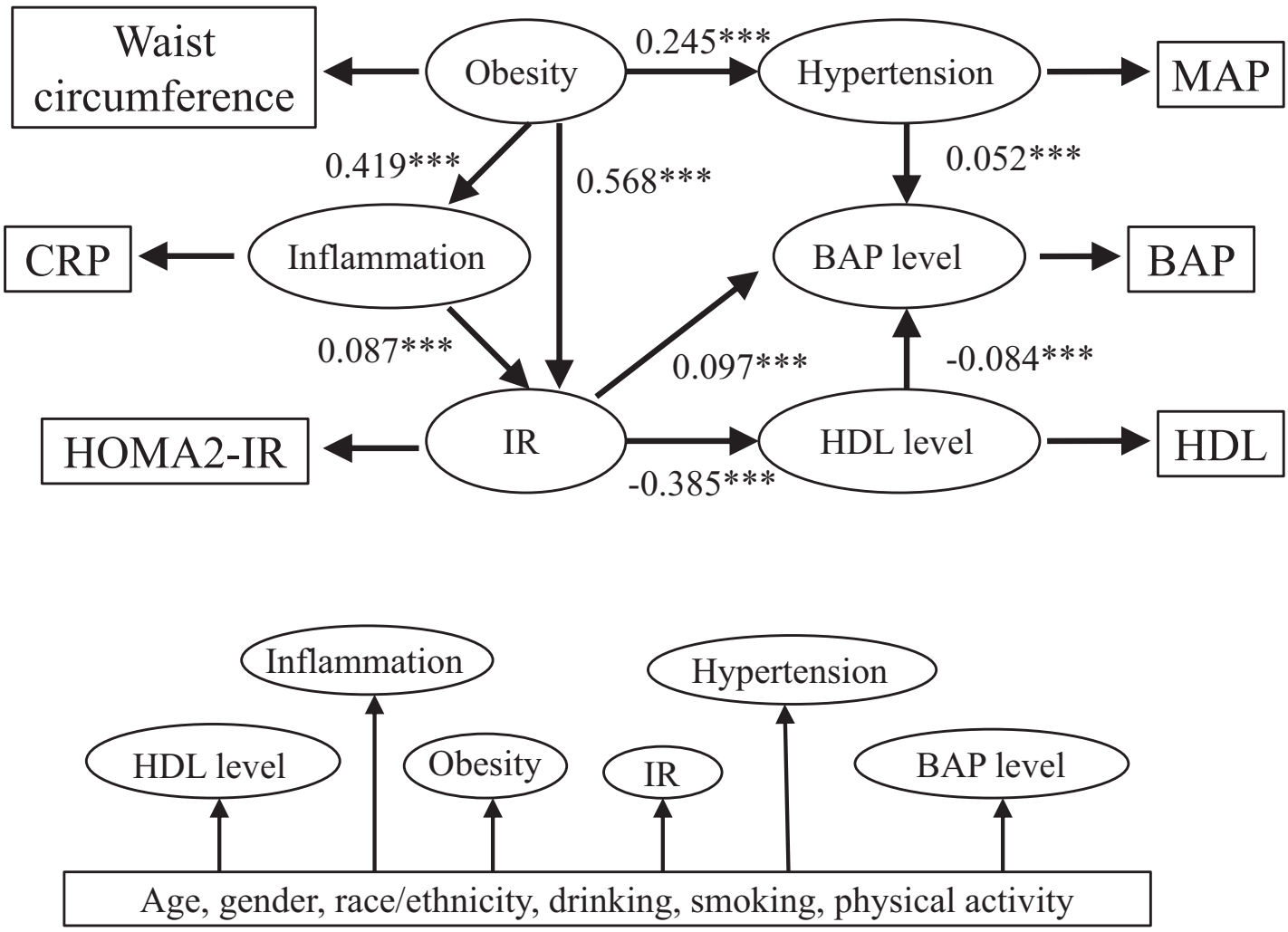

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