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Insulin Resistance is Associated With Total Bile Acid Level in Type 2 Diabetic and Nondiabetic Population

A Cross-Sectional Study

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Abstract: Bile acid metabolism was reported to be involved in glucose metabolism homeostasis. However, the exact relationship between bile acid and glucose metabolism as well as insulin sensitivity is not clarified. Therefore, we sought to investigate the association between insulin sensitivity and hyperbileacidemia in type 2 diabetic and nondiabetic population.

This community-based cross-sectional study included 9603 residents from Jiading, Shanghai, China, who were 40 years and older. Standardized questionnaire, anthropometric measurements and laboratory tests were conducted. Homeostasis model assessment of insulin resistance (HOMA-IR) $\geq$ 2.7 was defined as insulin resistance and fasting TBA $\geq$ 10 mmol/L was defined as hyperbileacidemia.

Multivariate stepwise regression analysis revealed that HOMA-IR, age, and male sex were positively associated with hyperbileacidemia in both nondiabetic and diabetic participants. In multivariate logistic models, participants with insulin resistance had significantly higher risk of hyperbileacidemia compared to those who have no insulin resistance, in both nondiabetic and diabetic population (nondiabetic: OR = 1.76; 95% CI 1.42–2.19; $P < 0.001$; diabetic: OR = 1.56; 95% CI 1.06 – 2.31; $P = 0.025$, respectively). Further adjustment for the HbA1c level in diabetic population did not change the significant association (OR = 1.59; 95% CI 1.06 – 2.40; $P = 0.024$). In nondiabetic participants, each 1-unit increment of HOMA-IR conferred an 18% higher risk of hyperbileacidemia (95% CI 1.04–1.35; $P = 0.013$), whereas in diabetic participants, this association was similar but not significant (95% CI 0.95–1.59; $P = 0.117$).

Insulin resistance was positively associated with hyperbileacidemia in both nondiabetic and diabetic population. The increase in the bile acid level in insulin-resistant population regardless of status of diabetes and glucose level indicated the important role of insulin resistance in the regulation of bile acid metabolism in human.

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Abbreviations: BMI = body mass index, CI = confidence interval, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HDL-c = high-density lipoprotein cholesterol, HOMA-IR = homoeostasis model assessment of insulin resistance, IGR = impaired glucose regulation, LDL-c = low-density lipoprotein cholesterol, OR = odds ratio, PPG = post-prandial glucose, SBP = systolic blood pressure, TC = total cholesterol, WC = waist circumference.

INTRODUCTION

Bile acid (BA) was demonstrated to exert variety of metabolic effects besides the known role in cholesterol homeostasis.[1] Increasing amount of studies have proposed that the bile acid (BA) was involved in glucose metabolism homeostasis.[1–4] Animal studies have denoted that glucose induces cholesterol bile acid synthesis via insulin signaling and epigenetic mechanisms.[5] Circulating insulin was reported to induce the CYP7A1 gene in diabetic mice, a gene mainly controlled BA synthesis in hepatocytes, which leads to higher level of circulating BA.[6–8] Meanwhile, BA could promote secretion of incretin in the digestive tract, which in turn improved glucose tolerance, promoted insulin release, and increased insulin sensitivity.[9–12]

It is important to note that to date most of these findings on the association between insulin sensitivity and bile acid have indeed been observed in mouse models.[13,14] Therefore, it is not well known whether alterations in BAs also occur in humans; inconsistent results were seen in the few human studies. In some studies, total bile acid (TBA) in serum or plasma was unchanged in type 2 diabetes (T2D) in comparison to controls.[13,15] Whereas in other study, BA were found increased in diabetic population.[15,16] Hence, given the unclear interaction between circulating insulin and BA in the progression of diabetes, it is important to understand the role of circulating insulin in BA in human. Therefore, the aim of our study was to investigate the association between insulin sensitivity and TBA concentrations

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in a Chinese population. Furthermore, we investigated this correlation in both diabetic and nondiabetic participants.

METHODS

Survey Design and Subjects

A population-based cross-sectional research randomly recruited 10,375 participants from Jiading district, Shanghai metropolitan area, China, between March 2010 and August 2010. All the participants were 40 years and older. Participants with missing data on glucose and insulin levels have been excluded (N = 18). Further exclusion occurred if participant took any hypoglycemic agents such as sulfonylurea, metformin, thiazide, diuretic, acarbose, or insulin (N = 754), as those agents could influence the exact relationship between bile acid and insulin sensitivity. Thus, the diabetic individuals included in the analysis were mostly newly diagnosed through our study. Eventually, a total of 9603 individuals (8463 nondiabetic and 1140 diabetic participants) were enrolled in the current analysis.

The study protocol was approved by the Institutional Review Board of the Rui-Jin Hospital affiliated to Shanghai Jiao-Tong University School of Medicine. Written informed consent was obtained from each participant before data collection.

Clinical Data Collection and Biochemistry Measurements

A standard questionnaire was administered to all participants for information collection on disease history, medication administration, and lifestyle risk factors.

All anthropometric measurements were performed by trained clinical staff adhered to standard protocols. Height and weight were recorded to the nearest 0.1 cm and 0.1 kg while participants were standing on the electronic weight device with light clothing and no shoes. BMI was then calculated as weight (kg) divided by squared height (kg/m^2). Waist circumference (WC) was measured to the nearest 0.1 cm at the umbilical level while participants were standing facing the staff with normal breathing. After 10 minutes of rest, blood pressure was measured in a sitting position 3 times with 1 minute interval. The averages of the triplicate readings of blood pressure were used for data analysis.

Glucose metabolism was measured using a simplified oral glucose tolerance test (OGTT) as described in our previous study.[17] Plasma glucose and insulin were measured by auto-analyzer Modular P800 (Roche, Basel, Switzerland) and Modular E170 (Roche), respectively. The insulin resistance index HOMA-IR was calculated as followed: HOMA-IR = fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5. Glycated hemoglobin (HbA1c) was measured by VARIANT II Hemoglobin Testing System (Bio-Rad Laboratories). Lipid profile including triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were examined by Modular E170 (Roche). Fasting total bile acid was examined using the enzyme cycle method by Bayer-1650 (German).

Definitions

In this study, diabetic participants were defined either with confirmed history of type 2 diabetes or in accordance with the 1999 World Health Organization (WHO) diagnostic criteria[18] (fasting plasma glucose [FPG] $\geq$ 7.0 mmol/L, and/or OGTT-2 h postload plasma glucose [PPG] $\geq$ 11.1 mmol/L). Impaired glucose tolerance (IGR) was defined as the FPG level between 6.0 mmol/L and 7.0 mmol/L, and/or OGTT-2 h PPG level between 7.8 mmol/L and 11.0 mmol/L. Dyslipidemia was defined as one or more of the following condition: hypercholesterolemia was defined as the TC level $\geq$ 6.22 mmol/L; high LDL-C level was $\geq$ 4.14 mmol/L; low HDL-C was $<$ 1.04 mmol/L; hypertriglyceridemia was defined as TG level $\geq$ 2.26 mmol/L.[19] General obesity was defined as a BMI of 30.0 or higher.[20] Central obesity was defined as waist circumference 90 cm or more in men and 80 cm or more in women.[21]

HOMA-IR >2.7 was defined as insulin resistance.[22] As diabetic population were all insulin resistant by definition, in diabetic population, HOMA-IR >2.7 was described as high insulin resistance, if <2.7, described as low insulin resistance.[23]

TBA between 0 and 10 μmol/L was considered normal level[24] and higher than 10 μmol/L was defined as hyperbileacidemia.[25]

Statistical Analysis

Skewed variables including TG and HOMA-IR were logarithmic transformed before analyses and presented as medians (interquartile range). Other normalized-distributed continuous variables were presented as mean ± standard deviation (SD). Categorical variables were expressed as numbers (proportions). Comparisons of proportions were performed with the χ^2 test.

Pearson's correlation and multiple stepwise linear regression were used to analyze the associations between influencing factors and TBA. The unadjusted (model 1) and multivariate adjusted logistic regression analyses were used to investigate association between TBA and insulin sensitivity. Model 1 included no covariates whereas model 2 included age, sex, BMI, hypertension, and hyperlipemia. Sex (male/female), hypertension status (yes/no), and dyslipidemia (yes/no) were used as categorical variables. All statistical tests were 2-sided, and P value < 0.05 was considered statistically significant. Statistical analysis was performed using SAS version 9.3 (SAS Institute Inc, Cary, NC).

RESULTS

Characteristics of the Study Population

The participants' characteristics were presented in Table 1. Among the total 9603 participants, 3632 (37.82%) were men. In nondiabetic population, the mean (±SD) age is 57.97 ± 9.64 years, the median of TBA is 4.3 μmol/L, and the median of HOMA-IR is 1.47. In the diabetic population, the mean age is 61.23 ± 9.71 years, the median of TBA level is 4.9 μmol/L, and the median of HOMA-IR is 2.78.

Both nondiabetic and diabetic populations were divided into 2 groups according to insulin sensitivity. Among nondiabetic population, those who had insulin resistance had significantly higher levels of BMI, waist circumference, SBP, DBP, TC, TG, LDL, FPG, PPG, proportion of IGR, and proportion of hyperbileacidemia than those who were not insulin resistant (all $P < 0.05$). Conversely, the level of HDL-c was higher in the noninsulin-resistant group ($P < 0.05$). There were no differences of age between the groups. Similar trend was also presented in diabetic patients except that there were no differences of TC or LDL-c level between the low-insulin-resistant and high-insulin-resistant counterparts.

Association Between Insulin Sensitivity and Total Bile Acid

The variables associated with TBA level demonstrated by Pearson's correlation and stepwise linear regression was shown

TABLE 1. Baseline Characteristics of the Study Population According to Insulin Sensitivity

	Fasting Insulin		
	Nondiabetic Population		
	HOMA-IR ≤2.7	**HOMA-IR>2.7**	***P***
Age, y	57.84 ± 9.71	57.97 ± 9.16	0.68
Male, N (%)	967 (51.0)	704 (36.0)	<0.001
BMI, kg/m^2	24.44 ± 2.96	27.48 ± 3.23	<0.001
Waist circumference, cm	80.69 ± 8.33	88.47 ± 8.46	<0.001
SBP, mm Hg	138.25 ± 19.60	145.53 ± 19.13	<0.001
DBP, mm Hg	81.85 ± 10.20	86.17 ± 10.03	<0.001
TC, mmol/L	5.28 ± 0.98	5.45 ± 1.03	<0.001
TG, mmol/L	1.25 (0.91–1.75)	1.80 (1.34–2.57)	<0.001
LDL-C, mmol/L	3.15 ± 0.84	3.27 ± 0.87	<0.001
HDL-C, mmol/L	1.36 ± 0.32	1.20 ± 0.27	<0.001
FPG, mmol/L	5.04 ± 0.53	5.46 ± 0.57	<0.001
PPG, mmol/L	6.51 ± 1.69	7.71 ± 1.75	<0.001
IGR, N (%)	1735 (23.96)	639 (52.33)	<0.001
Hyperbileacidemia, N (%)	617 (8.5)	161 (13.2)	<0.001
	Diabetic Population		
	HOMA-IR≤2.7	**HOMA-IR>2.7**	***P***
Age, y	61.96 ± 9.67	60.59 ± 9.47	0.02
Male, N (%)	254 (46.3)	224 (37.9)	0.004
BMI, kg/m^2	24.79 ± 3.07	27.85 ± 3.24	<0.001
Waist circumference, cm	82.89 ± 8.14	90.20 ± 8.43	<0.001
SBP, mm Hg	147.23 ± 19.08	151.05 ± 19.89	0.001
DBP, mm Hg	83.26 ± 10.31	86.59 ± 10.65	<0.001
TC, mmol/L	5.57 ± 1.17	5.62 ± 1.06	0.45
TG, mmol/L	1.51 (1.09–2.08)	1.98 (1.44–2.82)	<0.001
LDL-C, mmol/L	3.31 ± 0.91	3.35 ± 0.93	0.48
HDL-C, mmol/L	1.35 ± 0.35	1.20 ± 0.26	<0.001
FPG, mmol/L	6.52 ± 1.82	7.83 ± 2.58	<0.001
PPG, mmol/L	13.32 ± 4.07	15.54 ± 5.20	<0.001
Hyperbileacidemia, N (%)	73 (13.3)	104 (17.6)	0.045

Data were means ± SD or medians (interquartile range) for skewed variables, or proportions for categorical variables.

BMI = body mass index, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostasis model assessment of insulin resistance, LDL-C = low-density lipoprotein cholesterol, PPG = postprandial glucose, SBP = systolic blood pressure, TC = total cholesterol, TG = triglycerides, WC = waist circumference.

in Table 2. In nondiabetic population, Pearson's correlation indicated that HOMA-IR, age, male sex, WC, BMI, and hypertension were positively correlated with level of TBA. After performing multivariate stepwise linear regression analysis, HOMA-IR (β=0.045, $P < 0.001$), age (β=0.005, $P < 0.001$), and male sex (β=0.113, $P < 0.001$) were demonstrated to be positively associated with TBA (Table 2). Similarly in diabetic population, HOMA-IR, age, male sex, WC, BMI and hypertension showed positive significant correlation with TBA. Multivariate stepwise linear regression analysis demonstrated that HOMA-IR (β=0.105, $P < 0.001$), age (β=0.008, $P < 0.001$), and male sex (β=0.123, $P = 0.002$) were positively associated with TBA.

Multivariate-adjusted ORs of hyperbileacidemia with insulin resistance were presented in Table 3. Among nondiabetic participants, the unadjusted model showed that insulin resistance conferred a 63% higher risk of hyperbileacidemia compared to those without insulin resistance (OR = 1.63; 95% CI 1.36–1.96; $P < 0.0001$). After adjusting for age, sex, WC, BMI, hypertension, and dyslipidemia, this association remained significant (OR = 1.76; 95% CI 1.42–2.19; $P < 0.0001$). Each quartile increment of HOMA-IR conferred an 18% (95% CI 1.04–1.35; $P = 0.013$) higher risk of hyperbileacidemia (Table 3). In diabetic participants, those with insulin resistance also conferred an increased risk of hyperbileacidemia (OR = 1.39; 95% CI 1.01–1.93; $P = 0.046$). After adjusting for confounding factors, those with insulin resistance still yield a 56% higher risk of hyperbileacidemia compared to those without insulin resistance (95% CI 1.06–2.31; $P = 0.025$) (Table 3). Interestingly, further adjustment of HbA1c level in diabetic population did not affect this significant association (OR = 1.59; 95% CI 1.06–2.40; $P = 0.024$). Each quartile increment of HOMA-IR conferred a 23% higher risk, although it has not reached statistical significance (95% CI 0.95–1.59; $P = 0.117$).

TABLE 2. Pearson's Correlation and Multiple Stepwise Linear Regression Analysis of Risk Factors Associated With log (tba) Level

	Pearson's Correlation		Stepwise Linear Regression Model	
Nondiabetic population				
Variable	*r*	*P*	β ± SE	*P*
Log (HOMA-IR)	0.037	0.0006	0.045 ± 0.009	<0.001
Age, y	0.085	<0.001	0.005 ± 0.0007	<0.001
Sex (male = 1, female = 2)	−0.081	<0.001	−0.113 ± 0.014	<0.001
WC, cm	0.065	<0.001		
BMI, kg/m^2	0.025	0.020		
Hypertension (YES = 2, NO = 1)	0.042	0.0001		
Dyslipidemia (YES = 2, NO = 1)	−0.0001	0.99		
Diabetic population				
	r	***P***	**β ± SE**	***P***
Log (HOMA-IR)	0.111	0.0002	0.105 ± 0.024	<0.001
Age (years)	0.107	0.0003	0.008 ± 0.002	<0.001
Sex (male = 1, female = 2)	−0.075	0.012	−0.123 ± 0.040	0.002
WC, cm	0.125	<0.001		
BMI, kg/m^2	0.070	0.018		
Hypertension (YES = 2, NO = 1)	0.063	0.033		
Dyslipidemia (YES = 2, NO = 1)	0.032	0.278		

β = Regression coefficient; *r* = correlation coefficient. BMI = body mass index, HOMA-IR = homeostasis model assessment of insulin resistance, WC = waist circumference.

DISCUSSION

This study demonstrated that the total bile acid level was positively associated with insulin resistance in both nondiabetic and diabetic population aged 40 years or older. In the multiple stepwise regression analysis, insulin resistance was demonstrated to be positively associated with hyperbileacidemia in both groups. Furthermore, this association was demonstrated to be independent of status of diabetes, indicating the important role of insulin resistance in the regulation of bile acid metabolism in both diabetic and non-diabetic participants.

At present, the physiologic mechanism of relationship between insulin resistance and hyperbileacidemia has not been fully clarified. In rodents model, insulin signaling is known to inactivate the forkhead box transcription factor O1 (FoxO1),[26] the later were reported to inhibit cholesterol 7α-hydroxylase (CYP7A1), a key enzyme in bile acid synthesis, by blocking the HNF4 interaction with PGC-1[27] suggesting that insulin inhibit FoxO1 binding to the CYP7A1 gene promoter and results in induction of CYP7A1 gene expression and bile acid synthesis.[5] Other studies demonstrated that insulin would increase level of BA due to insulin resistance characteristic of diabetes through elevating the hydroxylase.[5,14,28] Furthermore, some bile salt hydrolase, which was reported to specifically degrade bile acid,[29] is inactive in the gut microbial environment of mice with obesity and diabetes.[30] In turn, BA could improve insulin sensitivity via reducing endoplasmic reticulum (ER) stress.[31] Insulin were also reported to induce the CYP7A1 gene and bile acid synthesis in primary human hepatocytes,[6–8] which were in line with our results in population research. The result of our study that the bile acid level increases in insulin-resistant population indicated the important role of insulin resistance in the regulation of bile acid metabolism in human.

Our result was also consistent with other human studies. It has been reported that bile acid pool and fecal bile acids are elevated in diabetic patients with uncontrolled hyperglycemia.[32] Haeusler et al[33] revealed that in their healthy subjects, insulin resistance was associated with increased BA (cholic acid, deoxycholic acid, and their conjugated forms). In T2DM patients, BA was revealed to be nearly 2-fold higher compared with healthy subjects. Interestingly the fasting insulin had no significant relationship with BA in 35 diabetic patients of their study. In our study, TBA was positively associated with insulin resistance. The possible explanation of this difference may be attributable to the different sample size and variability of study population. Meanwhile, in Haeusler's study, diabetic patients were taking hypoglycemic medications, which may affect insulin sensitization, whereas in our study, we excluded participants using hypoglycemic agents, leaving those with newly diagnosed diabetes in order to control the effect of hypoglycemic agents on this relationship. More importantly, our results were partially in accordance with Taylor's study[4] which demonstrated a positive correlation between urinary total bile acid excretion and HbA1c in diabetes patients. Moreover, in our study, the association between insulin resistance and TBA in diabetic population were not affected by the HbA1c level, indicating that the change of bile acid level in dysglycemic status largely due to insulin sensitivity rather than the glucose

TABLE 3. The Risk of Hyperbileacidemia According to Insulin Sensitivity in Nondiabetic and Diabetic Participants

	HOMA-IR≤2.7	HOMA-IR>2.7	1-Unit Increment of HOMA-IR
Nondiabetic population			
Model 1	ref	1.63 (1.36–1.96)	1.10 (1.00–1.22)
Model 2	ref	1.76 (1.42–2.19)	1.18 (1.04–1.35)
Diabetic population	HOMA-IR≤<2.7	HOMA-IR>2.7	1-unit increment of HOMA-IR
Model 1	ref	1.39 (1.01–1.93)	1.15 (0.94–1.41)
Model 2	ref	1.56 (1.06–2.31)	1.23 (0.95–1.59)

Model 1 unadjusted. Model 2 Adjusted for age, sex, general obesity, central obesity, hypertension, and dyslipidemia.

level. Therefore, our results, together with Taylor's, indicated that there might be a compensatory increase in bile acid signaling to maintain glucose homeostasis. Further studies were warranted to elucidate the exact role of insulin resistance in BA levels and more importantly.

The results of the Pearson's correlation showed the positive relationship between hypertension and bile acid level. So far, no studies have been found concerning the exact relationship between bile acid and hypertension. Previous studies showed that LDL-c can be reduced through increasing the fecal bile acid waste and by compensatory hepatic upregulation of bile acid synthesis to protect against atherosclerosis.[28,34] Thus, it could be deducted that the real connection between bile acid and hypertension may be mediated by the relationship between atherosclerosis and hypertension.

We also note several limitations in our study. First, limitation of such an association study resides in its correlative nature not allowing conclusion on causality. Prospective studies are needed to clarify their precise interrelationship. Second, the secondary BA types were not available in our study, the exact association between insulin sensitivity and each secondary BA type was not clearly known. Some researchers deduced that the effects of BA type changes may be dissociable from the effects of altering total BA levels, further studies to provide information of BA types should be conducted to clarify the detailed relationship. Third, antibodies of diabetes, including antibodies to insulin (IAA), glutamic acid decarboxylase (GAA or GAD) and protein tyrosine phosphatase (IA2 or ICA512), were not examined in our study. Thus, we could not clearly distinguish type 1 from type 2 diabetes; thus the association in T2DM patients might be underestimated, as Type 1 diabetes (T1DM) has been shown to be a disease characterized by immune-mediated destruction of the insulin-secreting cells of the pancreas. However, the prevalence of type 1 diabetes is very low worldwide; it comprises the majority of cases of diabetes seen in childhood and ~5% to 10% of all cases of diabetes mellitus in the USA.[35] Zhou et al reported that prevalence of latent autoimmune diabetes in adult (LADA) distinguished from type 2 diabetes in China is only 6.1% in men and 5.5% in women.[36] Furthermore, we excluded patients who used hypoglycemic agents; therefore less patients with type 1 diabetes could be included in the final analysis.

In conclusion, our study revealed that insulin resistance was significantly positively associated with hyperbileacidemia in middle-aged and elderly Chinese population, independent of status of diabetes and glucose levels. This study provides additional evidence to the correlation of TBA with insulin resistance in both diabetic and nondiabetic participants, indicating the important role of insulin resistance in regulation of bile acid metabolism. It warrants further experimental and longitudinal studies to determine the exact causal relationship between bile acid and insulin resistance.

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