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Insulin Resistance is Associated with Increased Concentrations of NT-pro-BNP in Rheumatoid Arthritis: IL-6 as a Potential Mediator

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Abstract

Objectives—To examine the hypothesis that insulin resistance (IR) decreases circulating concentrations of NT-pro brain natriuretic peptide (BNP).

Background—Obesity, despite being a risk factor for heart failure (HF), is paradoxically associated with lower concentrations of BNP, a marker of myocardial stress. Low BNP in obesity is postulated to be due to IR; however, it has been difficult to define the role of IR independent of obesity. IR in rheumatoid arthritis (RA) is increased, independent of obesity, thus allowing potential mechanistic insights into the relationship between IR and BNP.

Methods—We measured demographic factors, traditional cardiovascular risk factors, body mass index (BMI), markers of inflammation (interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor α (TNF α)), NT-pro-BNP, and IR by the homeostatic model assessment (HOMA) in 140 patients with RA and 82 control subjects. Patients with heart failure and coronary artery disease were excluded. We used multiple linear regression models to examine the relationship between HOMA and NT-pro-BNP in RA and controls, and in RA alone, the additional effect of inflammation.

Results—As previously reported, NT-pro-BNP concentrations were higher in RA (median 80.49 pg/mL, IQR (23.67–167.08 pg/mL) than controls (17.84 pg/mL (3.28–36.28 pg/mL)) ($P < 0.001$), and the prevalence of IR, defined by $\text{HOMA} > 2.114$, was higher among RA than controls (53% vs. 15%, $P > 0.001$). HOMA was positively correlated with NT-pro-BNP ($\rho = 0.226$, $P = 0.007$) in RA, but not in controls ($\rho = -0.154$, $P = 0.168$). In a multivariable model adjusted for age, race and sex, we found that increasing HOMA was statistically associated with increasing NT-pro-BNP concentrations in RA ($P = 0.001$), but not controls ($P = 0.543$) (P for interaction = 0.036). In RA subjects, when IL-6 was further included in the model, IL-6 ($P = 0.0014$), but not HOMA ($P = 0.43$), remained significantly associated with NT-pro-BNP, suggesting that IL-6 may be mechanistically involved in the relationship between IR and NT-pro-BNP in RA.

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Conclusions—We conclude that in patients with RA, insulin resistance is associated with higher, rather than the expected lower, concentrations of NT-pro-BNP and that this may be related to increased IL-6.

BACKGROUND

Cardiac B-type natriuretic peptides (BNP and NT-pro-BNP) are increased in heart failure (HF), and are widely used for diagnosis and prognosis determination (1,2). Even in populations without HF, higher BNP concentrations (although lower than those present in HF) provide information about future cardiovascular risk, including future risk of HF (3–5).

Obesity is an independent risk factor for HF (6,7), but is paradoxically associated with lower BNP levels in large population studies, an observation that has been termed “BNP deficiency” in obesity (8,9). The mechanisms underlying the lower concentrations of BNP associated with obesity are not clear, but insulin resistance (IR), which may itself contribute independently to increased cardiovascular risk (10–12), is thought to play a role (13). It is, however, difficult to determine if IR lowers BNP, because obesity and IR frequently co-exist and are mechanistically associated (14,15). A clinical condition in which IR occurs independent of obesity would provide insight into the relationship between IR and BNP concentrations.

Rheumatoid arthritis (RA) is a systemic inflammatory disease associated with increased cardiovascular disease and HF burden (16–19), increased concentrations of BNP (20), and frequent IR (21,22). The mechanisms underlying IR in RA differ from those in the general population, in that inflammation, as reflected by circulating IL6 and TNF α concentrations, in particular, is associated with IR, independent of obesity (21). Thus, we hypothesized that the relationship between BNP, IR, and inflammation in RA would allow us to define whether IR *per se* decreased concentrations of BNP, as is currently thought to be the case (13).

METHODS

Patients and Control Subjects

The study was approved by the Institutional Review Board (IRB) of Vanderbilt University Medical Center, and all subjects were provided written informed consent. Subjects were participants in an ongoing study of cardiovascular risk in RA, and detailed methods have been described previously (19,21). Consecutive eligible patients >18 years of age who met the American College of Rheumatology classification for RA (23) and control subjects without RA were studied. Patients were recruited from an RA registry, by referral from area rheumatologists, and by local advertisement. Control subjects were recruited from among the patient’s acquaintances, by local advertisement, and from a database of volunteers maintained by the Vanderbilt Clinical Research Center. Individuals with HF, defined as receiving treatment for a current diagnosis of HF, or fulfillment of at least 2 of three following criteria: a previous history of heart failure, use of digoxin, or use of a diuretic, were excluded from the study. Also, subjects who had a history of angina, myocardial

infarction, stroke, coronary artery stent placement, balloon angioplasty, or coronary artery bypass were excluded from this study.

Data Collection

Clinical and demographic information was acquired via structured interview, questionnaires, physical examination, laboratory tests, electron beam computed tomography (CT), and review of medical records. Current and historic medication use was determined from information provided by patients, and from review of medical records. Blood pressure was recorded as the average of 2 measurements obtained 5 minutes apart after the subject had rested in a supine position for at least 10 minutes. Subjects were considered hypertensive if they were taking antihypertensive agents, or if measured systolic blood pressure was greater than 140 mm Hg and/or diastolic pressure was greater than 90 mm Hg. RA disease activity was assessed using the Disease Activity Score based on evaluation of 28 joints (DAS28). The DAS28 is a validated composite index including a 28 joint count for tenderness and swelling, erythrocyte sedimentation rate (ESR), and the patient's overall assessment of wellbeing (24). Presence of metabolic syndrome was based on the National Cholesterol Education Program Adult Treatment Panel III definition (25).

Fasting blood samples were collected for determination of total cholesterol, high-density lipoprotein (HDL) and low-density (LDL) cholesterol, triglycerides, and homocysteine at the Vanderbilt University Medical Center laboratory. In patients with RA, CRP was measured by the hospital laboratory, except for 40 patients in whom CRP was measured by enzyme-linked immunosorbent assay (ELISA) (Millipore). TNF α , interleukin-6 (IL-6), insulin, and NT-pro-BNP concentrations were measured by multiplex ELISA (Linco Research/Millipore, St. Louis, MO). Insulin sensitivity was determined using the homeostasis model assessment (HOMA) index (fasting serum insulin (uU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5). Based on the Study of Inherited Risk of Coronary Atherosclerosis, a HOMA index of >2.114 was selected as the lower limit of the top quartile of a nondiabetic population, and thus indicative of IR (26).

Statistical Methods

Continuous variables are reported as median with interquartile range [IQR] and categorical variables as frequencies and percentages. To compare continuous values between RA patients and controls we used the Wilcoxon rank-sum test and to compare categorical variables the chi-square test. For preliminary assessment of the relationship between NT-pro-BNP, HOMA, and BMI we performed Spearman rank correlation analyses separately in RA patients and controls.

To assess whether the relationship between HOMA and NT-pro-BNP (dependent variable) was modified by disease status, we used multiple linear regression models including the cross-product term of disease status (RA or control) and HOMA while adjusting for age, sex, and race. We also performed similar regression analysis to test whether there was an association with BMI and NT-pro-BNP and effect modification by disease status while controlling for age, race and sex. Lastly, to account for hypertension, we performed a sensitivity analysis by adjusting the model for the presence of hypertension and systolic

blood pressure and by evaluating the significance of the adjusted model with hypertensive patients excluded.

Among patients with RA, to evaluate the role of inflammatory markers as potential mediators of the relationship between HOMA-IR and NT-pro-BNP; we additionally adjusted the model described previously for IL-6, TNF- α , and CRP, separately. Mediation effect was evaluated by degree of attenuation of the HOMA-IR effect by further adjusting for the inflammatory markers, and statistical significance for the mediation effect was carried out by formally testing for the indirect effect or average causal mediation effect, using bootstrapping techniques (27,28).

All calculations were performed using R version 3.01.0 (<http://www.r-project.org>) and two-sided P values less than 0.05 were considered statistically significant.

RESULTS

Study Subject Characteristics

Baseline characteristics of the control subjects (n=82) and RA patients (n=140) meeting criteria for the study, and who had HOMA and NT-pro-BNP measurements, are shown in Table 1. The two groups were of similar age, race and sex distribution. As previously reported, NT-proBNP levels, the prevalence of IR (53% RA vs. 15% controls), and markers of inflammation were higher in patients with RA compared to controls (all P values<0.001) (Table 1) (19,21).

Relationship between HOMA and NT-pro-BNP in RA and controls

In patients with RA, HOMA was significantly positively correlated with NT-pro-BNP ($\rho=0.226$, $P=0.007$), but not in control subjects ($\rho=-0.154$, $P=0.168$) (Figure 1). After adjusting for age, race and sex; HOMA remained significantly associated with NT-pro-BNP ($P=0.001$) in patients with RA, but there was no statistically significant association in controls (HOMA and NT-pro-BNP, $P=0.543$) (Figure 1). To assess whether the relationship between HOMA and NT-pro-BNP was modified by disease status (i.e., having RA), we used a multivariable regression analysis that included disease status (RA vs. control) and HOMA interaction while adjusting for age, race, and sex. The association between HOMA and NT-pro-BNP was significantly differential by disease status (P for interaction = 0.036).

In RA, NT-pro-BNP levels were highest in the insulin resistant RA patients (105.5 pg/mL [42.9–248.0 pg/mL]) compared to those who were insulin sensitive (49.1 pg/mL [14.9–120.4 pg/mL] ($P=0.006$)). BMI was not significantly correlated with NT-proBNP in RA patients ($\rho=-0.118$, $P=0.164$) or control subjects ($\rho=-0.07$, $P=0.524$) (Figure 2).

Role of inflammation in the association between HOMA and NT-pro-BNP in RA

Inflammatory markers including IL-6 ($\rho=0.59$, $P<0.001$), TNF α ($\rho=0.53$, $P<0.001$), and CRP ($\rho=0.28$, $P<0.001$) were strongly associated with insulin resistance in RA. Thus, we performed multivariable regression analyses in patients with RA to assess if the association between HOMA and NT-pro-BNP in RA might be due to the associated inflammation.

We assessed the positive association between HOMA and NT-pro-BNP in RA (for a log HOMA IQR difference of 2.68 units, $\beta=0.42$, 95% CI 0.17,0.68, $P=0.001$, adjusted for age, race and sex) for three potential mediating inflammation factors in separate models. We found that the association of HOMA and NT-pro-BNP was sustained, ($\beta=0.39$, 95% CI 0.08, 0.70, $P=0.015$; $\beta=0.36$, 95% CI 0.10, 0.62, $P=0.008$) in each model that included TNF α and CRP. In mediation assessment, there were no statistically significant indirect effects, indicating no mediation by TNF α or CRP ($P=0.63$, $P=0.07$). However, in the third model with IL-6, the association was no longer statistically significant (HOMA and NT-pro-BNP, $\beta=0.12$, 95% CI -0.18, 0.43, $P=0.430$) with a statistically significant indirect effect (0.264, 95% bias corrected interval: 0.121, 0.398, $P<0.01$) (Figure 3), suggesting potential mediation by IL-6.

In sensitivity analyses that further adjusted for hypertension and systolic blood pressure (or excluded subjects with hypertension and adjusted for systolic blood pressure) the results did not differ materially.

DISCUSSION

The major new finding of the study is that elevated circulating concentrations of NT-pro-BNP are associated with increasing insulin resistance in patients with RA. This is in contrast to the established pattern of lower BNP concentrations associated with IR and obesity in non-RA patients (7,8,9). Furthermore, just as IL-6 appears to be important in the pathogenesis of IR in RA, so too it appears to contribute to the relationship between IR and increased NT-pro-BNP concentrations in RA.

The majority of BNP is produced as a result of gene activation in response to myocardial wall stress and volume overload (29). The secreted pro-peptide undergoes cleavage to both biologically active and inactive remnants. Plasma measurements of either BNP or NT-pro-BNP have robust diagnostic efficacy in HF, and are predictive of cardiovascular outcomes (1,2,5). Levels of NT-pro-BNP below those that suggest HF, but within the range observed in this study, are predictive of future cardiovascular risk in subjects without HF, in both the general population and in RA (5,16,17,18,30).

The relationship between BNP, HF risk, and obesity presents a poorly understood paradox. Obesity is associated with an increased risk of heart failure (6,7), yet in large population studies, BNP levels are paradoxically lower in obese, compared to non-obese individuals (8,9). The underlying mechanism is not clear, but several mechanisms have been postulated to account for the lower concentrations of BNP observed in obesity; the most prominent are the influence of IR, hyperinsulinemia, and altered BNP clearance. BNP is cleared, in part, by binding to receptors on adipose tissue, and thus obesity has been suggested to increase clearance of BNP (5,6,7,8,9). In contrast, the N-terminal fragment, NT-proBNP, has no peripheral clearance receptors, and is cleared passively by organs with high blood flow (muscle, liver, kidney) (31,32). Like BNP, however, NT-pro-BNP is also lower in obesity, (5,8,9), suggesting a mechanism other than increased clearance by adipose tissue.

Several lines of evidence have suggested that IR may mediate lower BNP concentrations. For example, in the Framingham Heart Study, the inverse relationship between NT-pro-BNP and abdominal adipose tissue mass was stronger for visceral, compared to subcutaneous, adiposity (33). Furthermore, lower BNP concentrations have been associated with the metabolic syndrome and its components, even after statistical adjustment for obesity (9,34,35). In two large epidemiological studies, the Framingham Heart Study and the Malmö Diet and Cancer Study, the statistical relationship between obesity and lower NT-pro-BNP concentrations was markedly attenuated by adjustment for HOMA-IR; a finding the authors interpreted as suggestive that the reduction in BNP, associated with obesity, was largely mediated by IR (9,13). Insulin may itself be important since IR is associated with higher concentrations of insulin, and acute elevation of insulin concentrations decreased natriuretic peptide concentrations in some (36), but not all (37), studies.

Our findings inform the debate about the role of IR in mediating low BNP concentrations. We, and others, have previously shown that NT-pro-BNP concentrations are higher, and IR more frequent in RA, compared to control subjects (19,20,21). We used these observations to further define the relationship between IR and NT-proBNP concentrations. We did not find a strong association between obesity and NT-pro-BNP concentrations, likely because of the relatively small sample size (compared to larger epidemiologic studies). However, we found that IR was significantly associated with higher, rather than lower, concentrations of NT-proBNP in RA. If the lower BNP levels associated with obesity in other populations are attributable to increased IR, as has been suggested (9,13), this mechanism was either not functional in RA or was not sufficient to overcome the effect of inflammation on NT-pro-BNP.

Furthermore, our finding that the relationship between IR and higher NT-proBNP levels in RA was attenuated when we adjusted for IL-6 concentrations, suggest that IL-6 may be mechanistically involved. In keeping with this idea, IL-6 stimulated production of BNP in cardiac cells *ex vivo* (38). If similar processes occur *in vivo*, elevated IL-6 in RA may be a mechanism for myocardial BNP production. However, an argument against this interpretation is that IL-6 concentrations are higher in obesity, reflecting a condition of chronic, low level, inflammation (39,40). If IL-6 drives increased BNP production, then BNP levels would be expected to be higher, rather than lower, in obesity. We have previously shown that in patients with RA, IR is associated with increased IL-6 concentrations, independent of BMI. Therefore, RA may represent a situation in which IL-6, and perhaps other mediators of inflammation, increase both IR and BNP in an independent fashion.

Several limitations of this study should be considered. The cross-sectional cohort design allowed us to determine an association, but not a causal relationship, between IR, IL-6, and NT-pro-BNP levels in RA. Additionally, in order to limit the influence of cardiac hemodynamic factors on NT-pro-BNP levels, a history of significant cardiac disease was a specific exclusion criterion. Also, we did not perform cardiac imaging and therefore we cannot exclude the presence of undiagnosed cardiac structural abnormalities or left ventricular hypertrophy in the subjects studied. A sensitivity analysis, however, correcting for systolic blood pressure and excluding patients with hypertension, revealed that the

relationship between IR and NT-proBNP in RA patients remained significant with these adjustments, and did not change our conclusion.

We conclude that in patients with RA, insulin resistance is associated with higher, rather than the expected lower, concentrations of NT-pro-BNP and that this may be related to increased IL-6 concentrations.

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Figure 1. Non-linear smooth regression is used to illustrate the relationships between HOMA-IR and NT-pro-BNP in patients with RA patients and controls

Analysis using a multiple linear regression model that adjusted for age, race, and sex, showed that HOMA-IR was positively associated with NT-pro-BNP in patients with RA ($P=0.0014$), but not in control subjects ($P=0.543$), (P for interaction= 0.036).

Figure 2. Non-linear smooth regression is used to illustrate the relationships between BMI and NT-pro-BNP in patients with RA and controls

Analysis using a multivariable regression model that adjusted for age, race, and sex, showed that there was no statistically significant association between BMI and NT-pro-BNP in patients with RA or controls (both P values >0.2) (P for interaction=0.19).

Figure 3. Multivariable regression analyses examining the effect of inflammatory mediators on the relationship between HOMA and NT-pro-BNP in patients with RA

The relationship between HOMA and NT-pro-BNP, adjusted for age, race and sex in RA patients ($P=0.0012$) remained statistically significant after additional adjustment TNF α ($P=0.015$) and CRP ($P=0.008$). However, adjustment for IL-6 rendered the association between HOMA and NT-pro-BNP non-significant ($P=0.43$).

Table 1
Characteristics of Study Subjects

Parameter	Controls (n=82)	RA (n=140)	P Value [*]
Age (yr)	52	52	0.608
Sex (F/M)	67%/33%	73%/27%	0.361 ²
Caucasian (%)	83%	88%	0.306 ²
BMI (kg/m ²)	27.0	28.44	0.236
Hypertensive	30 (37%)	68 (49%)	
Systolic BP (mm Hg)	128.5 (113.8–136.4)	131.8 (115.5–144.1)	0.229
Diastolic BP (mm Hg)	72.25 (67.5–77.75)	73.0 (66.5–81.0)	0.345
Diabetes (%)	5%	9%	0.585
Metabolic Syndrome (%)	19%	36%	0.012
Insulin (mU/mL)	3.77	10.59	<0.0001
HOMA IR	0.826 (0.55–1.76)	2.358 (1.18–3.87)	<0.001
Insulin Resistant (HOMA>2.114)	13 (15%)	74 (53%)	
Obese (BMI>30 kg/m ²)	26 (32%)	60(43%)	
Cholesterol (mg/dL)	195 (171.0–216.0)	185 (158.8–210.2)	0.092
HDL(mg/dL)	45.0 (39.0–54.0)	43.5 (37.0–54.2)	0.538
LDL(mg/dL)	122.5 (104.2–145.0)	111.0 (89.0–134.2)	0.014
Triglycerides (mg/dL)	102.5 (72.5–130.8)	110.5 (79.8–152.8)	0.227
TNFα (pg/mL)	3.28 (2.36–4.62)	5.48 (2.63–11.02)	<0.001
CRP (mg/L)	0.55 (0.21–1.53)	4.0 (1.19–11.0)	<0.001
IL-6 (pg/mL)	4.25 (1.18–18.46)	13.94 (4.36–40.81)	<0.001
NT-proBNP (pg/mL)	17.84 (3.28–36.28)	80.49 (23.67–167.08)	<0.001

Continuous variables are presented as median and interquartile ranges (IQR). Categorical variables are presented as frequencies and percentages.

^{*} P values are for Wilcoxon rank sum test for continuous variables and Chi-squared test for categorical variables.