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Authors

Sanchez, Otto A
Lazo-Elizondo, Mariana
Zeb, Irfan
et al.

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Computerized tomography measured liver fat is associated with low levels of N-terminal pro-brain natriuretic protein (NT-proBNP). Multi-Ethnic Study of Atherosclerosis

Otto A. Sanchez[a,*], Mariana Lazo-Elizondo[b,c], Irfan Zeb[d], Russell P. Tracy[e], Ryan Bradley[f], Daniel A. Duprez[g], Hossein Bahrami[h], Carmen A. Peralta[i], Lori B. Daniels[j], João A. Lima[k], Alan Maisel[l], David R. Jacobs Jr.[m], Mathew J. Budoff[n]

[a] Department of Internal Medicine, Division of Renal Diseases and Hypertension, University of Minnesota, 717 Delaware, Mail Code 1932, Minneapolis, MN 55414

[b] Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD

[c] Department of Epidemiology and the Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[d] Department of Cardiology, Mount Sinai St. Luke's Roosevelt Hospital (Bronx-Lebanon Hospital Center)

[e] University of Vermont College of Medicine, Burlington, VT 05405

[f] National College of Natural Medicine, SW Porter Street, Portland, OR 97201

[g] Division of Cardiology, University of Minnesota, 420 Delaware St. SE. Mayo Mail Code 508, Minneapolis, MN 55455

[h] Stanford Cardiovascular Institute, Stanford University

[i] School of Medicine, University of California San Francisco, 33 Parnassus Ave, UC Hall, San Francisco, CA 94143

[j] Division of Cardiology, University of California, San Diego, Sulpizio Cardiovascular Center, 9434 Medical Center Drive, La Jolla, CA 92037

[k] Division of Cardiology, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, MD 21224

[l] School of Medicine, University of California, 3350 La Jolla Village Drive, Cardiology Section, mc 9111A, San Diego, CA 92161

[m] School of Public Health, Division of Epidemiology & Community Health, University of Minnesota, Minneapolis, MN

[n] Los Angeles Biomedical Research Institute, Division of Cardiology, Harbor-UCLA Medical Center, Los Angeles, CA

Abbreviations: NT-proBNP, N-terminal pro B-type natriuretic peptide; MESA, Multi-Ethnic Study of Atherosclerosis; NAFLD, nonalcoholic fatty liver disease; RP, relative prevalence; IL-6, interleukin-6; IP, inflection point; HU, Hounsfield units; GGT, gamma-glutamyl transferase; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; ATP III, Adult Treatment Panel III; CAC, coronary artery calcium; ICC, intraclass correlation coefficient; CVD, cardiovascular disease; PGC1A, peroxisome proliferator-activated receptor γ coactivator-1 α; NPR-A, B and C, natriuretic peptide receptor-A, B and C; COPD, chronic obstructive pulmonary disease.

* *Corresponding author at:* Division of Renal Disease and Hypertension. Department of Internal Medicine, University of Minnesota, 717 Delaware, Mail Code 1932, Minneapolis, MN 55414. Tel.: +1 612 626 2119 (Office); fax: +1 612 626 3840.

E-mail addresses: sanc0050@umn.edu (O.A. Sanchez), mlazo@jhu.edu (M. Lazo-Elizondo), izeb82@gmail.com (I. Zeb), russell.tracy@uvm.edu (R.P. Tracy), rbradley@ncnm.edu (R. Bradley), dupre007@umn.edu (D.A. Duprez), hbahrami@stanford.edu (H. Bahrami), CarmenAlicia.Peralta@ucsf.edu (C.A. Peralta), lbdaniels@ucsd.edu (L.B. Daniels), jlima@jhmi.edu (J.A. Lima), amaisel@ucsd.edu (A. Maisel), jacob004@umn.edu (D.R. Jacobs), mbudoff@labiomed.org (M.J. Budoff).

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ABSTRACT

Background and Aims. N-terminal pro B-type natriuretic peptide (NT-proBNP) is inversely associated with diabetes mellitus, obesity and metabolic syndrome. We aim to characterize the association between NT-proBNP and nonalcoholic fatty liver disease (NAFLD), a condition strongly associated with metabolic syndrome.

Methods. 4529 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) free of cardiovascular disease, without self-reported liver disease and not diabetic at their baseline visit in 2000–2002 were included in this analysis. NAFLD was defined by a liver attenuation <40 HU. Relative prevalence (RP) for NAFLD was assessed adjusted for age, race, and sex, percentage of dietary calories derived from fat, total intentional exercise, alcoholic drinks per week, and interleukin-6 by quintiles of NT-proBNP. Adjusted linear spline model was used to characterize a non-linear association between NT-proBNP and liver fat. The inflection point (IP) was the NT-proBNP concentration where there was a change in slope in the association between liver attenuation and NT-proBNP.

Results. RP for NAFLD decreased by 30% from the lowest to the highest quintile of NT-proBNP, $p = 0.01$. We observed an inverse linear association between NT-proBNP and liver fat, which plateaued (IP) at an NT-proBNP concentration of 45 pg/mL. Linear regression coefficient (SE) per unit of NT-proBNP less than and greater than or equal to IP was of 0.05 (0.02), $p = 0.001$ and 0.0006 (0.0008), $p = 0.5$, respectively; differences between slopes, $p < 0.0001$.

Conclusions. In this cross-sectional study of a community based multiethnic sample of non-diabetic adults, low levels of NT-proBNP are associated with greater prevalence of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as the accumulation of fat in the hepatic parenchyma (steatosis) with or without inflammation [1], is considered the hepatic component of the metabolic syndrome [2]. Not surprisingly, obesity, insulin resistance, metabolic syndrome and type 2 diabetes are associated cross-sectionally and prospectively with NAFLD [3,4]. Therefore, the accumulation of fat in the liver, the development of insulin resistance, and type 2 diabetes may share common risk factors.

Natriuretic peptides are inversely associated with percent body fat, fasting blood glucose and triglycerides [5–7]. Furthermore, natriuretic peptides have been shown to be inversely associated with incident diabetes [8,9]. These observations may stem from the metabolic effects of natriuretic peptides on lipoproteins [10], lipolysis, mitochondrial density and fat oxidation [11]. Given the common pathophysiological mechanisms of NAFLD with diabetes and other metabolic disorders, it is possible that natriuretic peptides have an effect on liver fat. In support of this assumption, Lazo et al. using liver enzymes as surrogate markers of liver fat demonstrated a U-shaped association between gamma-glutamyl transferase (GGT), and N-terminal pro B-type natriuretic peptide (NT-proBNP) [12].

The Multi-Ethnic Study of Atherosclerosis (MESA) measured baseline levels of NT-proBNP and estimated liver fat using computed tomographic imaging in a group of individuals free of cardiovascular disease. This allows for a quantitative assessment of the association between circulatory levels of NT-proBNP and the amount of liver fat.

The objective of this study is therefore to characterize the association between NT-proBNP and liver fat, as assessed by computed tomography, in a racially diverse group, without existing cardiovascular disease. We hypothesize that higher levels of NT-proBNP, but still within the "physiological range" are associated with less liver fat.

2. Methods

2.1. Study Subjects

This study included 6814 men and women MESA study participants of diverse ethnic and racial background (white, Africa-American, Chinese and Hispanics). They were recruited between July 2000 and August 2002, and were between 45 and 85 years of age and free of overt cardiovascular disease. Detailed description on the aims of the MESA study and the characteristics of this cohort is described in Refs [13,14]. The institutional review boards at all participating centers approved the study and written informed consent was obtained from every participant prior to data collection. For this cross sectional analysis data were restricted to 4529 MESA participants without self-reported liver disease, without diabetes at baseline and in whom NT-proBNP, gamma-glutamyl transpeptidase (GGT) and liver attenuation in Hounsfield units (HU) were measured at their baseline visit in 2000–2002.

2.2. Baseline Demographic, Anthropometric and Metabolic Characteristics

Among the anthropometric, cardiovascular and metabolic parameters that were included are: body mass index (BMI)

(computed as weight (kg)/height^2 (meters)), systolic and diastolic blood pressures; blood lipids, insulin, and glucose. Dietary energy and fat are derived by assigning tabulated weights to each food item in the food frequency questionnaire and summing [15]. Serum NT-proBNP was measured at the VA San Diego Healthcare System using an ElecSys 2010 analyzer (Roche Diagnostics, Indianapolis, IN) with intra-assay and interassay coefficients of variation of 1.3 and 4.8%, respectively [16]. We assessed insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as insulin (mU/L) × (glucose [mg/dL] × 0.055)/22.5 [17]. In addition, IL-6 concentration was used to adjust for inflammatory status and was measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS human IL-6 immuno-assay; R&D Systems, Minneapolis, MN).

2.2.1. *Diagnosis of Hypertension, Metabolic Syndrome, Diabetes, Low Estimated Glomerular Filtration Rate (eGFR) and Subclinical Cardiovascular Disease*

Hypertension was defined as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or the use of blood pressure lowering medications. Metabolic syndrome was defined according to the Adult Treatment Panel III report (ATP III) [18]. For Chinese individuals the definition of metabolic syndrome included those with waist circumference >90 cm and >80 cm for males and females, respectively. Diabetes was defined as self-reported physician diagnosis, fasting glucose ≥126 mg/dL or the use of insulin or oral hypoglycemic medications each assessed at study clinic examinations. Low eGFR was defined as a eGFR <60 mL/min/1.73 m^2 according to the EPI-CKD equation [19]. Subclinical cardiovascular disease was defined by the presence of at least two of the following: carotid plaque, left ventricular hypertrophy and a positive coronary artery calcium (CAC) score.

2.2.2. *Measurement of Liver Fat and Liver Enzyme*

Liver fat was estimated based on radiologic liver attenuation (Hounsfield units (HU)) using computerized tomographic imaging of the liver and the spleen. Liver attenuation is inversely associated with liver fat content, thus lower attenuation coefficients indicate greater fat content. A liver/spleen (L/S) attenuation ratio <0.8 [20] and a liver attenuation <42 HU are able to detect macrovesicular liver steatosis >30% with 100% specificity and sensitivity ranging from 73% and 82% [20]. Previous reports using data from the MESA study have used liver attenuation <40 HU and L/S ratio <1 to define NAFLD [4,21,22]. An L/S ratio <1 corresponds to mild steatosis (<9.9% fatty liver content) and a liver attenuation of <40 HU corresponds to a moderate to severe steatosis (10% to >25% fatty liver content) [23,24]. The methods used to acquire this information from the liver and spleen CT has been described in detail previously [4,25]. The interreader and intrareader intra-class correlation coefficients (ICC) for liver attenuation measurement were 0.96 and 0.99, respectively and the interreader and intrareader ICC for L/S was 0.99, for both measures [4]. GGT, another potential surrogate of fatty liver, was measured using methods previously described [26].

2.3. *Statistical Analysis*

2.3.1. *Descriptive Statistics*

Gender, race/ethnicity, sex, years of education, anthropometric, and metabolic parameters were used to characterize the sample at baseline by categories of liver attenuation <40 and ≥40 HU. Continuous variables with normal distributions are presented as mean ± SD and categorical variables as percentages. Variables with skewed distributions are log transformed and the mean log transformed value was exponentiated to obtain the geometric mean.

2.3.2. *Assessing the Relative Prevalence of NAFLD*

Due to the cross sectional nature of this analysis, the ratio of the prevalence of NAFLD in those exposed/prevalence of NAFLD in those not exposed is expressed as relative prevalence (RP) instead of relative risk. The RP of NAFLD (defined as an attenuation coefficient <40 HU or liver to spleen ratio <1) was assessed by quintiles of NT-proBNP adjusted for model 1 = age, race and sex; and model 2 = model 1 + percentage of dietary calories derived from fat, number of alcoholic drinks per week, total intentional exercise and IL-6. Model 3 included model 2 + metabolic syndrome, which may be on the causal pathway between NT-proBNP and the development of NAFLD. Further adjusting model 2 for BMI and waist circumference was performed to assess if the association between NAFLD and NT-proBNP was attenuated by BMI. NT-proBNP values differ according to sex [27] and presence of subclinical cardiovascular disease (CVD) [5,28]. For these reasons, a sex × NT-proBNP and also a subclinical CVD × NT-proBNP interaction term was assessed in model 2.

Sensitivity analyses were conducted excluding: 1) 422 heavier drinkers (i.e., males consuming >14 drinks/week and women consuming >7 drinks/week) [29], 2) 55 individuals with liver attenuations ≥80 HU (99th percentile) and 3) presence or absence of subclinical CVD.

2.3.3. *Associations Between Liver Attenuation and NT-proBNP*

The association between categories of baseline NT-proBNP as an independent variable and liver attenuation (HU) as a dependent variable was assessed using linear regression procedures adjusting for the models 1, 2 and 3 as described above (Fig. 2). The NT-proBNP values at which the slopes of the dependent variables had a substantial change, i.e., the inflection point, were determined using linear splines adjusted for model 2 with serial knots at NT-proBNP values every 5 pg/mL for intervals between 20 and 300 pg/mL. The NT-proBNP concentration at which the linear spline model had the highest R^2 was chosen as the inflection point [5]. Significance was set at $p < 0.05$. Statistical analysis was performed using SAS v 9.3 by SAS Institute, Cary, NC.

3. Results

3.1. *General Characteristics*

The mean (SD) liver attenuation of non-diabetic individuals without self-reported liver disease was 60.2 (11.6) HU with an

interquartile range from 55.5 to 67.0 HU. As shown in Table 1, compared to those without fatty liver, individuals with fatty liver were on average 3 years younger and had greater BMI, HOMA-IR, systolic and diastolic blood pressures; greater levels of blood triglycerides, fasting blood glucose, IL-6 values; and a higher percentage of dietary calories derived from fat; a greater percentage of them were heavier, alcohol drinkers, and had higher circulatory GGT levels. The prevalence of NAFLD was greater among white/Caucasians and Hispanics than among Chinese and African Americans. In addition, individuals with fatty liver were also more likely to have metabolic syndrome, have lower plasma NT-proBNP concentration, total intentional exercise per week and lower L/S ratio. There was no difference in the prevalence low eGFR (eGFR <60) between those with HU <40 or ≥40.

Table 1 – Demographic and metabolic characteristics and presence of subclinical disease by categories of liver attenuation coefficient <40 or ≥40 HU at baseline in individuals without diabetes and without self-reported liver disease.

N (%)	≥40 HU	<40 HU	
	4281 (94.5)	248 (5.5)	p value
Sex, % females	52.9	49.6	0.3
Age, years	62.7 (0.2)	59.1 (0.7)	<0.0001
Race			<0.0001
White	42.7	37.5	
Chinese	12.2	10.9	
African American	24.2	12.5	
Hispanics	20.9	39.1	
Education, %	83.2	77.8	0.02
Current smoker, %	12.2	12.1	1.0
HTN, %	44.4	48.8	0.2
Low eGFR, %	9.7	7.3	0.2
BMI, kg/m^2	27.8 (0.1)	31.9 (0.3)	<0.0001
Systolic blood pressure, mmHg	131.3 (0.3)	136.9 (1.4)	<0.0001
Diastolic blood pressure, mmHg	71.7 (0.1)	74.2 (0.6)	<0.0001
HDL-C, mg/dL	51.8 (0.2)	46.2 (0.9)	<0.0001
Triglycerides, mg/dL	126.5 (1.2)	170.3 (4.9)	<0.0001
Glucose, mg/dL	89.3 (0.2)	96.3 (0.6)	<0.0001
HOMA-IR, (mU/L * mmol/dL/22.5)	2.06 (0.02)	3.72 (0.08)	<0.0001
Interleukin-6, pg/mL	1.49 (0.02)	2.01 (0.08)	<0.0001
Percent calories from saturated fat, %	10.1 (0.05)	10.6 (0.2)	0.02
NT-proBNP, pg/mL	51.3 (49.9–52.8)	39.8 (35.4–44.9)	<0.0001
NT-proBNP >100 pg/mL, %	29.1	17.3	<0.0001
Heavy drinkers, %	9.2	11.3	0.3
Total intentional exercise, MET/min/week	1228 (1186–1272)	1031 (884–1202)	0.03
GGT, U/L	39.2 (0.5)	54.4 (1.9)	<0.0001
Liver/spleen	1.23 (0.004)	0.66 (0.018)	<0.0001
Metabolic syndrome, %	29.2	64.0	<0.0001
Subclinical CVD, %	26.8	28.8	0.6

Categorical variables are expressed as % and continuous variables are expressed as means (SE), except for NT-proBNP, which is the geometric mean (95% CI). Continuous variables were adjusted for age, race and gender, except when age was the dependent variable. HU = Hounsfield units. Education refers to the percentage of individuals who completed at least high school. HTN = individuals with hypertension. Low estimated glomerular filtration rate (eGFR) = eGFR, 60 mL/kg/min based on the CKD-EPI equation. BMI = body mass index kg/m^2. HDL-C = high density lipoprotein-cholesterol. GGT = gamma-glutamyl transpeptidase. Heavy drinkers were defined as males consuming >14 drinks/week and women consuming >7 drinks/week. Total intentional exercise is expressed as geometric mean (95% CI). Subclinical cardiovascular disease is defined as those individuals who had at least two of the following conditions: left ventricular hypertrophy, carotid plaque and coronary artery calcium >0 Agatston units.

3.2. *Relative Prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) by Quintiles of Baseline NT-proBNP*

Non-alcoholic fatty liver disease defined as a liver attenuation coefficient <40 HU represented 5.5% of the sample and was 3.6-fold (9.2% vs. 2.6%) more prevalent in the lowest quintile of NT-proBNP (range: 4.9–19.2 pg/mL) than at the highest quintile of NT-proBNP (≥135.5 pg/mL), Fig. 1. Relative prevalence adjusted for model 1 decreased across categories of NT-proBNP with a linear trend for each log unit of NT-proBNP of 0.81 (0.72–0.92), p = 0.001. Further adjusting for percentage of dietary calories derived from fat, total intentional exercise, alcoholic drinks per week and interleukin-6 did not substantially change the association between NAFLD and NT-proBNP. However, adding metabolic syndrome or BMI to model 2, the association between NAFLD and NT-proBNP was significantly weakened, p = 0.07 and p = 0.08 for adjustment for metabolic syndrome and BMI, respectively.

NAFLD defined as a liver/spleen ratio (L/S) <1 represented 16% of the sample and was 2.5-fold higher (23.7% vs. 9.5%) in the lowest quintile of NT-proBNP than in the highest quintile, Table 2. There was an inverse association in relative prevalence for NAFLD across quintiles of NT-proBNP following adjustment for model 1 and 2 covariates, p < 0.001 and p = 0.001, respectively. Further adjusting for metabolic syndrome or BMI (p = 0.01) did not substantially change the association between NAFLD and NT-proBNP, model 3 in Table 2.

Using either definition for NAFLD (<40 HU or L/S ratio <1), the association between relative prevalence of NAFLD and NT-proBNP was not different between sex, as reflected by the lack of a sex × NAFLD interaction, p = 0.09 and p = 0.7, respectively. Excluding individuals with alcohol consumption >14 drinks per week if males and >7 drinks per week if females, did not substantially change the association between NT-proBNP and relative prevalence of NAFLD for model 2, RP per unit increase in ln(NT-proBNP) = 0.83 (0.71–0.97), p = 0.02 and 0.83 (0.74–0.93), p = 0.002, respectively. There was no race × NAFLD interaction whether defining NAFLD as HU <40 or by an L/S ratio <1, p = 1.0 and p = 0.3, respectively. Similarly, there was no subclinical CVD × NAFLD interaction, p > 0.2. Excluding individuals with liver attenuation >80 HU did not substantially change the association between RP of NAFLD and NT-proBNP, RP per unit increase in ln(NT-proBNP) = 0.85 (0.74–0.98) and 0.84 (0.76–0.93), p < 0.03 for NAFLD and L/S <1, respectively. In addition, there was not a BMI category × NAFLD interaction, p = 0.6 and p = 0.8 when defining NAFLD as HU <40 or an L/S ratio <1, respectively.

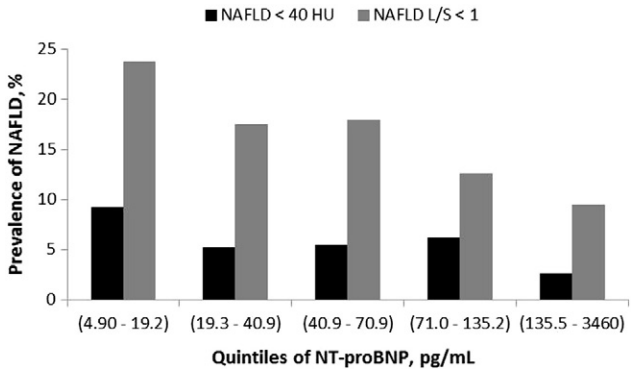

Fig. 1 – Prevalence of non-alcoholic fatty liver disease defined as a liver attenuation <40 HU or a liver to spleen ratio <1 by quintiles of NT-proBNP. Legend. HU = Hounsfield units. NAFLD = non-alcoholic fatty liver disease. Individuals with self-reported liver disease and diabetes were excluded.

3.3. *Association Between Liver Attenuation and NT-proBNP*

Linear regression coefficients between liver attenuation and log of NT-proBNP were 0.86 (0.18), p < 0.0001 when adjusted for model 1 covariates and 0.81 (0.21) when adjusted for model 2 covariates, p = 0.0002. Adjusting for metabolic syndrome (model 3) slightly weakened the association, 0.68 (0.21), p = 0.001. Further adjusting for BMI or waist circumference did not substantially change the association between liver attenuation and NT-proBNP. There was no low eGFR × NT-proBNP interaction for model 1 or 2, p = 0.9 and p = 0.5, respectively. Fig. 2 shows that the association between liver attenuation and NT-proBNP did not follow a linear pattern. Linear spline analysis adjusted for model 2 covariates showed that the inflection point (highest R^2 = 0.173) occurred at an NT-proBNP concentration of 45 pg/mL, corresponding to a liver attenuation of 60 HU. Linear regression coefficient below the inflection point was 0.05 (0.02) for every pg/mL increase in NT-proBNP, p = 0.001 and above the inflection point was 0.0006 (0.0008), p = 0.5. Linear slopes at NT-proBNP concentrations <45 and ≥45 pg/mL were different, p < 0.001.

4. Discussion

The results of this cross-sectional analysis show a positive linear association between liver attenuation in HU (less attenuation more fat) and baseline values of NT-proBNP. However, the association between NT-proBNP and liver attenuation is not linear and plateaus at NT-proBNP value ≥45 pg/mL. This study also shows an inverse association between baseline NT-proBNP and relative prevalence of fatty liver disease. Furthermore, the inverse association between relative risk for NAFLD and NT-proBNP persists after adjusting for age, race, sex, and percentage of calories derived from fat, total intentional exercise, number of alcoholic drinks per week and IL-6.

4.1. *Association Between Liver Fat and NT-proBNP*

Although plasma levels of NT-proBNP differ by race, race does not modify the association between NT-proBNP and NAFLD. This is similar to the result report by Al Rifai et al. [21] that found that the association between metabolic syndrome and NAFLD was not dependent on race. The greater prevalence of NAFLD when defining it as an L/S ratio <1 is related to the inclusion of individuals with mild liver disease. This is likely the explanation for the weakening of the association between NT-proBNP and risk of NAFLD when including metabolic syndrome in the model and defining NAFLD as a liver attenuation <40 HU (a more restrictive definition of NAFLD), but not when defining it as an L/S <1. The weakening of the association between NT-proBNP and NAFLD when adding metabolic syndrome to the model depends on the defining criteria for NAFLD and may suggest that metabolic syndrome is on the causal pathway for the development of NAFLD. However, further research is required to assess the dose response effect of natriuretic peptides on the metabolism and accumulation of fat in the liver and the development of NAFLD.

The plateauing of the positive association between NT-proBNP and liver attenuation at levels of NT-proBNP ≥45 pg/mL is most likely related to the reported upper limit in liver attenuation of individuals without clinical signs of liver disease [30,31]. In this study, the upper interquartile value of liver

Table 2 – Cross sectional adjusted relative prevalence for non-alcoholic fatty liver disease by quintiles of baseline NT-proBNP in non-diabetic individuals without self-reported liver disease.

		Quintiles of NT-proBNP, pg/mL						
	Range	4.9–19.2	19.3–40.9	41.0–70.9	71.0–135.2	135.5–3460		
NAFLD	n	888	902	929	919	891	Linear trend	p value
<40 HU	Model 1	1	0.63 (0.44–0.89)	0.70 (0.49–1.01)	0.67 (0.46–0.99)	0.40 (0.24–0.68)	0.81 (0.72–0.92)	0.001
	Model 2	1	0.64 (0.41–1.00)	0.78 (0.51–1.21)	0.72 (0.45–1.15)	0.48 (0.26–0.88)	0.82 (0.71–0.96)	0.01
	Model 3	1	0.66 (0.43–1.02)	0.78 (0.51–1.2)	0.74 (0.47–1.18)	0.54 (0.29–1.00)	0.88 (0.76–1.01)	0.07
<1	Model 1	1	0.77 (0.61–0.98)	0.81 (0.63–1.03)	0.58 (0.44–0.77)	0.46 (0.33–0.63)	0.82 (0.75–0.89)	<0.0001
	Model 2	1	0.83 (0.62–1.12)	0.86 (0.63–1.17)	0.6 (0.42–0.85)	0.49 (0.33–0.74)	0.87 (0.78–0.97)	0.001
	Model 3	1	0.87 (0.65–1.15)	0.86 (0.64–1.16)	0.63 (0.45–0.89)	0.54 (0.37–0.81)	0.87 (0.78–0.97)	0.01

HU = Hounsfield units. Relative risk adjusted by model 1 = age, race, sex. Model 2 = model 1 + percentage of dietary calories derived from fat, total intentional exercise, alcoholic drinks per week and interleukin-6. Model 3 = model 2 + metabolic syndrome. NAFLD = non-alcohol fatty liver disease defined as an average liver attenuation coefficient <40 HU or a liver to spleen ration <1. Linear trend is per unit of log (NT-proBNP).

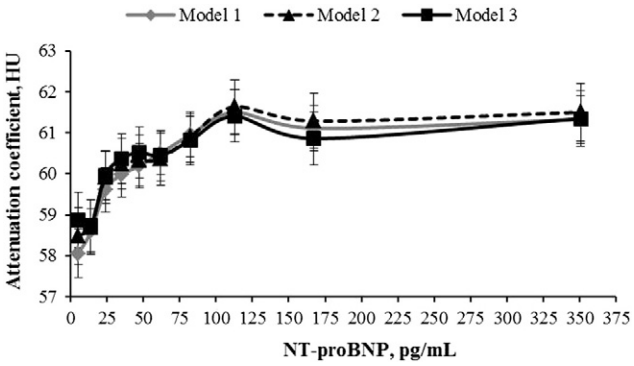

Fig. 2 – The association between liver attenuation coefficient (HU) and deciles of baseline NT-proBNP in non-diabetic individuals without self-reported liver disease. Legend. Model 1 adjusted for age, race and sex. Model 2 = model 1 + percentage of calories derived from fat, total intentional exercise per week, number of alcoholic drinks per week and, IL-6. Model 3 = model 2 + metabolic syndrome at MESA baseline. HU = Hounsfield units.

attenuation for those non-diabetics and without self-reported liver disease was of 67 HU, which is near to the corresponding liver attenuation value (60 HU) at the NT-proBNP inflection point. The association between NT-proBNP and liver fat follows a linear pattern along the range of values that describe healthy and fatty liver tissue. Liver attenuation values greater than 67 HU in liver CT scans may reflect areas of abnormal liver tissue and beyond that HU value the association between NT-proBNP and liver attenuation is no longer significant.

4.2. *Potential Biological Mechanism*

The biologically active B-type natriuretic peptide (BNP) and the inactive NT-proBNP are released on an equimolar basis by the heart due to myocyte stretching and cleavage of the precursor peptide proBNP by the enzymes corin and/or furin [32]. Low plasma NT-proBNP has been associated in cross sectional studies with greater BMI [5,33], increased visceral adipose tissue [6], presence of metabolic syndrome [34,35] and in longitudinal studies low levels of NT-proBNP have shown to be predictive of incident diabetes [8,36–38]. The epidemiological associations between natriuretic peptides with obesity and incident diabetes could result from reverse causality. However, a Mendelian randomization study found that individuals with a genetic variant BNP, which was associated with elevated plasma levels of BNP, were less likely to develop incident diabetes [37]. In addition to epidemiological evidence, animal and human models have shown that atrial natriuretic peptide (ANP) and BNP have metabolic actions that can explain the associations observed in epidemiological studies. Cellular actions of natriuretic peptides occur by binding to natriuretic peptide (NP) receptors A and B (NPR-A and NPR-B, respectively). Binding of NP to its receptors leads to activation of the gene that codes for the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC1A) [39] and stimulates an increase in mitochondrial density, oxygen consumption and an increase in insulin sensitivity [11,40] and increased lipolysis in adipose tissue independently from catecholamine induced lipolysis [41,42]. These metabolic actions may provide a biological explanation for the accumulation of fat and the development of NAFLD associated with low levels of NT-proBNP.

NPs are cleared from the circulation by natriuretic peptide receptor-C (NPR-C) [43,44]. NP receptors have been detected in adipose tissue, cardiac and skeletal muscles, kidneys and liver [45,46]. It was further shown that insulin concentration decreased NPR-A and NPR-B in adipose tissue and increased NPR-C, whereas fasting induced the opposite effect [45]. Therefore, any event that increases insulin concentration can lower natriuretic peptide levels (due to increased clearance) and decrease the ability of natriuretic peptides to exert their cellular actions due to lower numbers of NPR-A and NPR-B.

4.3. *Strengths and Limitations*

The strength of this analysis is that it included a large sample of individuals of diverse ethnic and racial background free of CVD and a wide range of liver attenuation coefficients. Another strong point is the very high intra- and interreader correlation for the determination of HU and the low intra-assay and interassay coefficient of variation for NT-proBNP. The weakness is the cross-sectional and observational nature of this analysis, which prevents the ability to establish a causal effect for NT-proBNP in the development of NAFLD. However, a Mendelian randomization study showed an inverse association between NT-proBNP and incidence diabetes [37]. The result from that study provides further support for the hypothesis that natriuretic peptides may be an important factor to consider in the development of type 2 diabetes mellitus. Also, although the inverse association between HU by computed tomography and fat liver storage has been well documented, using magnetic resonance imaging to evaluate the difference in resonance frequency between water and fat may have a better biophysical basis to evaluate fatty liver [47]. A number of participants had conditions which could increase plasma levels of NT-proBNP such as chronic obstructive pulmonary disease (COPD) [48], subclinical CVD [49] and low eGFR [50] and confound the association between NT-proBNP and NAFLD. While the prevalence of COPD was not assessed at baseline, the prevalence of current or former smokers was similar in those with and without NAFLD. Similarly, the prevalence of those with low eGFR or subclinical CVD was not different between those with and without NAFLD and the lack of an interaction between NT-proBNP and subclinical CVD or with low eGFR supports the results that NT-proBNP is an independent risk factor associated with NAFLD.

5. Future Research and Potential Clinical Implications

There is increasing epidemiological and experimental evidence describing associations between low levels of natriuretic peptides and disorders of glucose and fat metabolism. Whether

there is a cause and effect relationship between low levels of natriuretic peptides and the development of obesity, metabolic syndrome and overt type 2 diabetes have yet to be clarified. Furthermore, the mechanism(s) that is involved in lowering blood levels of natriuretic peptides has only been scarcely studied. Blood levels of NT-proBNP may have important clinical applications in order to identify, prevent and treat individuals at risk of metabolic disorders of glucose and fat.

5.1. *Conclusions*

This cross-sectional analysis has shown that low concentrations of NT-proBNP (<19.3 pg/mL) are an independent risk factor associated with the presence of NAFLD and that for NT-proBNP values <45 pg/mL there is an inverse association between NT-proBNP and amount of liver fat.

Author Contribution

Proposed the topic and wrote the manuscript and performed statistical analysis: Otto A. Sanchez. Analysis and writing the manuscript: Mariana Lazo, Lori B Daniels, Daniel Duprez, Hossein Bahrami, Joao A. lima, Alan Maisel, Carmen A. Peralta, Russell P Tracy and Ryan Bradley.

Expertise in liver disease and imaging: Irfan Zeb and Mathew Budoff.

Statistical analysis and writing manuscript: David R Jacobs.

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Conflict of Interest

Otto A. Sanchez, no disclosures. Daniel Duprez, consultant to Novartis Astra Zeneca. Mariana Lazo, no disclosures. Irfan Zeb, no disclosures. Russell P Tracy, no disclosures. Ryan Bradley, no disclosures. Hossein Bahrami, no disclosures. Lori B Daniels, consultant to Alere; speaking fees from Critical Diagnostics and Roche. Joao A. Lima, consultant to Toshiba Medical Systems, Bracco. Alan Maisel, consultant to Alere, BG Medicine, Brahms, Critical Diagnostics, EFG diagnostics, Novartis, Abbott. Carmen A. Peralta, no disclosures. David R Jacobs, no disclosures. Mathew Budoff, consultant to General Electric.

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