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Original article

Low N-terminal pro-brain natriuretic peptide levels are associated with non-alcoholic fatty liver disease in patients with type 2 diabetes

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ABSTRACT

Aim. – Natriuretic peptides (NPs) have emerged as important regulators of lipid metabolism. Reduced levels of NPs are reported in obesity and in patients with type 2 diabetes (T2D). This NP deficiency may affect their ectopic fat distribution and lead to high risk of non-alcoholic fatty liver disease (NAFLD).
Methods. – In this cross-sectional study, the association between N-terminal pro-B-type natriuretic peptide (NT-proBNP) and liver fat content was quantified using 1H-magnetic resonance spectroscopy in 120 patients with T2D.
Results. – NAFLD (defined as liver fat content $\geq$ 5.6%) was found in 57 (48%) of the T2D patients, who also had significantly lower NT-proBNP (P = 0.002) levels compared with patients without NAFLD, but did not differ as regards the presence of cardiovascular disease (CVD) or in kidney function. After adjusting for potential confounders (age, gender, HbA_{1c}, BMI, HOMA2-IR, CVD, eGFR), the odds ratio for the presence of NAFLD was increased by 2.9 (P = 0.048) for NT-proBNP levels $<$ 45 ng/L. In a multivariable linear regression model, the relationship with NT-proBNP was further analyzed as a continuous variable, and was independently and inversely associated with increasing liver fat content after full adjustment (P = 0.031).
Conclusion. – Reduced plasma NT-proBNP levels are independently associated with high liver fat content in patients with T2D. The present study suggests that NP deficiency may play a role in the development of NAFLD in T2D.

Introduction

Increased liver fat content, defined as non-alcoholic fatty liver disease (NAFLD), is highly prevalent among patients with type 2 diabetes (T2D) and associated with subsequently poor prognoses primarily due to increased risk of cardiovascular disease (CVD) [1,2]. Increased myocardial insulin resistance, decreased perfusion and corresponding accumulation of ectopic fat in the heart are possible mechanisms of this increased CVD risk in patients with NAFLD [3,4]. However, the central role of dysfunctional adipose tissue in the setting of NAFLD has been established in T2D [5–7]. According to the fatty-acid overspill hypothesis, the capacity to oxidize excess fatty acids in adipocytes is eventually compromised, leading to increased storage as ectopic fat in the liver [5,6,8].

Although increased liver fat is the most common cause of incidentally discovered elevated liver transaminases, most patients with NAFLD have liver enzyme levels within the normal reference range [9]. Therefore, imaging modalities are widely accepted for diagnosing NAFLD, with ^{1}H-magnetic resonance (MR) spectroscopy and MR imaging (MRI)-derived proton density fraction considered the gold standards for quantifying liver fat content [10,11], as both are more sensitive and accurate than other imaging modalities [12]. Natriuretic peptides (NPs), which are mainly used as

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biomarkers in CVD, have emerged in recent years as key regulators of metabolic processes [13,14], and potential causality between NP deficiency in obesity and risk of T2D has been suggested [15,16].

In fact, growing evidence from clinical and experimental studies suggests that A- and B-type NPs play a central role in regulating lipid metabolism by stimulating lipid oxidation [17–19]. The potent metabolic effects of B-type NP (BNP) are mediated by two receptors in adipocytes: NP receptor A (NPR-A); and NP clearance receptor C (NPR-C) [17,20]. The decreased NPR-A/NPR-C ratio observed in T2D is mechanistically linked to impaired NP response [21,22]. The effects of NPs on lipid mobilization and energy expenditure are mediated by increased fat oxidation rates through upregulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) [23,24]. Therefore, NP deficiency in T2D could play a role in the progression of impaired metabolism. Notably, in mouse models fed high-fat diets, BNP infusion reduced fat deposits in the liver, decreased the size of hypertrophied adipocytes and improved insulin resistance [15]. In addition, the important role of BNPs in stimulating synthesis of insulin-sensitizing adipocytokine adiponectin has already been demonstrated [25].

Thus, our hypothesis was that reduced levels of B-type NPs in patients with T2D are associated with increased liver fat. The objective was therefore to investigate the relationship between N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations and liver fat content in T2D patients, and also to evaluate the impact of insulin resistance and adipocyte function on the potential link between NT-proBNP and NAFLD.

Materials and methods

Study design and population

Patients were recruited at the diabetes outpatient clinic at the University Hospital of Herlev, Denmark, from an investigator-initiated, double-blind, placebo-controlled clinical trial, Mineralocorticoid Receptor Antagonists in Type 2 Diabetes (the MIRAD study) (ClinicalTrials.gov number NCT02809963), which randomized 140 T2D patients with known CVD or at high risk. For the present study, 120 T2D patients with complete ^{1}H-MR spectroscopy and plasma NT-proBNP measurements at baseline were included.

Patients were eligible for the initial study if they had T2D (diagnosed at least 3 months prior to enrollment) and were receiving stable glucose-lowering and antihypertensive therapy according to European Society of Cardiology (ESC)/European Association for the Study of Diabetes (EASD) guidelines [26]. Further inclusion criteria were a history of CVD (> 3 months prior to enrollment), defined as previous myocardial infarction (MI), significant stenosis on coronary angiography (CAG), previous stroke or peripheral artery disease (PAD), or high CVD risk as identified by NT-proBNP levels ≥ 70 ng/L or the presence of albuminuria (urinary albumin/creatinine ≥ 30×10^{-3} mg/g).

The main exclusion criteria were heart failure with a reduced left ventricular ejection fraction (LVEF) of < 40%, severe liver disease and impaired kidney function with an estimated glomerular filtration rate (eGFR) < 40 mL/min/1.73 m^2, according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [27]. A full list of the inclusion and exclusion criteria for the MIRAD study is presented in Table S1 (see supplementary materials associated with this article online). All patients gave their written informed consent prior to inclusion in the study.

MRI and MR spectroscopy

MR measurements were performed with a 3T Achieva MRI system (Philips Medical Systems International BV, Best, The Netherlands). Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were measured by MRI using a 1-cm-thick slice taken at the middle of the third lumbar vertebra (L3), as described by Jürs et al. [28]. Liver fat content was measured by ^{1}H-MR spectroscopy with a single voxel (11 mm × 11 mm × 11 mm) positioned at the right lobe of the liver, avoiding vascular structures. The acquired spectra were fitted to obtain peak areas, and liver fat signal content was calculated as: [fat peak area at echo time (TE) = 0 ms]/(fat peak area at TE = 0 ms + water peak area at TE = 0 ms) × 100. Peak areas at TE = 0 ms were corrected for T2 relaxation effects for each peak using an exponential least-squares fitting algorithm for peak areas with a series of TEs [29]. NAFLD was defined as per recommendations as liver fat content ≥ 5.6% [10].

Dual-energy X-ray absorptiometry (DEXA)

Total fat mass was measured with a whole-body scan using a DEXA Discovery scanner (Hologic, Inc., Bedford, MA, USA).

Biochemistry and biomarkers

After a minimum of 8 h of fasting and 30 min of rest in a supine position, patients' blood pressure was measured and morning blood samples collected from an antecubital vein. Triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using the enzymatic technique (VITROS 5,1 FS Chemistry System, Ortho Clinical Diagnostics, Raritan, NJ, USA); interassay coefficients of variability (CVs) were 11%, 5% and 2%, respectively. Plasma NT-proBNP concentrations were measured by chemiluminescence immunometric assay (IMMULITE 2000; Siemens Healthineers, Erlangen, Germany). Intra-assay variations were 5.4% and 3.1% at concentrations of 35.6 ng/L and 146 ng/L, respectively, and interassay variations were 6.4% and 4.4% at the same concentrations; patients with NT-proBNP concentrations below the detection limit (< 20 ng/L) were assigned this value. Plasma aliquots for measurements of glycerol, free fatty acid (FFA) and adiponectin were stored at −80 °C until analyses were performed by spectrophotometry, using validated in-house endpoint enzyme-based assays. The intra-assay CV for glycerol was 1.2% and the interassay CV was 3.9%; the corresponding rates for FFA were 0.6% and 2.2%. Adiponectin was measured by validated in-house time-resolved immunofluorometric assays based on commercial reagents with human adiponectin radioimmunoassay (RIA) kits (125T; MilliporeSigma, Burlington, MA, USA; ref: HADP-61HK, lot. No. 3044042C). Intra-assay and interassay CVs for adiponectin were 2.8% and 2.6%, respectively. Insulin resistance was measured using the updated computerized version of homoeostasis model assessment for insulin resistance (HOMA2-IR), which incorporates proinsulin secretion and takes into account variations in hepatic and peripheral glucose resistance [30].

Statistical analyses

Variables are presented as means ± SD, medians (25th and 75th percentiles) or numbers (percentages) as appropriate. Normal distribution of continuous variables was assessed by creating histograms and evaluating linearity in QQ plots. All variables with skewed distributions were log-transformed to create normal distributions prior to statistical analysis; this included liver fat content, NT-proBNP, glycerol and FFA. Patients were compared according to the presence of NAFLD, using analysis of variance (ANOVA) for normally distributed variables and the Wilcoxon signed-rank test for non-normally distributed variables. A chi^2 test was performed to investigate differences in categorical variables.

Patients were divided according to quartiles of NT-proBNP to compare the prevalence of NAFLD. A multivariable linear regres-

sion model with backward elimination was used to adjust for potential confounders affecting the association between NT-proBNP and liver fat content, and to determine parameters independently associated with liver fat content. A cut-off NT-proBNP value of < 45 ng/L, as previously reported by Sanchez et al. [31], was used in the multivariable logistic analyses to assess the association with NAFLD in the present T2D study population. The association between NT-proBNP levels and liver fat content as continuous variables was also evaluated in multivariable linear regression analyses. Models were adjusted for the same covariables of age, gender, presence of CVD and eGFR as potential confounders of NT-proBNP levels, as well as the clinically relevant metabolic parameters of HbA_{1c}, body mass index (BMI) ≥ 30 kg/m^2 and HOMA2-IR.

Sensitivity analyses were performed to evaluate the role of lipid metabolism by replacing BMI with total fat mass and with adiponectin. In supplementary logistic regression analyses, the predictive value of NT-proBNP for NAFLD was further evaluated by dividing patients according to CVD status. In addition, a formal interaction analysis between NT-proBNP and CVD was performed in this multivariable model. The ability of NT-proBNP to detect NAFLD was analyzed using receiver operating characteristic (ROC) curve statistics to evaluate the area under the curve (AUC), sensitivity, specificity, and positive (PPVs) and negative predictive values (NPVs), using Youden's criteria to determine the cut-off level of 42 ng/L. Collinearity was assessed, using the variance inflation factor, between NT-proBNP and adiponectin, HOMA2-IR and BMI, and between HbA_{1c} and HOMA2-IR. No significant collinearity was found, whereas a high intercorrelation between VAT and liver fat content was observed.

Two-tailed *P*-values < 0.05 were considered statistically significant, and all analyses were performed using SAS Enterprise Guide version 7.11 software (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

Of the 120 T2D patients included in this study, 87 (73%) were men, mean age was 63 years and mean BMI was 30.3 kg/m^2, with a mean HbA_{1c} of 7.5% (59 mmol/mol). Almost half (45%) the patients had a history of CVD, the median (interquartile range, IQR) level of NT-proBNP was 68 (37–163) ng/L, and 37 (31%) had albuminuria (urinary albumin/creatinine ratio ≥ 30×10^{-3} mg/g). Median (IQR) liver fat content was 5.2% (1.2–11.6), and 57 (48%) patients had NAFLD. Baseline characteristics and medications according to the presence of NAFLD are presented in Table 1 and 2. The NAFLD group was younger and had lower levels of NT-proBNP with, as expected, higher levels of alanine aminotransferase (ALT) as well as HOMA2-IR, total cholesterol and triglycerides, whereas adiponectin and HDL cholesterol levels were lower. Moreover, waist circumference, BMI and VAT were higher among these T2D patients with NAFLD. However, no differences in CVD, eGFR, systolic blood pressure, albuminuria, total fat mass, FFA and glycerol were observed between the groups (Table 1). Patients with CVD were equally distributed between both groups according to previous MI (19% vs. 14%), CAG (21% vs. 14%), stroke (9% vs. 11%) and PAD (5% vs. 16%; $P > 0.050$ for all comparisons). After applying an upper limit for ALT of 40 U/L, 23 patients in the NAFLD group exceeded this value compared with 9 patients in the group without NAFLD ($P = 0.002$).

NAFLD according to NT-proBNP

Dividing patients into NT-proBNP quartiles demonstrated that the prevalence of NAFLD was twofold higher (73% vs. 37%) in the lower vs. upper quartiles, respectively ($P = 0.010$; Fig. 1). In

Table 1
Characteristics of patients with and without non-alcoholic fatty liver disease (NAFLD).

	With NAFLD (*n* = 57)	Without NAFLD (*n* = 63)	*P*
Demographics			
Age (years)	60 ± 8	66 ± 9	< 0.001
Gender: male (%)	43 (75%)	44 (70%)	0.543
Duration of type 2 diabetes (years)	8 (5, 13)	14 (7, 17)	0.012
Cardiovascular disease[a]	40%	49%	0.362
Systolic blood pressure (mmHg)	146 ± 19	147 ± 18	0.727
Diastolic blood pressure (mmHg)	86 ± 10	81 ± 11	0.018
Body mass index (kg/m^2)	31.2 ± 3.1	29.5 ± 4.2	0.014
Waist circumference (cm)	108 ± 10	103 ± 11	0.003
Visceral fat volume (cm^3)	307 ± 96	234 ± 92	< 0.001
Subcutaneous fat volume (cm^3)	241 ± 81	240 ± 100	0.916
Total fat mass (kg)	27.0 ± 5.5	25.9 ± 7.7	0.327
Number of drinks (12 g of alcohol) per week	2 (0.5, 5)	2 (0, 7)	0.390
Biochemistry			
HbA_{1c} [% (mmol/mol)]	7.5 ± 3.2 (59)	7.5 ± 3.6 (59)	0.856
HOMA2-IR	2.9 ± 1.4	2.0 ± 1.0	< 0.001
Urinary albumin/creatinine ratio (mg/g)	16 (6, 41)	13 (5, 43)	0.733
Alanine aminotransferase (U/L)	40 ± 17	30 ± 9.7	< 0.001
High-sensitivity C-reactive protein (mg/L)	1.6 (0.8, 2.7)	1.2 (0.6, 3.4)	0.407
Estimated glomerular filtration rate (mL/min/1.73 m^2)	89 ± 17	84 ± 19	0.109
Estimated glomerular filtration rate 40–60 mL/min [*n* (%)]	3 (5)	7 (11)	0.329
Cholesterol (mmol/L)	3.9 ± 1.1	3.4 ± 0.7	0.006
Low-density lipoprotein cholesterol (mmol/L)	1.8 ± 0.6	1.8 ± 0.6	0.466
High-density lipoprotein cholesterol (mmol/L)	0.96 ± 0.23	1.07 ± 0.35	0.048
Triglyceride (mmol/L)	2.1 (1.4, 2.8)	1.1 (0.9, 1.5)	< 0.001
N-terminal pro-brain natriuretic peptide (ng/L)	55 (26, 111)	94 (54, 181)	0.002
Adiponectin (μg/mL)	0.8 (0.7, 1.2)	1.3 (0.9, 1.8)	< 0.001
Free fatty acid (μmol/L)	588 ± 197	573 ± 229	0.417
Glycerol (μmol/L)	66 ± 25	61 ± 28	0.161

Data are presented as means ± SD, medians (25th quartile, 75th quartile) or *n* (%).
HOMA2-IR: updated homoeostasis model assessment for insulin resistance.
[a] Myocardial infarction, coronary angiography, stroke, peripheral artery disease.

Table 2
Medications taken by patients with and without non-alcoholic fatty liver disease (NAFLD).

	With NAFLD (n = 57)	Without NAFLD (n = 63)	P
Antidiabetics			
Metformin	88%	78%	0.229
Dipeptidyl peptidase-4 inhibitor[a]	30%	18%	0.133
Sodium–glucose cotransporter type-2 inhibitor[b]	18%	14%	0.803
Sulphonylurea	14%	8%	0.380
Glucagon-like peptide-1 analogue	26%	35%	0.329
Insulin	40%	56%	0.104
Antihypertensives			
Angiotensin-converting enzyme inhibitor	32%	25%	0.544
Angiotensin-II receptor blocker	46%	52%	0.471
Beta-blocker	40%	32%	0.347
Calcium-blocker	35%	35%	1.000
Diuretic	49%	44%	0.714
Lipid-lowering[c]	88%	92%	0.546

[a] Sitagliptin, vildagliptin, linagliptin.
[b] Dapagliflozin, empagliflozin.
[c] Statins, fibrates, ezetimibe.

contrast, no difference in the presence of CVD according to quartile was observed (P = 0.209). Mean eGFR was 93 mL/min in the lower quartile vs. 79 mL/min in the upper quartile (P = 0.004). As expected, patients were younger, aged 56 years in the lower vs. 67 years in the upper NT-proBNP quartile ($P < 0.001$). Using the NT-proBNP cut-off value of 45 ng/L in a logistic regression model adjusted for age, gender, HbA_{1c}, BMI ($\geq$ 30 kg/m^2), HOMA2-IR, CVD and eGFR, the odds ratio (OR) for presence of NAFLD was increased by 2.9 (P = 0.048) for NT-proBNP levels < 45 ng/L (Table 3). When the presence of CVD was further evaluated, the risk of NAFLD for patients without CVD was markedly increased with an OR of 8.2 (P = 0.011), whereas there was no apparent increased risk for patients with CVD and an OR of 0.72 (P = 0.741), using the same cut-off NT-proBNP value of < 45 ng/L. The test for a formal interaction between CVD and NT-proBNP was, however, not statistically significant (P = 0.678). Finally, the diagnostic performance of NT-proBNP in detecting NAFLD showed an AUC of 0.66 (0.56, 0.76), with a sensitivity of 47%, specificity of 84%, PPV of 73% and NPV of 63%.

Association of liver fat content with NT-proBNP

Evaluation of the relationship between NT-proBNP levels and liver fat content has demonstrated that, after multivariable adjustment in a linear regression model for age, gender, HbA_{1c}, BMI, HOMA2-IR, CVD and eGFR, NT-proBNP levels were inversely and independently associated with liver fat content (P = 0.031). Also, in this model, HOMA2-IR ($P < 0.001$) and BMI $\geq$ 30 kg/m^2 (P = 0.039) remained associated with liver fat content. Adjusting for glucose-lowering medication, including glucagon-like peptide (GLP)-1 analogues, and the use of lipid-lowering medication did not change the results of the main model (Table 3). A high intercorrelation between VAT and liver fat content (r = 0.44, $P < 0.001$) was found and, therefore, not included in the multivariable models.

In the sensitivity analyses, when the role of total fat mass was evaluated by replacing BMI with total fat mass in the multivariable linear model, the independent association between NT-proBNP and liver fat remained significant (P = 0.019). The role of insulin sensitivity in adipose tissue was further addressed by adding adiponectin to the model, which attenuated the association between NT-proBNP and liver fat content (P = 0.075). Adiponectin showed a clear inverse association with liver fat content (P = 0.001) and a positive association with NT-proBNP ($P < 0.001$), yet the latter was associated with neither VAT (P = 0.538) nor total fat mass (P = 0.537). There was, however, an inverse univariate association between NT-proBNP and eGFR (r = −0.23, P = 0.006) and BMI (r = −0.18, P = 0.036). Moreover, while NT-proBNP was associated with age (r = 0.37, $P < 0.001$), there were no significant correlations with HbA_{1c} (P = 0.119), HOMA2-IR (P = 0.702), gender (P = 0.472) and the presence of CVD (P = 0.262).

Discussion

The present cross-sectional study shows, for the first time, that reduced NP levels are associated with liver fat content in patients with T2D. A more than twofold increased risk of NAFLD was observed in patients with NT-proBNP levels < 45 ng/L, which has been reported as the cut-off level for NAFLD in subjects without diabetes [31]. In addition, low NT-proBNP levels remained independently associated with liver fat content after adjusting

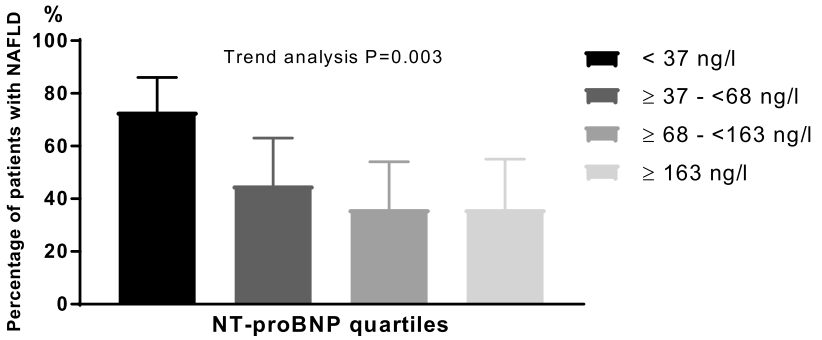

Fig. 1. Percentages of patients with non-alcoholic fatty liver disease (NAFLD) according to N-terminal pro-B-type natriuretic peptide (NT-proBNP) quartiles.

Table 3
Multivariable linear and logistic analyses.

Linear regression				Logistic regression			
Dependent variable: liver fat content % (log)				Dependent variable: non-alcoholic fatty liver disease			
	B	95% CI	*P*		OR	95% CI	*P*
NT-proBNP (log2)	−0.17	−0.33, −0.02	0.031	NT-proBNP < 45 ng/L	2.91	1.01, 8.37	0.048
HOMA2-IR	0.38	0.23, 0.54	< 0.001	HOMA2-IR	2.17	1.39, 3.40	< 0.001
BMI ≥ 30 kg/m^2	0.43	0.02, 0.84	0.039	BMI ≥ 30 kg/m^2	2.44	0.97, 6.13	0.059

Multivariable models adjusted for age, gender, cardiovascular disease (myocardial infarction, coronary angiography, stroke, peripheral artery disease), estimated glomerular filtration rate (mL/min/1.73 m^2) and HbA_{1c}, none of which were significantly associated with the dependent variable.
CI: confidence interval; NT-proBNP: N-terminal pro-brain natriuretic peptide; HOMA2-IR: updated homoeostasis model assessment for insulin resistance; BMI: body mass inde.

for BMI and HOMA2-IR as potential confounders of liver fat content, and also after adjusting for age, CVD and renal function, all of which are known to influence circulating levels of NT-proBNP. However, including the adipocytokine adiponectin in the main models attenuated the association, suggesting that the link between NPs and liver fat might be mediated by beneficial effects on adipocyte metabolism in patients with T2D.

T2D patients with NAFLD were younger, with larger waist circumferences, BMIs and VAT volumes and were also, as expected, more insulin-resistant and dyslipidaemic with significantly lower adiponectin levels. Patients with NAFLD also displayed higher levels of ALT, with a greater number of them having values above the upper reference limit, although only 23 of the 57 patients with NAFLD had ALT levels > 40 U/L. Thus, our present results support the previously suggested lack of predictive ability of liver enzymes with respect to NAFLD. Indeed, differences in NT-proBNP levels could not be explained by either a higher prevalence of CVD or impaired kidney function in patients with NAFLD, as there was no difference in their prevalence between our groups.

Few cohorts have reported on the association between NPs and liver fat content and, to the best of our knowledge, the present study is the first to investigate the relationship in T2D. Results from the Multi-Ethnic Study of Atherosclerosis (MESA), which included a large cohort of healthy participants with no diabetes or CVD, reported that NAFLD [defined as a liver attenuation coefficient < 40 HU on computed tomography (CT) imaging] was 3.6 times more prevalent in the lowest vs. highest quintile of NT-proBNP [31]. Also, the inverse association between liver fat and NT-proBNP in that study reached a plateau, suggesting a non-linear association with a cut-off at 45 pg/mL. Using the same cut-off value for NT-proBNP in the present T2D population resulted in a more than twofold greater risk of NAFLD for patients with reduced NT-proBNP levels, with only HOMA2-IR remaining in the model as an independent predictor.

The present data are further supported by the Dallas Heart Study, which demonstrated an inverse association between both BNP and NT-proBNP and liver fat using ^{1}H-MR spectroscopy in a cohort predominantly (90%) free of a T2D diagnosis [18]. As with the present study, this relationship remained significant after adjusting for BMI and insulin resistance. Therefore, these findings can be extended to a population with established T2D, high risk of NAFLD and more severe insulin resistance and, notably, with known or high risk of CVD, suggesting that NP deficiency could play a role in the progression of NAFLD in T2D patients at high CVD risk. In addition, no relationship was observed between NT-proBNP and body fat measurements of VAT and SAT as assessed by DEXA, which are in contrast to data from the Framingham Heart Study (third-generation study) and the Malmö Diet and Cancer Study (cardiovascular arm), which reported an association with VAT and SAT using multidetector CT [13,32]. The present study's sample size and minor variability in VAT measurements among patients with T2D may explain part of this discrepancy. Also, it should be noted that participants eligible for inclusion in the Malmö Diet and Cancer Study and Framingham Heart Study were excluded if they had either prevalent CVD or diabetes.

In addition to epidemiological evidence, experimental studies in both animals and humans have investigated the link between NPs and liver fat content to elucidate the underlying mechanism. Mice overexpressing BNP are protected from diet-induced obesity and insulin resistance, which might be explained by the BNP-induced uncoupling protein (UCP)-1 expression in adipose tissue stimulating energy expenditure [33,34]. Furthermore, deletion of the natriuretic peptide receptor (NPR)-C in adipose tissue is protective against diet-induced obesity and hepatic steatosis in mice [20], thereby supporting the potential impact of the reduced NPR-A/NPR-C ratio and corresponding NP deficiency in T2D and high risk of NAFLD.

The ability of BNP to stimulate adiponectin expression and secretion also supports the idea that the beneficial lipid metabolic effects of NPs might be mediated by the insulin-sensitizing properties of adiponectin [25]. Indeed, the present study likewise supports this by demonstrating a positive association between NT-proBNP and adiponectin, as including this adipocytokine in multivariable models suggested an impact on the association with NAFLD. Reduced levels of NPs in T2D have also been demonstrated to decrease NP-stimulated lipolysis, lipid oxidation and, thus, lipid mobilization [17,23]. However, no differences in FFA and glycerol (as measures of whole-body lipolysis) were observed between our NAFLD groups. In a study by Ryden et al. [35], no differences in atrial NP-stimulated lipolysis were reported across five groups with increasing Adult Treatment Panel (ATP) III scores. Also, NPR-C expression gradually increased with BMI, which confers resistance to the lipolytic effect of NPs, thereby showing that the metabolic syndrome has a strong impact on NP biological activities in adipose tissue and particularly lipolysis [36].

While an increased de novo lipogenesis in patients with high liver fat has been demonstrated, no differences in plasma FFA compared with patients with low liver fat were found [37]. Interestingly, investigating the metabolic effects of inhibition of the NP metabolizing enzyme neprilysin demonstrated only increased lipolysis in abdominal SAT, but no effect on whole-body lipolysis or plasma FFA concentrations, indicating that increased NP availability in adipose tissue may have contributed to the metabolic improvement [38].

The clinical perspectives of the present study should also be considered as there is an unmet need for a biomarker to quantify liver fat content, particularly given that ^{1}H-MR spectroscopy is expensive, and expertise is required to perform such an analysis. However, even though the specificity and PPV are relatively acceptable, the low sensitivity and poor overall performance does not allow recommending single measurements of NT-proBNP to predict NAFLD in T2D, based on the data thus far. Nevertheless, in recognition of the need for biomarkers to detect NAFLD and given the strong association between NT-proBNP and liver fat content,

future studies in larger populations of T2D patients without CVD, and perhaps in combination with other potential biomarkers, may well provide additional clinically relevant data.

In the meantime, the present data support the notion that NPs have therapeutic potential in patients with T2D. Several ongoing studies are investigating NAFLD as a therapeutic target, and the beneficial effects of pioglitazone in a randomized clinical trial have already been demonstrated [39], although some concerns as regards safety and risk of heart failure limit its use. There is thus an unmet need for therapeutics in T2D patients with NAFLD, while studies indicate that NPR-C may be a candidate drug target [40].

Several limitations in the present study should be noted. First, it was not designed to measure differences in adipose tissue lipolysis, whole-body lipolysis, basal lipolysis or stimulated lipolysis and, therefore, the impact of potentially impaired NP-stimulated lipolysis was not investigated. Second, as the cross-sectional study design prevents any conclusions on causality, only associations can be made, although the findings of an effect of B-type NPs on liver fat in T2D can generate hypotheses. Also, the study sample size may be a limitation for measurements of body composition, although this was not a primary objective. Third, as no liver biopsies were performed, there can be no conclusions as to the relationship between NT-proBNP and severity of liver disease or presence of non-alcoholic steatohepatitis (NASH).

On the other hand, it may be considered a strength of the present study that liver fat content was measured in 120 patients with T2D using gold-standard proton MR spectroscopy.

In conclusion, the present study shows that reduced levels of NT-proBNP are independently and inversely associated with an increased risk of NAFLD in a population of T2D patients at high CVD risk. The results suggest that an impaired NP response in T2D is part of the mechanism behind the development of NAFLD.

Ethics

The study was carried out in accordance with the latest revision of the Helsinki Declaration. The Danish Medicines Agency (EudraCT 2015-002519-14), Capital Regional Scientific Ethics Committee (H-15009313) and Danish Data Protection Agency (2012-58-0004) approved the trial, which was monitored by the GCP unit, Copenhagen, Denmark. The GCP unit also monitored study activities and verified their compliance with existing regulations and the consistency between the CRF and source data.

The corresponding author has the raw data.

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Authors' contributions

MLJ, MS, PR, JF and CK designed and planned the trial. MLJ, MRH, EC, JR, FD and MS collected and analyzed the data. MLJ wrote the paper. MS, PR, JR, FD, MRH, EC, JF and CK provided feedback on the initial draft. The final version of the paper was approved by all authors. MLJ is responsible for its submission.

Disclosure of interest

MLJ, JR, MRH, EC and FD declare that they have no competing interest.
MS: Lecture fees and advisory board member: Boehringer Ingelheim and Novartis.
PR: Personal fees (consulting): Novartis, Relypsa, AstraZeneca, Grünenthal, Stealth Peptides, Fresenius, Vifor Fresenius Medical Care Renal Pharma, Vifor and CTMA; lecture fees: Bayer and CVRx; cofounder: CardioRenal.
JF: Advisory board member: Novo Nordisk.
CK: Lectures fees: AstraZeneca, MSD, Boehringer Ingelheim, Pfizer and Novo Nordisk. Advisory board member: Novo Nordisk, Astra Zeneca and MSD.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.diabet.2018.11.003.

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