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Circulating Pro-Neurotensin in gestational diabetes mellitus

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Abstract *Background and aims:* Pro-Neurotensin (NT), a stable surrogate parameter of NT, has recently been introduced as a peptide predicting the development of obesity, diabetes mellitus, cardiovascular diseases, and cardiovascular mortality. However, regulation of Pro-NT in gestational diabetes mellitus (GDM) remains uninvestigated.
Methods and results: Pro-NT was quantified in 74 women with GDM, 74 healthy, gestational age-matched, pregnant controls, as well as in a second cohort comprising of 74 healthy, non-pregnant control women, using a chemiluminometric sandwich immunoassay. Pro-NT was correlated to measures of obesity, hypertension, glucose and lipid metabolism, renal function, and inflammation.

Mean ± standard deviation of circulating Pro-NT levels were not significantly different in women with GDM (100.2 ± 75.7 pmol/l) as compared to healthy, pregnant controls (103.2 ± 37.4 pmol/l) and healthy, non-pregnant female controls (105.9 ± 38.9 pmol/l) ($p = 0.661$). Homeostasis model assessment of insulin resistance (HOMA-IR) and creatinine positively correlated with serum Pro-NT in multivariate regression analysis. In contrast, free fatty acids (FFA) were inversely correlated with circulating Pro-NT. Results sustained adjustment for pregnancy status.
Conclusions: Circulating Pro-NT is not independently associated with GDM, but is with HOMA-IR, creatinine, and FFA even after adjustment for pregnancy status.

Introduction

Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy contributing to an increased risk of acute and chronic complications in both mother and newborn [1]. A central component in the development of GDM is insulin resistance [2]. In the last decades, several cytokines contributing to the pathogenesis of insulin resistance in both type 2 diabetes (T2D) and GDM were identified [3–5]. Thus, the adipokine adiponectin is decreased in T2D and is an independent and negative

Abbreviations: $AUC_{Glucose}$, Area under the glucose curve; BMI, Body mass index; CV, Coefficient of variation; FFA, Free fatty acids; GDM, Gestational diabetes mellitus; HbA1c, Glycated hemoglobin A1c; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; NT, Neurotensin; OGTT, Oral glucose tolerance test; T2D, Type 2 diabetes.

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predictor of GDM (reviewed in Refs. [3,4]). Furthermore, the metabolically adverse hepatokine fetuin B is also increased in both T2D and GDM [6,7]. Circulating cytokines, therefore, might mediate metabolically adverse effects and contribute to the pathogenesis of insulin resistance states, i.e. T2D and GDM.

Neurotensin (NT) is a peptide predominantly-secreted by distinct N cells in the intestine [8]. NT exerts different cardiovascular effects on heart rate, blood pressure, and cardiac contractility (reviewed in Ref. [9]). Furthermore, NT is involved in the central regulation of appetite [10] and increases plasma insulin when infused intrapancreatically [11]. However, reliable determination of circulating NT in biological fluids is complicated by the instability of NT *in vivo* and *in vitro* [12]. Therefore, a sandwich immunoassay for the detection of Pro-NT 1–117, the stable NT precursor fragment in human blood, was developed [12]. Since Pro-NT is produced in stoichiometric amounts relative to NT it serves as a robust surrogate marker for the release of NT [12]. Pro-NT was recently positively associated with markers of obesity [13], new-onset obesity [14], nonalcoholic fatty liver disease [15], and diabetes mellitus [16]. Most importantly, circulating Pro-NT was not only linked to these metabolic disease states but also predicted incident cardiovascular diseases [16,17] and mortality [16] in the Malmö Diet and Cancer Study and the Framingham Heart Study. Taken together, using Pro-NT as a surrogate parameter, NT appears to be a metabolically adverse peptide linking obesity and diabetes mellitus with cardiovascular disease and mortality.

In contrast to the regulation of Pro-NT in these metabolic disease states, there are no studies investigating Pro-NT in GDM. Therefore, we have measured serum levels of Pro-NT concentrations in 74 women with GDM during pregnancy as compared to 74 gestational age-matched, healthy pregnant controls using the same immunoassay as in the previously published cohorts [14,16,17]. Furthermore, Pro-NT was quantified in 74 healthy, non-pregnant control women matched for age. Pro-NT was correlated to measures of obesity, hypertension, indices of glucose metabolism, lipid metabolism, renal function, and inflammation.

We hypothesize that Pro-NT is increased in women with GDM and is independently associated with markers of impaired metabolic status.

Methods

Study participants

For this cross-sectional study, we have analyzed data collected from a population of pregnant women with GDM as compared to healthy pregnant controls, as well as from an additional population comprising of healthy, non-pregnant women. Briefly, 148 pregnant women were recruited from the outpatient care unit of the Department of Endocrinology and Nephrology, University of Leipzig between 2006 and 2011 [7,18,19]. Age and body mass index (BMI) before pregnancy did not differ between women with GDM and healthy pregnant controls. Additionally, a control cohort of 74 healthy, non-pregnant women matched for age was extracted out of a sample from a self-contained population of the Sorbs from Eastern Germany [20,21]. In brief, about 1050 Sorbs were enrolled in the study between 2005 and 2007. Importantly, Veeramah and co-workers demonstrated that the Sorbs are only a minor genetic isolate and are, therefore, comparable to the GDM and non-GDM, pregnant control groups [21].

Investigations in the total cohort (74 GDM, 74 pregnant controls, 74 non-pregnant controls) included standardized questionnaires to assess past medical history and family history, anthropometric parameters, as well as a 75 g, 2 h oral glucose tolerance test (OGTT). In all pregnant women, 74 women were classified as patients with GDM according to the 2012 American Diabetes Association criteria [22], whereas 74 gestational age-matched pregnant women with normal glucose tolerance served as pregnant controls. In all 74 non-pregnant women, the OGTT was used to rule out a T2D. Area under the glucose curve ($AUC_{Glucose}$) was determined using the trapezoidal rule. The studies were approved by the local Ethics Committee and all subjects gave written informed consent before taking part.

Assays

All blood samples were obtained after an overnight fast. Blood specimens were immediately centrifuged and frozen at −80 °C until analyses were performed. Serum levels of Pro-NT were then quantified by sphingotec (sphingotec GmbH, Hennigsdorf, Germany) using a previously validated chemiluminometric sandwich immunoassay [12]. During measurement, about two samples were assayed in quadruples on each plate. Using our own validation analyses of this Pro-NT assay, intra-assay coefficient of variation (CV) was 3.6% and inter-assay CV was 4.3%. To investigate associations of Pro-NT with markers of obesity, serum concentrations of leptin and adiponectin were determined with commercially available ELISA kits (Mediagnost, Reutlingen, Germany). As a marker of inflammation, high sensitivity C reactive protein was quantified. Fasting insulin was determined with a two-site chemiluminescent enzyme immunometric assay for the LIAISON automated analyzer (DiaSorin, Saluggia, Italy). Intra- and inter-assay CVs of the insulin assay were <6.5%, respectively. All other parameters including lipid, i.e. high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, triglycerides (TG), and free fatty acids (FFA); renal, i.e. creatinine, and glucose markers, i.e. glycated hemoglobin A1c (HbA1c) and glucose levels during the OGTT, were measured by standard laboratory methods in a certified laboratory using the Cobas Modular Analyzer Series (Roche, Basel, Switzerland).

Statistical analysis

SPSS software version 24.0 (IBM, Armonk, NY) was used in all statistical analyses. Prior to statistical analyses, distribution was tested for normality for all variables using Shapiro–Wilk W test and non-normally distributed parameters were logarithmically transformed. Group

differences between women with GDM, healthy pregnant control women, as well as non-pregnant controls, were assessed by one-way ANOVA or unpaired Student's t-test, respectively. Univariate correlations were performed using Spearman's rank correlation method. In a second step, multivariate linear regression analysis was performed to identify independent relationships. In multivariate regression analysis, only parameters that correlated significantly with Pro-NT in univariate analysis were included. Additionally, pregnancy status was added to the same model instead of the group variable in a second analysis. A p-value of <0.05 was considered as statistically significant in all analyses.

Given the total sample size of 222 and an α of 0.05, we would be able to detect an effect size of f = 0.21 with a power of 80% in the one-way ANOVA.

Results

Baseline characteristics (N = 222)

In the total sample comprising of 74 women with GDM, 74 healthy, pregnant controls, as well as 74 healthy, non-pregnant control women of a second cohort, mean ± standard deviation serum levels of Pro-NT were 108.6 ± 53.7 pmol/l. Clinical characteristics of the subgroups, i.e. women with GDM, pregnant control women, and non-pregnant controls, are shown in Table 1. Mean Pro-NT levels were not significantly different in women with GDM (100.2 ± 75.7 pmol/l) compared to healthy, pregnant (103.2 ± 37.4 pmol/l) and non-pregnant controls (105.9 ± 38.9 pmol/l) (p = 0.661) (Table 1). In contrast to Pro-NT, markers of glucose homeostasis, dyslipidemia, renal function, and inflammation significantly differed between the three subgroups ($p < 0.05$) (Table 1). When study participants with extreme levels of Pro-NT in our cohort were analyzed separately, women in the fourth quartile of serum Pro-NT concentrations had higher total cholesterol, LDL cholesterol, and creatinine as compared to women in the first quartile (Table 2). In contrast, women with circulating Pro-NT in the fourth quartile had significantly lower FFA as compared to subjects in the first quartile (Table 2).

Univariate correlations (N = 222)

Pro-NT positively correlated with gestational age at blood sampling in pregnant women, as well as with HbA1c and creatinine in the entire cohort ($p < 0.05$) (Table 3). In contrast, there was a negative correlation of Pro-NT with FFA (p = 0.005) (Table 3). When correlating Pro-NT with

Table 1 Baseline characteristics of the entire study population (women with GDM, pregnant matched controls, non-pregnant matched controls).

	GDM	Pregnant controls	Non-pregnant controls	p
N	74	74	74	
Pro-NT [pmol/l] (lg)	100.2 ± 75.7	103.2 ± 37.4	105.9 ± 38.9	0.661
Age [years] (lg)	31.3 ± 5.3	29.9 ± 5.0	29.6 ± 3.0	0.134
Gestational age at blood sampling [days] (lg)	201 ± 38	199 ± 39	–	0.792[b]
Gestational age at delivery [days] (lg)	272 ± 12	273 ± 16	–	0.746[b]
Infant birth weight [g] (lg)	3416 ± 545	3468 ± 706	–	0.991[b]
BMI [kg/m^2] (lg)	25.7 ± 6.1	24.0 ± 4.7	23.4 ± 3.5	**0.038**[a]
Systolic blood pressure [mmHg] (lg)	121 ± 14	125 ± 16	121 ± 10	0.295
Diastolic blood pressure [mmHg]	74 ± 11	76 ± 10	77 ± 8	0.151
HbA1c [%] (lg)	5.5 ± 0.8	5.4 ± 0.6	5.1 ± 0.3	**0.001**[a]
HbA1c [mmol/mol] (lg)	36.2 ± 8.8	35.2 ± 6.3	32.5 ± 3.5	**0.001**[a]
Glucose 0 $h_{(OGTT)}$ [mmol/l] (lg)	4.9 ± 1.5	4.2 ± 0.4	4.9 ± 0.4	**< 0.001**[a]
Glucose 1 $h_{(OGTT)}$ [mmol/l] (lg)	10.2 ± 1.8	7.6 ± 1.1	–	**< 0.001**[b]
Glucose 2 $h_{(OGTT)}$ [mmol/l] (lg)	8.6 ± 2.1	6.2 ± 1.2	4.8 ± 1.0	**< 0.001**[a]
$AUC_{Glucose}$ [mmol/l] (lg)	16.9 ± 2.7	12.8 ± 1.5	12.0 ± 1.8	**< 0.001**[a]
FI [pmol/l] (lg)	85.4 ± 60.8	59.0 ± 27.8	34.7 ± 21.2	**< 0.001**[a]
HOMA-IR (lg)	2.66 ± 2.40	1.54 ± 0.77	1.29 ± 0.76	**< 0.001**[a]
Total cholesterol [mmol/l] (lg)	6.73 ± 1.34	6.49 ± 1.38	4.86 ± 0.89	**< 0.001**[a]
HDL cholesterol [mmol/l] (lg)	1.85 ± 0.47	1.90 ± 0.38	1.78 ± 0.42	0.167
LDL cholesterol [mmol/l] (lg)	4.00 ± 1.30	3.95 ± 1.26	2.84 ± 0.94	**< 0.001**[a]
TG [mmol/l] (lg)	2.47 ± 1.07	2.36 ± 0.94	1.01 ± 0.72	**< 0.001**[a]
FFA [mmol/l] (lg)	0.56 ± 0.22	0.50 ± 0.24	0.46 ± 0.21	**0.010**[a]
Creatinine [μmol/l] (lg)	47.6 ± 8.0	49.4 ± 16.5	64.4 ± 9.2	**< 0.001**[a]
hsCRP [mg/l] (lg)	6.03 ± 7.29	4.84 ± 3.33	2.02 ± 3.55	**< 0.001**[a]
Adiponectin [mg/l] (lg)	7.2 ± 4.0	7.1 ± 2.8	17.5 ± 5.0	**< 0.001**[a]
Leptin [μg/l] (lg)	29.2 ± 18.5	24.5 ± 11.9	14.8 ± 8.4	**< 0.001**[a]

Baseline characteristics of the entire study population comprising women with GDM (N = 74), pregnant and gestational age-matched controls (N = 74), as well as non-pregnant and age-matched controls (N = 74; total N = 222). $AUC_{Glucose}$, Area under the glucose curve; BMI, Body mass index; FFA, Free fatty acids; FI, Fasting insulin; GDM, Gestational diabetes mellitus; HbA1c, Glycated hemoglobin A1c; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance; hsCRP, High sensitivity C reactive protein; LDL, Low-density lipoprotein; OGTT, oral glucose tolerance test; Pro-NT, Proneurotensin; TG, Triglycerides. Values for mean ± standard deviation are shown (lg) indicates non-normally distributed parameters as assessed by Shapiro–Wilk test.

[a] and boldface indicate $p < 0.05$ as assessed by one-way NOVA or unpaired Student's t-test, respectively.

[b] Indicates that comparisons between groups were only performed in women during pregnancy, i.e. women with GDM and pregnant controls.

Table 2 Baseline characteristics of a subset of the study population comprising of women with extreme levels of Pro-NT as considered as belonging to the 1st vs. the 4th quartile of serum Pro-NT concentrations.

	Pro-NT Quartile I	Pro-NT Quartile IV	p
N	56	55	
GDM [N (%)]	20 (35)	21 (38)	0.845
Pregnancy status [N (%)]	40 (71)	37 (67)	0.684
Pro-NT [pmol/l] (lg)	60.4 ± 10.6	173.6 ± 66.3	**< 0.001**[a]
Age [years] (lg)	29.8 ± 4.8	30.6 ± 4.6	0.311
BMI [kg/m^2] (lg)	25.4 ± 5.4	24.3 ± 5.4	0.216
Systolic blood pressure [mmHg] (lg)	122 ± 15	122 ± 12	0.786
Diastolic blood pressure [mmHg]	76 ± 11	76 ± 10	0.737
HbA1c [%] (lg)	5.3 ± 0.7	5.4 ± 0.7	0.172
HbA1c [mmol/mol] (lg)	34.1 ± 8.1	35.9 ± 8.0	0.148
Glucose 0 $h_{(OGTT)}$ [mmol/l] (lg)	4.6 ± 0.7	4.9 ± 1.6	0.276
Glucose 2 $h_{(OGTT)}$ [mmol/l] (lg)	6.5 ± 2.0	6.7 ± 2.2	0.738
$AUC_{Glucose}$ [mmol/l] (lg)	13.9 ± 2.9	14.1 ± 3.4	0.863
FI [pmol/l] (lg)	55.9 ± 38.3	63.7 ± 42.3	0.124
HOMA-IR (lg)	1.64 ± 1.18	2.10 ± 2.05	0.060
Total cholesterol [mmol/l] (lg)	5.76 ± 1.13	6.45 ± 1.79	**0.044**[a]
HDL cholesterol [mmol/l] (lg)	1.82 ± 0.44	1.91 ± 0.44	0.247
LDL cholesterol [mmol/l] (lg)	3.29 ± 1.04	3.97 ± 1.52	**0.024**[a]
TG [mmol/l] (lg)	2.07 ± 1.27	1.91 ± 1.07	0.527
FFA [mmol/l] (lg)	0.56 ± 0.20	0.50 ± 0.28	**0.044**[a]
Creatinine [μmol/l] (lg)	51.7 ± 12.1	56.3 ± 10.8	**0.019**[a]
hsCRP [mg/l] (lg)	5.61 ± 7.87	3.67 ± 3.91	0.081
Adiponectin [mg/l] (lg)	9.6 ± 5.5	11.5 ± 6.2	0.058
Leptin [μg/l] (lg)	21.4 ± 13.5	25.6 ± 15.9	0.244

Baseline characteristics of a subset of the study population comprising of women with extreme levels of Pro-NT as considered as belonging to the 1st vs. the 4th quartile of serum Pro-NT concentrations. Values for mean ± standard deviation are shown (lg) indicates non-normally distributed parameters as assessed by Shapiro–Wilk test.

[a] and boldface indicates $p < 0.05$ as assessed by Student's t-test or Chi-squared test, respectively.

FFA in the three subcohorts, FFA were significantly and negatively related to circulating Pro-NT only in women with GDM (Table 4).

Multivariate regression analysis (N = 222)

In multivariate linear regression analysis, HOMA-IR remained a positive predictor of circulating Pro-NT levels after adjustment for age, group (GDM vs. pregnant controls vs. non-pregnant controls), HDL cholesterol, LDL cholesterol, TG, FFA, creatinine, as well as leptin ($p < 0.001$) (Table 3). Furthermore, creatinine was also positively associated with Pro-NT levels in our cohort ($p = 0.026$) (Table 3). In contrast, FFAs were negatively and independently associated with circulating Pro-NT ($p = 0.014$) (Table 3).

When pregnancy status instead of the group variable was included in the model, HOMA-IR ($p < 0.001$) and creatinine ($p = 0.015$) remained an independent and positive predictor of Pro-NT (Table 5). Furthermore, FFAs were still independently and negatively associated with circulating Pro-NT ($p = 0.017$) (Table 5).

Discussion

In the present study, we demonstrated that circulating Pro-NT is not significantly different in women with GDM compared to healthy pregnant and non-pregnant controls. In the total cohort, FFAs significantly and inversely associated with Pro-NT. Further, HOMA-IR and creatinine significantly and positively predicted Pro-NT.

Previously, it was shown in the Malmö Diet and Cancer Study that Pro-NT positively associated with the development of new-onset obesity [14] and diabetes mellitus [16]. In our cohort used in the present study, we could not detect a significant difference in Pro-NT between women with GDM compared to pregnant and non-pregnant controls. However, there was an independent association between Pro-NT and HOMA-IR as a marker of insulin resistance. This is in accordance with data from Barchetta and co-workers demonstrating a positive correlation between Pro-NT and HOMA-IR in a cohort comprising of patients with nonalcoholic fatty liver disease [15]. In contrast, there was no significant association between Pro-NT and metabolically active adipokines including leptin and adiponectin. Furthermore, Pro-NT was not significantly related to markers of obesity. Based on these findings using a highly validated assay [14,16,17], we conclude that Pro-NT is not a marker of GDM status, an adverse adipokine profile, and obesity in pregnancy but is related to markers of insulin resistance, i.e. HOMA-IR.

Aside from its role in regulating glycemia, NT has been implicated in promoting intestinal lipid uptake in mice [14]. In light of these findings, our observation of a negative association between Pro-NT with FFAs seems counterintuitive [14]. FFAs have, however, been found to

Table 3 Univariate correlations and multivariate regression analysis with serum Pro-NT in the entire study population (women with GDM, pregnant matched controls, non-pregnant matched controls).

Dependent variable	Univariate correlations		Multivariate regression analysis	
	–	–	Pro-NT (lg)	
	r	p	β	p
Age [years] (lg)	0.085	0.205	0.090	0.177
Group	–	–	−0.040	0.678
Gestational age at blood sampling [days] (lg)	**0.192**	**0.020**[a‡]	–	–
Gestational age at delivery [days] (lg)	−0.160	0.064[‡]	–	–
Infant birth weight [g] (lg)	−0.141	0.104[‡]	–	–
BMI [kg/m^2] (lg)	−0.071	0.299	–	–
Systolic blood pressure [mmHg] (lg)	0.008	0.903	–	–
Diastolic blood pressure [mmHg]	0.028	0.685	–	–
HbA1c [%] (lg)	**0.148**	**0.029**[a]	–	–
HbA1c [mmol/mol] (lg)	**0.148**	**0.029**[a]	–	–
Glucose 0 h$_{(OGTT)}$ [mmol/l] (lg)	0.018	0.787	–	–
Glucose 2 h$_{(OGTT)}$ [mmol/l] (lg)	−0.025	0.721	–	–
AUC$_{Glucose}$ [mmol/l] (lg)	0.001	0.985	–	–
FI [pmol/l] (lg)	0.091	0.177	–	–
HOMA-IR (lg)	0.121	0.071	**0.304**	**< 0.001**[b]
Total cholesterol [mmol/l] (lg)	0.109	0.106	–	–
HDL cholesterol [mmol/l] (lg)	0.112	0.097	0.112	0.111
LDL cholesterol [mmol/l] (lg)	0.105	0.120	0.122	0.094
TG [mmol/l] (lg)	−0.063	0.347	−0.059	0.536
FFA [mmol/l] (lg)	**−0.191**	**0.005**[a]	**−0.175**	**0.014**[b]
Creatinine [μmol/l] (lg)	**0.189**	**0.005**[a]	**0.182**	**0.026**[b]
hsCRP [mg/l] (lg)	−0.105	0.120	–	–
Adiponectin [mg/l] (lg)	0.089	0.149	–	–
Leptin [μg/l] (lg)	0.129	0.055	−0.056	0.494

Univariate correlations with serum Pro-NT and multivariate regression analysis in the entire study population comprising of women with GDM (N = 74), pregnant and gestational age-matched controls (N = 74), as well as non-pregnant and age-matched controls (N = 74; total N = 222). Multivariate regression analysis was calculated for Pro-NT (lg, dependent variable) adjusted for age (lg), group (GDM vs. pregnant controls vs. non-pregnant controls), HOMA-IR (lg), HDL cholesterol (lg), LDL cholesterol (lg), TG (lg), FFA (lg), creatinine (lg), as well as leptin (lg). Non-normally distributed variables as assessed by Shapiro-Wilk-test were logarithmically transformed prior to multivariate testing (lg). r- and p-values, as well as standardized β-coefficients and p-values, are given. Abbreviations are indicated in Table 1.

[‡] Indicates that correlation analyses were only performed in women during pregnancy, i.e. women with GDM and pregnant controls.

[a] and boldface indicate significant correlation as assessed by Spearman's correlation method.

[b] Indicates significant correlation in multivariate analysis.

increase during pregnancy [23]. In line with this, we found that pregnant women in our cohort (N = 148) had higher FFA levels compared to non-pregnant control women (N = 74) (p = 0.031, data not shown) despite the fact that they did not differ in terms of age. Thus, pregnancy status itself might significantly contribute to the observed effects. However, it should be noted that FFAs survived as an independent predictor of Pro-NT when the multivariate analysis was adjusted for pregnancy status. Thus, further studies in larger sample sets need to be performed to determine whether a relationship exists between age-, BMI-, and (pregnancy-independent) FFAs with Pro-NT.

Table 4 Univariate correlations of Pro-NT with FFA in the entire study population (N = 222), as well as in the subcohorts, i.e. women with GDM, pregnant matched controls, non-pregnant matched controls (N = 74, respectively).

		Pro-NT			
		Entire cohort	GDM	Pregnant controls	Non-pregnant controls
		N = 222	N = 74	N = 74	N = 74
FFA (mmol/l)	r	**−0.191**	**−0.272**	−0.178	−0.126
	p	**0.005**[a]	**0.020**[a]	0.130	0.310

Univariate correlations of Pro-NT with FFA in the entire study population (N = 222), as well as in the subcohorts, i.e. women with GDM, pregnant matched controls, non-pregnant matched controls (N = 74). r- and p-values are given. Abbreviations are indicated in Table 1.

[a] and boldface indicates significant correlation as assessed by Spearman's correlation method.

In addition to HOMA-IR and FFAs, we found that creatinine positively correlated with Pro-NT even after multivariate adjustment. These results are virtually the same when pregnancy status is included in the multivariate model instead of the grouping variable, i.e. GDM vs. pregnant controls vs. non-pregnant controls. Shulkes and co-workers previously demonstrated that patients with end-stage renal disease have increased plasma NT levels and decreased metabolic clearance rate of NT compared to control patients [24]. Further studies need to determine whether or not Pro-NT is a marker and/or involved in the pathogenesis of renal dysfunction/damage potentially linking Pro-NT with cardiovascular diseases and mortality [16,17]. It should be noted that pregnant patients with preeclampsia have elevated creatinine and also show an adverse cardiometabolic status [25]. Since we have excluded women

Table 5 Multivariate regression analysis with serum Pro-NT in the entire study population (women with GDM, pregnant matched controls, non-pregnant matched controls).

	β	p
Age [years] (lg)	0.085	0.201
Pregnancy status	0.123	0.316
HOMA-IR (lg)	**0.295**	**< 0.001**[a]
HDL cholesterol [mmol/l] (lg)	0.098	0.169
LDL cholesterol [mmol/l] (lg)	0.111	0.131
TG [mmol/l] (lg)	−0.103	0.322
FFA [mmol/l] (lg)	**−0.168**	**0.017**[a]
Creatinine [μmol/l] (lg)	**0.218**	**0.015**[a]
Leptin [μg/l] (lg)	−0.061	0.460

Multivariate regression analysis in the entire study population comprising of women with GDM (N = 74), pregnant and gestational age-matched controls (N = 74), as well as non-pregnant and age-matched controls (N = 74; total N = 222). Multivariate regression analysis was calculated for Pro-NT (lg, dependent variable) adjusted for pregnancy status, HbA1c (lg), FFA (lg), and creatinine (lg). Non-normally distributed variables as assessed by Shapiro-Wilk-test were logarithmically transformed prior to multivariate testing (lg). Standardized β-coefficients and p-values are given. Coefficients corresponding to pregnancy status assume that non-pregnant women are coded such that pregnant women have a larger value. Abbreviations are indicated in Table 1.

[a] and boldface indicates significant correlation in multivariate analysis.

with preeclampsia from the present cohort, future studies need to investigate Pro-NT regulation in preeclampsia.

Some limitations of our study need to be emphasized: Our study has been performed in a cross-sectional design, and, therefore, causality cannot be established. Furthermore, sample size is rather low in the three subgroups. On the other hand, extensive phenotyping was performed at a high level of standardization by a trained study team in both cohorts, i.e. pregnant women with GDM or pregnant controls and the Sorbs. Furthermore, all samples of both cohorts were analyzed in a single laboratory.

In conclusion, serum concentrations of Pro-NT do not significantly differ between women with GDM compared to pregnant and non-pregnant controls but does independently associate with HOMA-IR, creatinine, and FFAs.

Conflicts of interest

None declared.

Author contributions

T.E., A.T., and M.F. designed the study, researched data, and wrote the manuscript. S.K., A.H., D.S., and J.K. analyzed the data and reviewed/edited the manuscript. M.B., M.S., and P.K. contributed to the interpretation of the data and reviewed/edited the manuscript.

Guarantors

Dr. Thomas Ebert, Dr. Anke Tönjes, and Professor Mathias Fasshauer are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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The authors of this manuscript have nothing to declare.

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