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Fibulin-1 is associated with cardiovascular risk in non-obese, non-diabetic individuals

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ABSTRACT

Background. Fibulin-1 (FBLN1) is an extracellular matrix protein that appears in blood vessels. Recent studies have confirmed its role in atherogenesis, vascular complications and cardiovascular disease in chronic diseases such as diabetes mellitus. The aim of this study was to evaluate the association between serum fibulin-1 and biochemical indicators of cardiovascular risk: lipid profile, apolipoproteins B, AI, vitamin 25(OH)D and high sensitivity troponin T (hs-cTnT) in non-diabetic subjects.

Materials and methods. The study consisted of 120 normoglycaemic, non-smoking, non-obese Caucasian subjects aged 25–40 (66 women and 54 men). Serum FBLN1 and plasma fasting glucose, lipid profile, C-reactive protein, insulin, glycated haemoglobin, apolipoproteins B100 and AI, total 25(OH)D, and hs-cTnT measurements were performed. Anthropometric parameters, HOMA-IR and atherogenic index (apoB:apoAI) were calculated. Carotid intima-media thickness (IMT) was measured using an ultrasound method. Subjects were divided by FBLN1 tertiles.

Results. FBLN1 was significantly higher in women than in men (1.06 vs. 0.96; $p < 0.05$). FBLN1 positively correlated, when adjusted for age, BMI and blood pressure, with lipid profile, atherogenic index, apolipoprotein B ($R = 0.28$; $p < 0.016$), and hs-cTnT ($R=0.39$; $p < 0.003$) and negatively with 25(OH)D. ApoB and hscTnT were significantly associated with fibulin-1 concentration and, together with 25(OH)D, explained 19% of its variation. FBLN1 ≥1.0 ng/mL predicted atherogenic risk with OR = 3.11 and 4.26 for having elevated apolipoprotein B and hs-TnT.

Conclusions. Fibulin-1 might be a promissing risk factor for cardiovascular risk in young, non-obese, non-diabetic individuals, but this requires further investigation.

Key words: fibulin-1, extracellular matrix proteins, cardiovascular disease, high sensitivity troponin T

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Introduction

The fibulins are a family of seven proteins associated with elastic extracellular matrix fibres and basement membranes. The characteristic feature in the structure of fibulins is a series of epidermal growth factor EGF-like modules, followed by specific fibulin-type module at the C-terminus of the peptide chain, which determines their properties [1].

Fibulin-1 (FBLN1) occurs in four variants (Fig. 1). This protein binds to tropoelastin and is associated with extracellular matrix fibres that contain elastin and fibrillins 1 and 2. FBLN1 is also related to elastin in the core of mature fibres in the skin and blood vessels. FBLN1 is highly expressed during the development of the cardiovascular system (e.g. cardiac valvuloseptal and aortic-arch vessel formation) and is produced by cardiac mesenchymal cells and primordial vascular smooth muscle cells (VSMCs) [2]. Fibulin-1 may play a role in the development or progression of cardiovascular disease [3]. Recent studies have highlighted the role of FBLN1 in several human pathologies, such as myocardium dysfunction, cancer and retinopathy. However, there has only been one study reporting its relation to cardiovascular risk markers [3].

The aim of our study was to evaluate the association between serum fibulin-1 and biochemical indicators of cardiovascular risk in young non-obese, normoglycaemic subjects.

Subjects, materials and methods

The study included 120 normoglycaemic, non-obese, non-smoking, Caucasian subjects with normal

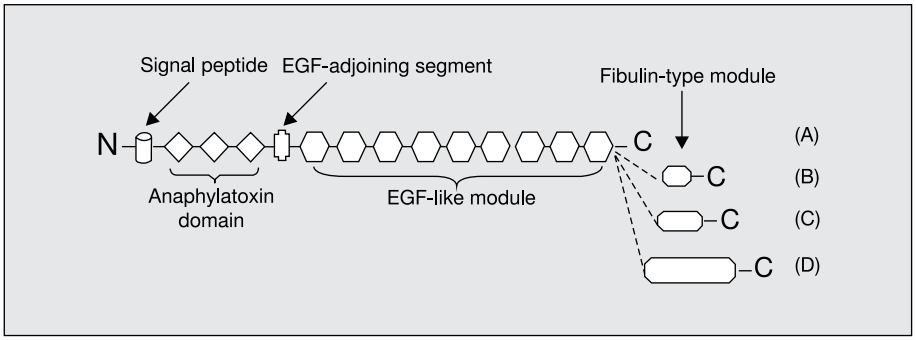

Figure 1. Fibulin-1 (FBLN1) and its variants (A–D) [2].

blood pressure, aged 25–40 (66 women and 54 men) attending a routine medical examination who agreed to participate in the study. Written informed consent was obtained from each participant and the study was approved by the Bioethics Committee at Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland.

Fasting venous blood was drawn in the early morning into tubes containing sodium fluoride, EDTA and clotting activator in order to obtain plasma, whole blood and serum, respectively. Almost all laboratory tests were performed immediately after sample collection, otherwise the remaining serum was stored deep-frozen (–80°C) in small aliquots for further assays.

Laboratory tests were performed in the Department of Laboratory Medicine, Collegium Medicum at Nicolaus Copernicus University. Fasting plasma glucose, serum total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), C-reactive protein (CRP), apolipoproteins B100 and AI (apoB, apoAI) and glycated haemoglobin (HbA_{1c}) were measured using an Abbott ARCHITECT ci8200 analyser. Serum insulin, total 25-(OH)D and high sensitivity troponin T (hs-cTnT) were measured using a Roche Cobas e411 analyser. The limit of detection for hs-cTnT was 3 pg/mL. Fibulin-1 (FBLN1) was assayed by ELISA (Wuhan EIAab Science) with a limit of detection of 0.1 ng/mL. LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C), homeostatic model assessment (HOMA-IR), atherogenic index apoB:apoAI, body mass index (BMI), and waist-hip ratio (WHR) were calculated. The following values for routine laboratory parameters were considered normal: plasma glucose 3.33–5.5 mmol/L (60–99 mg/dL), insulin 2.6–24.9 μIU/mL, CRP < 1 mg/L, TC < 4.91 mmol/L (< 190 mg/dL), TG < 1.69 mmol/L (< 150 mg/dL), HDL-C > 1.03 mmol/L for men and > 1.16 mmol/L for women (> 40 and > 45 mg/dl, respectively), LDL-C < 2.97 mmol/L (< 115 mg/dL), non-HDL-C < 3.75 mmol/L (< 145 mg/dL), 25(OH)D ≥ 30 ng/mL, hs-cTnT < 14 pg/mL (99th percentile for healthy individuals). An HbA_{1c} concentration of 29–42 mmol/mol Hb (4.8–6.0%) was accepted as a reference range for subjects without diabetes. The value of $HbA_{1c} \leq 31.0$ mmol/mol Hb, used in further calculations, was equal to estimated average glucose of ≤ 5.5 mmol/L.

Systolic and diastolic blood pressures (BP) were measured twice at 1–2 minutes intervals according to standard procedures by trained personnel and < 130/85 mm Hg was accepted as normal [4]. Carotid intima-media thickness (IMT) as a marker of subclinical atherosclerosis was measured using an ultrasound method in the Department of Nephrology, Hypertension and Internal Diseases at Collegium Medicum NCU. Values < 0.9 mm were considered normal.

Statistics

Data is presented as mean ± standard deviation (normal distribution) or median and the 25th and 75th percentile (non-Gaussian distribution). Statistical methods: t-Student's test, U-Mann-Whitney test and multivariable regression analysis were performed using Statistica 9.0 for Windows (Stat Soft Inc.). $P < 0.05$ was considered statistically significant.

Results

The clinical and biochemical characteristics of the study group are presented in Table 1. Significantly higher values of most parameters, except total cholesterol, LDL-C and CRP, were found in men. On the other hand, women had significantly higher HDL-C, apoAI and 25(OH)D. Serum concentration of fibulin-1 was in the range of 0.13 to 13.56 ng/mL and was higher in women. Of the study group, 16% were overweight (25–27.8 kg/m^2), 32% had hypercholesterolaemia, 34.5% had moderately increased CRP, and 45% had moderate 25(OH)D deficiency (10–20 ng/mL). Taking into consideration estimated average glucose of 5.5 mmol/L equal to HbA_{1c} of 31 mmol/mol Hb, in 58% of our normoglycaemic subjects the HbA1c concentration was over that cut-off.

Table 1. Clinical and biochemical characteristics of the study group

Parameters	All (n = 120)	Women (n = 66)	Men (n = 54)
Age [years]	30.8 ± 4.7	30.8 ± 4.9	31.03 ± 4.5
BMI [kg/m^2]	22.7 (20–25)	21 (20–23)	24.5 (23–26.5)**
Systolic BP [mmHg]	117.8 ± 9.5	114 ± 9.2	123 ± 7.2**
Diastolic BP [mmHg]	80 ± 9.6	78.3 ± 8.6	82 ± 10.4*
Glucose [mmol/L]	4.98 ± 0.39	4.89 ± 0.36	5.11 ± 0.41**
HbA_{1c} [mmol/mol]	32 (30–36)	32 (29–33)	34 (31–37)*
Insulin [μIU/mL]	7.62 ± 3.53	6.92 ± 2.7	8.53 ± 4.25*
HOMA-IR	1.56 (1.17–2.07)	1.43 (1.12–1.73)	1.69 (1.23–2.47)*
TC [mmol/L]	4.89 (3.21–5.33)	4.92 (4.39–5.25)	4.91 (4.31–5.37)
HDL-C [mmol/L]	1.42 (1.16–1.58)	1.53 (1.37–1.73)	1.19 (1.06–1.5)**
LDL-C [mmol/L]	2.94 (2.45–3.41)	2.76 (2.3–3.23)	3.02 (2.61–3.67)
TG [mmol/L]	0.92 (0.72–1.33)	0.85 (0.68–1.07)	1.16 (0.85–1.68)**
non-HDL-C [mmol/L]	3.44 (2.92–3.90)	3.36 (2.76–3.67)	3.72 (3.18–4.08)*
apoB [g/L]	0.76 (0.65–0.88)	0.73 (0.62–0.82)	0.82 (0.69–0.90)*
apoAI [g/L]	1.45 (1.31–1.64)	1.54 (1.41–1.74)	1.32 (1.19–1.53)**
apoB:apoAI	0.51 (0.41–0.61)	0.45 (0.38–0.53)	0.61 (0.52–0.74)**
CRP [mg/L]	0.6 (0.3–1.6)	0.5 (0.3–1.3)	0.7 (0.4–1.45)
25(OH)D [ng/mL]	18.4 (12.9–24.1)	20.1 (15.1–29.3)	15.3 (10.7–22.1)*
hs-cTnT [pg/mL]	4.72 (3.54–6.18)	4.67 (3.18–6.13)	5.07 (4.15–7.49)
IMT [mm]	0.55 (0.5–0.6)	0.5 (0.5–0.6)	0.6 (0.5–0.65)*
FBLN1 [ng/mL]	**1.05 (0.88–1.22)**	**1.06 (0.96–1.22)**	**0.96 (0.71–1.17)***

Statistically significant differences between women vs. men: *p < 0.05; **p < 0.001

Table 2. Correlation of serum FBLN1 and selected cardiovascular risk markers (log-transformed variables)

	FBLN1			
Parameter	**Unadjusted**		**Adjusted for age, BMI and blood pressure**	
	R	**p**	β	**p**
25(OH)D	–0.35	0.003	–0.23	0.015
TC	0.35	< 0.001	0.26	0.01
LDL-C	0.33	< 0.001	0.25	0.02
non-HDL-C	0.36	< 0.001	0.27	0.001
apoB	0.34	< 0.001	0.28	0.016
apoB:apoAI	0.22	0.014	0.19	0.02
hs-cTnT	0.42	< 0.001	0.39	0.003

Weak or moderate correlation of serum fibulin-1 concentration and biochemical cardiovascular risk markers was observed which remained significant when adjusted for age, BMI and blood pressure (Tab. 2). The strongest correlation was found for FBLN1 and hs-cTnT (β = 0.39; p = 0.003), apoB (β = 0.28; p = 0.016) and non-HDL-C (β = 0.27; p = 0.001). Multivariable regression analysis was performed to assess the independent contributions of different variables to serum fibulin-1 concentration. The best model, with three independent variables: 25(OH)D, apoB and hscTnT, explained 19% of fibulin-1 concentration (R^2 = 0.19; F = 8.31; p < 0.001) (Tab. 3). In this model, two independent variables, apoB and hs-cTnT, correlated weakly but significantly with fibulin-1.

Table 3. Multivariable regression analysis on serum fibulin-1

Model	Independent variable	Adjusted R^2	F	β	p
FBLN1 +		0.19	8.31		< 0.001
	25(OH)D			–0.17	0.08
	apoB			0.22	0.02
	hs-cTnT			0.26	0.01

Table 4. Odds ratio for atherogenic profile in subjects with FBLN1 over 1.0 ng/mL

	FBLN1 ≥ 1.0 ng/mL		
	OR	95% CI	p
apoB ≥ 0.9 g/L	3.1	1.1–9.0	0.03
non-HDL-C ≥ 3.75 mmol/L	2.5	1.1–5.7	0.025
hs-cTnT ≥ 4.75 pg/mL	4.3	1.9–9.7	< 0.001

Afterwards, the distribution of selected cardiovascular risk factors was analysed according to fibulin-1 concentration in tertiles. Parameters such as apoB ≥ 0.9 g/L (≥ 90 mg/dL), non-HDL-C ≥ 3.75 mmol/L (≥ 145 mg/dL), 25(OH)D >10–20 ng/mL and HbA_{1c} > 31 mmol/mol Hb were used to define the risk. The prevalence of subjects with elevated apoB and non-HDL-C (Fig. 2) was 2.3-fold (12% vs. 28%) and 1.7-fold (24% vs. 40%) higher in the third tertile of fibulin-1 concentration, compared to the first one. The percentage of individuals with moderate 25(OH)D deficiency increased from 32% in the first tertile of fibulin-1 concentration to 53% in the third tertile, whereas no differences were observed among subjects with HbA1c concentration over 31 mmol/mol (Fig. 3).

Fibulin-1 concentration ≥ 1.0 ng/mL predicted atherogenic risk with OR 3.1 (95% CI: 1.1–9.0; p = 0.03), 2.5 (95% CI: 1.1–57; p = 0.025) and 4.3 (95% CI: 1.9–9.7; p < 0.001) for having elevated apoB, non-HDL-C and hs-cTnT.

Disscusion

Fibulin-1 seems to play an important role not only in the coagulation process, but also in atherogenesis and is found in atherosclerotic lesions [5]. Two recent studies have highlighted the significance of FBLN1 in cardiovascular disease and vascular complications during chronic kidney disease and diabetes [3, 6]. The results presented in this study, performed in young and apparently healthy normoglycaemic non-obese subjects, showed a significant association of serum FBLN1 with atherogenic risk markers. Moreover, FBLN1 was related to high sensitivity cardiac troponin T which might reflect silent ischaemic disorders characterised by cTnT leakage and potential future coronary risk. Obtained results also indicated a negative association between serum FBLN1 and 25-hydroxyvitamin D concentration. Vitamin 25(OH)D insufficiency has long been known to be related to a higher risk of cardiovascular diseases.

Interestingly, in our study we have not found any association of fibulin-1 with HbA_{1c}, whereas the association with glucose was weak and observed only in tertiles of FBLN1 (glucose in the 1st tertile of FBLN1 was 4.8 vs. 5.2 mmol/L in the 3rd tertile; p < 0.015; results not shown). These findings, in part, contradict the results of others [3, 6]. The study by Cangemi et al. [6] showed a higher expression of FBLN1 and its concentration in plasma in diabetic individuals compared to controls. The protein was detected in arterial wall by immunochemistry

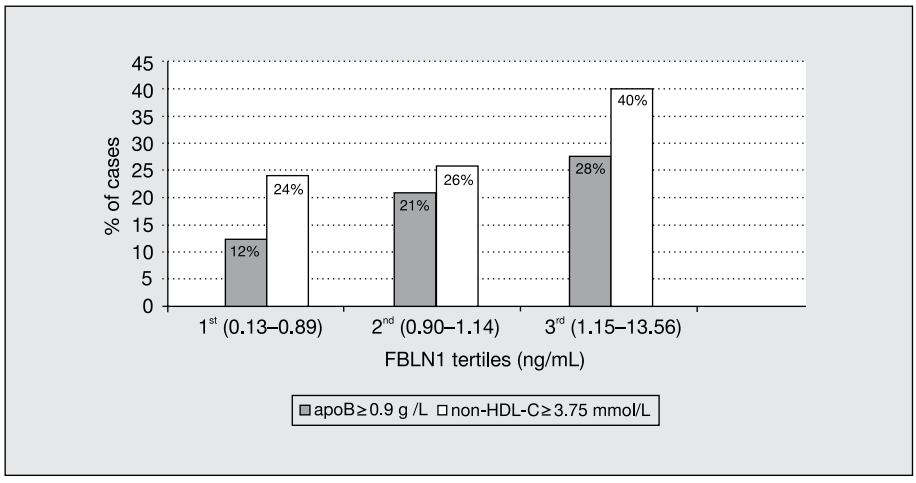

Figure 2. Prevalence of subjects with elevated apoB and non-HDL-C across FBLN1 tertiles

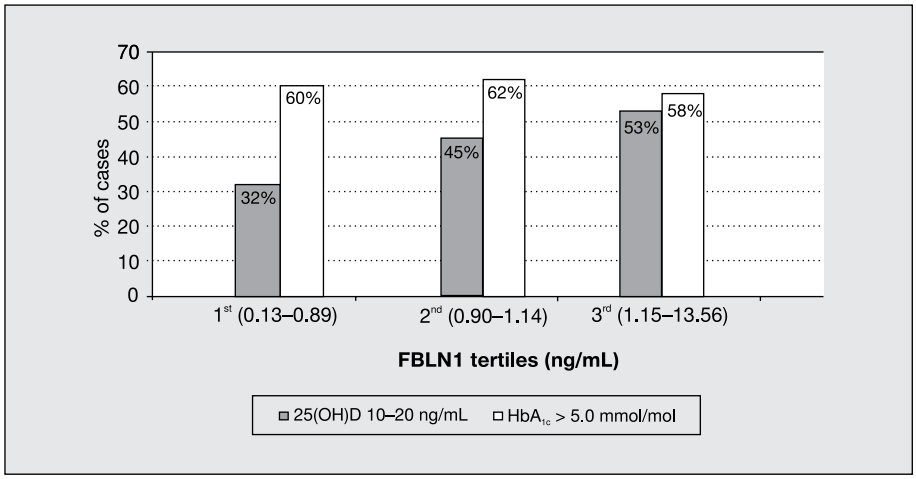

Figure 3. Prevalence of subjects with moderate 25(OH)D deficiency and HbA_{1c} > 5.0 mmol/mol Hb across FBLN1 tertiles

staining, and its highest levels were found in external elastic membranes in both diabetic and healthy individuals. In T2DM subjects, FBLN1 was correlated with blood pressure but not carotid IMT. Moreover, the relationship between plasma FBLN1, glucose and HbA_{1c} was found, but not with lipid profile. Fibulin-1 has been associated with overall and cardiovascular mortality in T2DM patients in a 15-year follow-up. A correlation between FBLN1 and NT-pro-BNP has also been confirmed in clinically healthy African men in the SAfrEIC study after adjustments for age, BMI, blood pressure, heart rate and estimated creatinine clearance [7], although the authors did not investigate the association of fibulin-1 with other biochemical parameters and measures of arterial stiffness.

Scholze et al. showed significantly increased values of plasma FBLN-1 in patients with chronic kidney disease with and without diabetes compared to controls [3]. Fibulin-1 was strongly positively correlated with HbA_{1c} and fibrinogen, creatinine, urea, and eGFR and negatively with heart rate. In this study, the correlation of FBLN-1 with HbA_{1c} and eGFR was performed only in 12 patients. In multivariable regression analysis, FBLN1 was associated with diabetes in CKD patients ($\beta = 0.40$; $p = 0.005$).

In our study, young non-obese normoglycaemic subjects with normal blood pressure were included; this may explain these discrepancies. Using multiple linear regression analysis, we have shown that apoB and hscTnT are significantly associated with fibulin-1 concentration and, together with 25(OH)D, explain 19% of its variation. Additionally, fibulin-1 was found to predict atherogenic risk reflected by elevated apoB, non-HDL-C and hs-cTnT. Even if the relationship between fibulin-1 and IMT was not found, it seems that in nondiabetic individuals the impact of atherogenic risk markers on FBLN1 may be stronger than the effect of glycaemia.

The lack of large population-based studies looking into the role of fibulin-1 as a potential biomarker of cardiovascular risk in clinically healthy, non-diabetic individuals makes it impossible to confirm our findings. We hope that planned follow-up studies on a larger sample will provide valuable information for a better assessment of FBLN1 in relation to cardiovascular risk markers.

Conclusions

Fibulin-1 seems to be a promising indicator of atherogenic risk in apparently healthy, non-diabetic subjects, but this requires further investigation in population-based studies.

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