Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study

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Aims	Growth-differentiation factor-15 (GDF-15) is emerging as an independent prognostic biomarker in patients with cardiovascular (CV) disease. Little is known about the pathophysiological basis for the close association of GDF-15 to future CV events. We hypothesized that GDF-15 is related to underlying CV pathologies.
Methods and results	To relate the levels of GDF-15 to indices of CV dysfunction and disease in elderly individuals, serum levels of GDF-15 were measured in 1004 subjects aged 70 years from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Carotid intima-media thickness and plaque burden, and left ventricular (LV) geometry and function were assessed by ultrasound. Endothelial function was evaluated in forearm resistance vessels and in the brachial artery by venous occlusion plethysmography and ultrasound imaging, respectively. Elevated levels of GDF-15 were related to several CV risk factors (male gender, current smoking, body mass index, waist circumference, diabetes, fasting glucose, triglycerides, and low HDL cholesterol). After adjustment for CV risk factors, increased levels of GDF-15 were associated with reduced endothelium-dependent vasodilation in resistance vessels, plaque burden, LV mass and concentric LV hypertrophy, reduced LV ejection fraction, and clinical manifestations of coronary artery disease and heart failure.
Conclusion	GDF-15 carries information on CV dysfunction and disease that is not captured by traditional CV risk factors in elderly individuals.
Keywords	GDF-15 • Biomarker • Endothelial function • Atherosclerosis • Hypertrophy • Heart failure

Introduction

Growth-differentiation factor-15 (GDF-15) is a distant member of the transforming growth factor-β cytokine superfamily that was initially cloned from an activated macrophage cell line.[1] While GDF-15 is weakly expressed in most tissues under physiological conditions,[2] its expression levels may increase in response to pathological stress associated with inflammation or tissue injury. Along that line, GDF-15 has been detected in macrophages in human carotid atherectomy specimens.[3] GDF-15 is also expressed in the myocardium of patients with acute myocardial infarction (MI).[4] It has recently been shown that the circulating levels of

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GDF-15 are elevated and closely related to mortality in patients with non−ST-elevation acute coronary syndrome (NSTE-ACS) or chronic heart failure.[5,6] GDF-15 adds prognostic information to clinical risk indicators and established biomarkers in NSTE-ACS and heart failure, indicating that GDF-15 may provide insight into a distinct pathophysiological axis.[5,6]

While GDF-15 is emerging as a prognostic marker in patients with established CV disease, little is known about the (patho)biological information that is carried by this biomarker in unselected individuals from the general population. It has previously been shown that elderly subjects with no apparent CV disease or other illness have lower levels of GDF-15 when compared with patients with NSTE-ACS or chronic heart failure.[7] GDF-15 levels are significantly related to C-reactive protein in these individuals, thus supporting an association between GDF-15 and inflammation.[7] A relation of GDF-15 to C-reactive protein has also been observed in elderly women with no previous evidence of CV disease.[8] Increasing levels of GDF-15 were associated with the risk of future CV events in that study. Notably, this effect was independent of traditional CV risk factors and additive to that of C-reactive protein.[8]

In the present investigation, we first characterized the relations of GDF-15 to traditional CV risk factors in a community-based cohort of subjects aged 70 years from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study.[9] Based on the standardized assessment of vascular function, carotid artery atherosclerosis, LV geometry and function, and CV diagnoses in these subjects, we then explored different mechanisms by which high levels of GDF-15 could be associated with signs or symptoms of CV dysfunction and morbidity.

Methods

Study population

All subjects aged 70 years living in the community of Uppsala, Sweden, were eligible for the study. The subjects were chosen from the register of community living and were invited in a randomized order from the start of the study in April 2001 to the last included subject in June 2004. The subjects received an invitation letter within 2 months of their 70th birthday in order to standardize for age. Of the 2025 subjects invited, 1016 subjects were investigated giving a participation rate of ∼50%. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave informed consent.

Baseline investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits, and regular medication. Reported hospital treated MIs, coronary revascularizations, and strokes were verified by hospital records. All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After recordings of height, weight, and waist circumference, an arterial cannula was inserted in the brachial artery for blood sampling, and later regional infusions of vasodilators. Lipid variables, fasting blood glucose, and creatinine were measured by standard laboratory techniques. Glomerular filtration rate (GFR) was estimated using the four-variable Modification of Diet in Renal Disease Study equation.[10] Almost half of the cohort reported any CV medication (45%), with anti-hypertensive medication being the most

prevalent (32%). Fifteen per cent reported the use of statins, while insulin and oral anti-glycemic drugs were reported in 2 and 6%, respectively. Diabetes mellitus was defined as a self-reported history of diabetes or fasting blood glucose of ≥6.2 mmol/L. Hypertension was defined as anti-hypertensive treatment or a blood pressure >140/90 mmHg at the examination.

Carotid artery ultrasound evaluation

The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, CA, USA) as described previously.[11] In brief, the common carotid artery, the bulb and the internal carotid artery were visualized and the occurrence of plaque was recorded on both sides. The intima-media thickness (IMT) was evaluated in the far wall in the common carotid arteries 1−2 cm proximal to the bulb; the mean value from both sides is reported. A plaque was considered to be present if the IMT was locally thickened by more than 50% when compared with the surrounding IMT.

Forearm blood flow measurements

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden) with the strain-gauge technique as reported previously.[9] An arterial cannula was placed in the brachial artery. Resting FBF was measured 30 min after cannula insertion, and local intra-arterial drug-infusions were given during 5 min for each dose with a 20 min washout period between the drugs. The infused dosages were 25 and 50 μg/min of acetylcholine (Clin-Alpha, Läufelfingen, Switzerland) and 5 and 10 μg/min of sodium nitroprusside (Abbott, Maidenhead, UK). The drugs were given in random order at a maximum rate of 1 mL/min. Endothelium-dependent vasodilation was defined as FBF during infusion of 50 μg/min of acetylcholine minus resting FBF divided by resting FBF. Endothelium-independent vasodilation was defined as FBF during infusion of 10 μg/min of sodium nitroprusside minus resting FBF divided by resting FBF.

Brachial artery ultrasound examination

The brachial artery was assessed by external B-mode ultrasound imaging 2−3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer), as described previously.[9] A cuff was placed below the elbow and inflated to a pressure at least 50 mmHg above systolic blood pressure for 5 min. Flow-mediated dilation was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus diameter at rest divided by the diameter at rest.

Echocardiography

Two-dimensional echocardiography was performed with an Acuson XP124 cardiac ultrasound unit, as described previously.[12] Left ventricular dimensions were measured with M-mode. Ejection fraction was determined according to the method of Teicholtz. Left ventricular mass was determined according to the Penn convention and indexed for height$^{2.7}$ to obtain LV mass index. Four geometric subgroups were defined according to Simone et al.[13]

Biochemistry

The concentration of GDF-15 was determined by a recently established and validated immunoradiometric assay.[7] The assay has a theoretical detection limit of 20 ng/L and allows reliable analyte quantification over a wide concentration range (∼200−50 000 ng/L).

Serum samples that had not previously been thawed were available from 1004 subjects for the present study.

High-sensitivity C-reactive protein was measured by an ultrasensitive particle-enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was determined with a sandwich immunoassay on an Elecsys 2010 instrument (Roche Diagnostics, Mannheim, Germany).

Statistics

Baseline characteristics are presented as proportions or medians with interquartile range. Pearson's correlations were used to evaluate the relations between the levels of GDF-15 and CV risk factors and biochemical parameters. Pearson's correlations and linear regressions were used to evaluate the relations of GDF-15 to different CV diagnoses and measures obtained by carotid artery ultrasound evaluation, forearm blood flow measurements, brachial artery ultrasound examination, and echocardiography. The regressions are reported unadjusted and adjusted for traditional risk factors (gender, current smoking, hypertension, diabetes, and LDL and HDL cholesterol). The predictive value of GDF-15, the Framingham risk score, endothelium-dependent vasodilation, and IMT for any CV diagnosis was assessed by simple and multiple logistic regression analysis. The values of GDF-15, C-reactive protein, NT-proBNP, and endothelium-dependent and -independent vasodilations were not normally distributed and transformed to their natural logarithms (ln) before all analyses. All P-values reported are two-sided and were regarded as statistically significant if <0.05. No adjustments for multiplicity were made, as the results are to be considered exploratory. All calculations were performed with SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

GDF-15 levels in the study population ranged from 450 to 9338 ng/L with a median (interquartile range) value of 1134 (948−1390) ng/L.

GDF-15 in relation to risk factors and biochemical markers of cardiovascular risk and disease

GDF-15 levels were significantly related to male gender, current smoking, body mass index, waist circumference, and diabetes. GDF-15 was also related to fasting blood glucose and triglycerides, and inversely related to LDL and HDL cholesterol. Moreover, GDF-15 was significantly associated with C-reactive protein, NT-proBNP, and a reduced GFR (*Table 1*). In a multiple regression model, male gender ($P < 0.001$), current smoking ($P < 0.001$), diabetes ($P < 0.001$), triglycerides ($P = 0.027$), low LDL ($P < 0.001$) and low HDL cholesterol ($P < 0.001$), C-reactive protein ($P < 0.001$), NT-proBNP ($P < 0.001$), and a reduced GFR ($P < 0.001$) were independently related to GDF-15 ($R^2=0.32$).

GDF-15 in relation to vascular function

Endothelium-dependent and -independent vasodilations in forearm resistance vessels declined with increasing levels of GDF-15 (*Table 2*). Flow-mediated dilation in the brachial artery, however, was not related to GDF-15. After adjustment for risk factors, endothelium-dependent vasodilation remained inversely and significantly related to GDF-15 (*Table 2*).

GDF-15 in relation to carotid intima-media thickness and plaque burden

GDF-15 levels were significantly related to carotid artery IMT, but this relation lost significance following adjustment for risk factors (*Table 2*). The proportion of subjects with no carotid plaques decreased, whereas the proportion of subjects with plaques in one or both carotid arteries increased with increasing levels of GDF-15 (*Figure 1A*; $P < 0.001$, Pearson's rho, 0.13). The relation of GDF-15 to carotid plaque burden remained significant following adjustment for CV risk factors ($P = 0.031$).

GDF-15 in relation to left ventricular geometry and function

GDF-15 levels were significantly related to increased LV mass index and end-diastolic diameter, and to a reduced LV ejection fraction (*Table 2*). After adjustment for CV risk factors, GDF-15 remained significantly associated with increased LV mass index and reduced LV ejection fraction, whereas the relation to LV end-diastolic diameter was attenuated ($P = 0.07$) (*Table 2*). The proportion of subjects with normal LV geometry declined with increasing levels of GDF-15; conversely, the proportion of subjects with concentric LV remodelling and concentric hypertrophy increased with increasing levels of GDF-15 (*Figure 1B*; $P < 0.001$, Pearson's rho, 0.17). The association of GDF-15 to LV geometry remained significant following adjustment for risk factors ($P < 0.001$).

Prevalence of cardiovascular diagnoses in relation to GDF-15

When all CV diagnoses were combined, a highly significant relation to GDF-15 levels was observed, also following adjustment for CV risk factors (*Table 3*). A similar picture emerged when GDF-15 levels were related to the prevalence of established coronary artery disease (combined endpoint of previous angina pectoris, hospital-treated MI, and/or previous coronary revascularization) (*Table 3*). Looking at individual diagnoses, increasing levels of GDF-15 were significantly related to previous angina pectoris, hospital-treated MI, previous coronary revascularization, history of heart failure, and hospital-treated stroke. Following adjustment for CV risk factors, the same picture was seen except that the relationship between GDF-15 levels and stroke was no longer significant.

Finally, the predictive value of GDF-15 for any CV diagnosis was tested in the context of the Framingham risk score and the CV disease surrogate markers, endothelium-dependent vasodilation and IMT. Out of these four variables, only GDF-15 predicted prevalent CV disease (*Table 4*). GDF-15 remained significantly related to CV disease, after adjustment for the Framingham risk score, endothelium-dependent vasodilation, and IMT (*Table 4*).

Discussion

This study shows that the circulating levels of GDF-15 are related to several indices of CV dysfunction and clinical manifestations of

Table 1 GDF-15 levels in relation to cardiovascular risk factors and biochemical risk and disease indicators

	Total sample (n = 1004)	1st Quartile GDF-15 (<948 ng/L, n = 250)	2nd Quartile GDF-15 (948−1134 ng/L, n = 252)	3rd Quartile GDF-15 (1135−1390 ng/L, n = 252)	4th Quartile GDF-15 (>1390 ng/L, n = 250)	Rho	P-value
Cardiovascular risk factors							
Male gender (%)	50.0	38.0	51.6	50.4	60.0	0.15	<0.001
Current smoking (%)	10.8	3.2	4.0	12.7	23.2	0.23	<0.001
Hypertension (%)	72.2	68.8	71.8	73.0	75.2	0.04	0.18
Systolic BP (mmHg)	148 (134−164)	148 (135−164)	148 (135−164)	148 (136−162)	148 (134−165)	−0.01	0.81
Diastolic BP (mmHg)	78 (72−86)	78 (72−86)	80 (72−85)	80 (72−86)	78 (72−86)	−0.02	0.48
Body mass index (kg/m^2)	26.6 (24.0−29.7)	25.8 (23.6−28.4)	26.3 (23.6−29.0)	27.1 (24.6−29.8)	27.4 (24.3−30.6)	0.15	<0.001
Waist circumference (cm)	91.0 (84.0−98.0)	88.0 (81.0−94.0)	91.0 (83.0−97.0)	92.0 (86.0−100.0)	94.0 (86.0−103.0)	0.21	<0.001
Diabetes mellitus (%)	11.8	8.4	6.3	7.5	24.8	0.26	<0.001
Clinical chemistry							
Glucose (mmol/L)	5.0 (4.6−5.4)	5.0 (4.7−5.4)	4.9 (4.7−5.4)	4.9 (4.6−5.3)	5.2 (4.7−6.0)	0.25	<0.001
Triglycerides (mmol/L)	1.14 (0.87−1.51)	1.05 (0.81−1.47)	1.05 (0.84−1.43)	1.19 (0.92−1.48)	1.28 (0.97−1.69)	0.17	<0.001
LDL cholesterol (mmol/L)	3.33 (2.79−3.93)	3.45 (2.90−3.98)	3.41 (2.89−4.05)	3.36 (2.88−3.89)	3.12 (2.48−3.82)	−0.13	<0.001
HDL cholesterol (mmol/L)	1.4 (1.2−1.8)	1.6 (1.3−1.9)	1.5 (1.3−1.8)	1.4 (1.2−1.7)	1.3 (1.1−1.6)	−0.26	<0.001
C-reactive protein (mg/dL)	1.21 (0.62−2.34)	0.92 (0.54−1.84)	1.05 (0.56−1.88)	1.37 (0.71−2.55)	1.58 (0.93−3.49)	0.25	<0.001
NT-proBNP (ng/L)	110 (65−181)	100 (59−151)	102 (61−166)	108 (66−177)	137 (78−342)	0.26	<0.001
GFR (mL/min)	74.2 (61.5−89.3)	79.6 (66.3−94.8)	75.5 (63.3−89.3)	73.8 (60.6−86.7)	66.4 (56.2−82.6)	−0.24	<0.001

Data are presented as percentages (%) or median (interquartile range). P-values were calculated from regression analyses with GDF-15 as the dependent variable. GDF-15, C-reactive protein, and NT-proBNP were ln transformed before analysis.
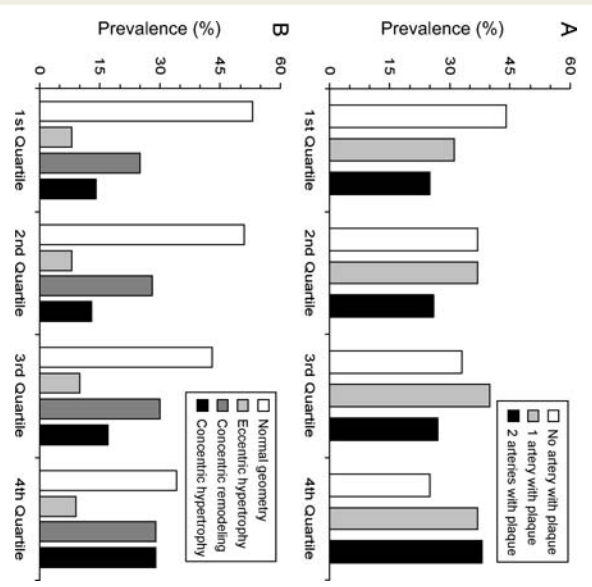
BP, blood pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; GFR, glomerular filtration rate. Rho, Pearson's correlation coefficient. Current smoking, n = 1003; systolic and diastolic BP, n = 1000; waist circumference, n = 993; glucose, triglycerides, HDL cholesterol, and NT-proBNP, n = 1001; LDL cholesterol and GFR, n = 999; C-reactive protein, n = 1002; all other variables, n = 1004.

Table 2 GDF-15 levels in relation to vascular and cardiac structure and function

	Total sample	1st Quartile GDF-15 (<948 ng/L)	2nd Quartile GDF-15 (948–1134 ng/L)	3rd Quartile GDF-15 (1135–1390 ng/L)	4th Quartile GDF-15 (>1390 ng/L)	Rho	P-value	Adjusted for risk factors P-value
Vascular function and IMT								
EDV (%)	460 (311–674)	514 (366–727)	489 (335–714)	454 (300–639)	398 (259–587)	−0.16	<0.001	<0.001
EIV (%)	329 (220–467)	351 (231–495)	341 (245–504)	318 (219–453)	298 (188–428)	−0.13	<0.001	0.15
FMD (%)	4.44 (2.50–6.90)	4.30 (2.44–6.67)	4.88 (2.50–6.90)	4.44 (2.63–6.67)	4.35 (2.50–7.14)	0.00	0.97	0.71
IMT (mm)	0.87 (0.77–0.98)	0.85 (0.75–0.99)	0.84 (0.76–0.94)	0.89 (0.79–0.99)	0.90 (0.81–1.01)	0.11	<0.001	0.14
LV geometry and function								
LVMI (g/m$^{2.7}$)	41.3 (33.4–50.8)	38.6 (32.2–48.0)	39.0 (32.1–47.8)	42.4 (33.5–51.3)	46.8 (37.1–54.7)	0.21	<0.001	<0.001
LVEDD (mm)	47 (44–50)	46 (44–49)	47 (43–50)	46 (43–51)	47 (45–51)	0.14	<0.001	0.07
LVEF (%)	68 (63–72)	69 (66–72)	68 (63–72)	68 (62–72)	66 (60–71)	−0.19	<0.001	<0.001

Data are presented as median (interquartile range). GDF-15, EDV, and EIDV were ln transformed before the analyses. P-values were derived from regression analyses with GDF-15 as the dependent variable. Risk factors include gender, current smoking, hypertension, diabetes, LDL and HDL cholesterol.
EDV, endothelium-dependent vasodilation (n = 855); EIV, endothelium-independent vasodilation (n = 872); FMD, flow-mediated dilation (n = 978); IMT, intima-media thickness (n = 944); LV, left ventricular; LVMI, left ventricular mass index (n = 912); LVEDD, left ventricular end-diastolic diameter (n = 914); LVEF, left ventricular ejection fraction (n = 826). Rho, Pearson's correlation coefficient.

Figure 1 (A) Proportion of subjects without carotid artery plaques or with plaques in one or both carotid arteries according to quartiles of GDF-15 (n = 236, n = 231, n = 233, and n = 232 in the four strata). (B) Proportion of subjects with different left ventricular (LV) geometries according to quartiles of GDF-15 (n = 236, n = 229, n = 230, and n = 217 in the four strata).

CV disease in a large population sample of elderly individuals. Notably, the associations of GDF-15 to reduced endothelium-dependent vasodilation, LV mass index and concentric hypertrophy, reduced LV ejection fraction, clinical manifestations of coronary artery disease, and to heart failure were independent of traditional CV risk factors.

GDF-15 and cardiovascular risk factors

The median level of GDF-15 in the present population was lower when compared with the median values measured with the same assay in some diseased populations of a similar age,[5,6] but higher than the levels found in a younger healthy population sample.[7] GDF-15 levels were independently related to male gender, current smoking, and diabetes in the PIVUS cohort. Notably, the same independent relations have been observed in patients with NSTE-ACS.[5] In PIVUS, we also observed independent relations of GDF-15 to triglyceride levels, and to low LDL and low HDL cholesterol levels. Triglyceride-rich lipoproteins have been found to stimulate GDF-15 expression in human coronary artery smooth muscle cells.[14] Moreover, recent studies have shown that GDF-15 is expressed and secreted by human adipocytes upon exposure to oxidative stress,[15] and that mouse adipocytes can release paracrine factors that stimulate other cell types to produce GDF-15.[16] While these data support a link between GDF-15 and lipid metabolism, the exact roles of GDF-15 in this context need to be further explored.

GDF-15 levels in patients with NSTE-ACS and heart failure are related to circulating markers of inflammatory activity (C-reactive protein), myocardial dysfunction (NT-proBNP), and renal

Table 3 GDF-15 levels in relation to cardiovascular diagnoses

	Total sample	1st Quartile GDF-15 (<948 ng/L)	2nd Quartile GDF-15 (948–1134 ng/L)	3rd Quartile GDF-15 (1135–1390 ng/L)	4th Quartile GDF-15 (>1390 ng/L)	P-value	Adjusted for risk factors P-value
Any cardiovascular diagnosis	16.0	8.4	13.9	13.5	28.4	<0.001	<0.001
Coronary artery disease	12.3	6.4	10.7	10.3	21.6	<0.001	<0.001
Angina pectoris	8.2	4.0	8.8	6.0	14.1	<0.001	0.007
Hospital treated MI	6.7	3.2	4.0	5.6	14.1	<0.001	<0.001
PCI/CABG	5.4	1.6	4.4	4.8	10.8	<0.001	<0.001
Heart failure	3.5	1.6	1.2	2.0	9.3	<0.001	<0.001
Hospital-treated stroke	3.1	0.8	2.8	2.8	6.0	0.006	0.18

Data are presented as percentages. *P*-values were derived from regression analyses with GDF-15 as the dependent variable. Risk factors include gender, current smoking, hypertension, diabetes, LDL and HDL cholesterol. For all variables, *n* = 998 to 1004.
MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery.

Table 4 Predictive value of GDF-15 for any cardiovascular diagnosis

Parameter	Simple model OR (95% CI)	P-value	Multiple model OR (95% CI)	P-value
Framingham score	1.02 (0.97 to 1.07)	0.53	1.00 (0.94 to 1.07)	0.92
EDV	0.81 (0.58 to 1.13)	0.21	0.99 (0.69 to 1.41)	0.96
IMT	1.11 (0.39 to 3.21)	0.84	0.51 (0.13 to 1.91)	0.32
GDF-15	3.95 (2.49 to 6.25)	<0.001	4.08 (2.22 to 7.50)	<0.001

Simple and multiple logistic regressions, any CV diagnosis as outcome variable. All variables were treated as continuous variables. GDF-15 and EDV were ln transformed. The multiple models includes all four variables. ORs refer to a 1-point increase in 10-year risk calculated by the Framingham risk score, an increase of 1 unit in the ln scale for EDV and GDF-15, and a 1 mm increase in IMT.
OR, estimated odds ratio; CI, confidence interval; EDV, endothelium-dependent vasodilation; IMT, intima-media thickness.

dysfunction.[5,6] The present study confirmed these findings in an unselected cohort of elderly individuals. Considering its relation to C-reactive protein, GDF-15 seems to be, in part, a marker of inflammation, which is consistent with its expression by activated macrophages,[1,3] and the observation of increased circulating levels of GDF-15 in patients with chronic inflammatory disease, such as rheumatoid arthritis.[17] The relation of GDF-15 to NT-proBNP indicates that GDF-15 reflects, to some extent, cardiac pathologies (see below). Higher levels of GDF-15 with decreasing renal function suggest that GDF15 is cleared from the circulation by the kidneys and/or that GDF-15 synthesis is increased in renal disease, as it is suggested by animal models of renal injury.[18,19]

It should be emphasized that these relations of GDF-15 to CV risk factors and clinical and biochemical indicators of CV risk and disease explained only some of the variation in the GDF-15 level (the model R^2 value was 0.32), highlighting that GDF-15 provides additional, independent information.

GDF-15 and measures of endothelial function

High GDF-15 levels were associated with a reduced capacity for endothelium-dependent and -independent vasodilations in resistance vessels, but not with flow-mediated vasodilation in the brachial artery. It has previously been shown in this cohort, as well as in younger subjects, that endothelium-dependent vasodilation in resistance vessels and the brachial conduit artery are not well correlated.[9,20] As previously discussed,[21] methodological differences in determining vasoreactivity, as well as different mechanisms promoting vasodilation in these two vascular beds may explain this discrepancy. Another reason might be that flow-mediated vasodilation in the brachial artery becomes markedly reduced by age, also in healthy elderly individuals, and it might therefore be difficult to relate flow-mediated vasodilation to any physiological parameter when the range of values is narrow.[22] Furthermore, it has been shown that flow-mediated vasodilation is influenced by arterial stiffening,[23] a condition often found in the elderly.

Common causes of endothelial dysfunction, including the GDF-15-related CV risk factors smoking and diabetes, operate via generation of oxidant stress within the vessel wall.[24] Previous studies have shown a rapid decline in endothelium-dependent vasodilation following exposure to inflammatory stimuli,[25,26] and we have previously presented a close link between other inflammatory markers and endothelium-dependent vasodilation in resistance vessels in the present cohort.[21] Oxidant stress and pro-inflammatory cytokines induce GDF-15 expression in macrophages and in other cell types,[3,4,15,27,28] thus providing a potential

link between oxidant stress, inflammation, endothelial dysfunction, and increased circulating levels of GDF-15. It should be emphasized that the association between GDF-15 and reduced endothelium-dependent vasodilation persisted after adjustment for traditional CV risk factors. In contrast, GDF-15 lost its relation to endothelium-independent vasodilation after adjustment, suggesting an independent relation between GDF-15 and endothelial function, and not vasodilation in general.

GDF-15 and cardiovascular disease

GDF-15 levels were closely associated with carotid IMT and plaque burden and clinical manifestations of coronary atherosclerosis, including previous angina pectoris or MI, and a history of revascularization. With the exception of IMT, these associations remained highly significant following adjustment for CV risk factors, suggesting a link between GDF-15 and vascular pathologies that cannot be explained solely by the close association of GDF-15 to several CV risk factors.

Carotid IMT and plaque burden are both measures of atherosclerosis that are associated with CV risk factors and outcomes, and while both indices are closely related, they may have different risk factor associations.[29,30] Similar to GDF-15, cardiac troponin I has previously been shown in the PIVUS cohort to be more closely associated with carotid artery plaque burden when compared with IMT.[31]

Elevated levels of GDF-15 were related to LV mass, a concentric pattern of LV hypertrophy, LV end-diastolic diameter, reduced LV ejection fraction, and a history of heart failure. GDF-15 remained independently associated with LV mass, reduced LV ejection fraction, and a history of heart failure after adjustment for CV risk factors. Experimentally, cardiac myocytes produce GDF-15 in response to mechanical stretch, ischaemia, oxidative and nitrosative stress, and angiotensin II stimulation.[4,27,32] Consistent with these *in vitro* data, GDF-15 expression is induced in the heart in mouse models of MI, aortic stenosis, and heart failure.[4,33] Conceptually, these stimuli might also induce GDF-15 expression in the hypertrophied and failing human heart, and the circulating levels of GDF-15 may therefore reflect these processes.

It is intriguing that GDF-15 was independently related to LV mass (shown here for the first time) but not to hypertension (observed here and in patients with established CV disease).[5,34] Although blood pressure is probably the main driver of LV hypertrophy, hormones like insulin, renin, and aldosterone, and other circulating factors have been implicated in the pathogenesis of LV hypertrophy,[35] and future studies should address if GDF-15 has a pressure-independent effect on LV mass.

Notably, GDF-15 was predictive for the presence of any CV diagnosis even after correction for the Framingham risk score, endothelium-dependent vasodilation, and IMT, emphasizing the strong and independent relation of this biomarker to CV disease. It should be pointed out that GDF-15 is not specific for any *particular* CV disease, as increased levels of GDF-15 have been observed in patients with stable and unstable coronary artery disease, heart failure, acute pulmonary embolism, and idiopathic pulmonary arterial hypertension.[5,6,34,36,37]

Limitations of the study

The present sample is limited to Caucasians aged 70, so caution should be used in drawing conclusions for other ethnic and age groups.

The present study had a moderate participation rate. However, an analysis of non-participants showed that the present sample is fairly representative of the total population regarding most CV disorders and drug intake.[21]

Only some of the diagnoses (hospital-treated MI, coronary revascularization, and stroke) were verified by hospital records. Other diagnoses (angina and heart failure) were self-reported, and the prevalence of these conditions must be interpreted with some caution.

Owing to the cross-sectional design of our study, we are not able to ascertain a causal relationship between GDF-15 and CV pathologies, and GDF-15 could be a marker as well as a mediator of the different CV endpoints studied in the PIVUS population.

Conclusion

The present study identifies GDF-15 as an independent marker of cardiac and vascular dysfunction and disease, and provides new insight into the pathobiology of this emerging biomarker.

Funding

Conflict of interest: L.W., T.K., and K.C.W. have filed a patent and have a contract with Roche Diagnostics to develop a GDF-15 assay for CV applications.

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