

Circulating Concentrations of Growth-Differentiation Factor 15 in Apparently Healthy Elderly Individuals and Patients with Chronic Heart Failure as Assessed by a New Immunoradiometric Sandwich Assay

TIBOR KEMPF,¹ RÜDIGER HORN-WICHMANN,² GEORG BRABANT,² TIMO PETER,¹ TIM ALLHOFF,¹ GUNNAR KLEIN,¹ HELMUT DREXLER,¹ NINA JOHNSTON,³ LARS WALLENTIN,³ and KAI C. WOLLERT^{1*}

Background: Growth-differentiation factor 15 (GDF15) is a member of the transforming growth factor β (TGF- β) cytokine superfamily. There has been increasing interest in using circulating GDF15 as a biomarker in patients, for example those with cardiovascular disease.

Methods: We developed an IRMA that uses a polyclonal, affinity chromatography-purified goat anti-human GDF15 IgG antibody, assessed the preanalytic characteristics of GDF15, and determined circulating GDF15 concentrations in 429 apparently healthy elderly individuals and 153 patients with chronic heart failure (CHF).

Results: The assay had a detection limit of 20 ng/L, an intraassay imprecision of $\leq 10.6\%$, and an interassay imprecision of $\leq 12.2\%$. Specificity was demonstrated with size-exclusion chromatography, parallel measurements with polyclonal and monoclonal anti-GDF15 antibody, and lack of cross-reactivity with TGF- β . The assay was not appreciably influenced by the anticoagulant matrix or unrelated biological substances. GDF15 was stable at room temperature for 48 h and resistant to 4 freeze-thaw cycles. Apparently healthy, elderly indi-

viduals presented with a median GDF15 concentration of 762 ng/L (25th–75th percentiles, 600–959 ng/L). GDF15 concentrations were associated with age and with cystatin C and C-reactive protein concentrations. CHF patients had increased GDF15 concentrations that were closely related to disease severity.

Conclusion: The IRMA can detect GDF15 in human serum and plasma with excellent sensitivity and specificity. The reference limits and confounding variables defined for apparently healthy elderly individuals and the favorable preanalytic characteristics of GDF15 are expected to facilitate future studies of GDF15 as a biomarker in various disease settings, including CHF.

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Growth-differentiation factor 15 (GDF15)⁴ is a member of the transforming growth factor β (TGF- β) cytokine superfamily. GDF15 was originally cloned as macrophage-inhibitory cytokine 1 and later also identified as placental TGF- β , placental bone morphogenetic protein, nonsteroidal antiinflammatory drug-activated gene 1, and prostate-derived factor (1–5). Like other TGF- β -related cytokines, GDF15 is synthesized as a precursor protein that undergoes disulfide-linked dimerization. Proteolysis cleaves the correctly folded GDF15 precursor protein to release the N-terminal propeptide from the mature

Departments of ¹Cardiology and Angiology and ²Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany.

³Department of Cardiology and Uppsala Clinical Research Center, University of Uppsala, Uppsala, Sweden.

*Address correspondence to this author at: Abt. Kardiologie und Angiologie, Medizinische Hochschule Hannover, Carl-Neuberg Str. 1, 30625 Hannover, Germany. Fax 49-511-532-5412; e-mail wollert.kai@mh-hannover.de.

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⁴Nonstandard abbreviations: GDF15, growth-differentiation factor 15; TGF- β , transforming growth factor β ; CHF, chronic heart failure; rh, recombinant human; SWISCH, Swedish Women and Men and Ischemic Heart Disease; CRP, C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

GDF15 peptide, which is then secreted as a disulfide-linked dimer with an M_r of $\sim 28\,000$ (1, 6, 7). In tumor cells, unprocessed or partially processed forms of GDF15 may also be secreted, and these forms remain bound to the extracellular matrix and undergo extracellular processing (8).

GDF15 is weakly produced under baseline conditions in most tissues (1, 9). In response to pathologic or environmental stress, however, GDF15 production may sharply increase. For example, experimental carbon tetrachloride poisoning and cryoinjury strongly induce GDF15 expression in the liver and brain, respectively (10, 11). We and others recently reported that GDF15 production increases markedly in the heart in mouse models of myocardial infarction and heart failure (12, 13). Cell culture experiments indicate that GDF15 is involved in the execution of cell survival and cell death programs in neurons and tumor cell lines (14–18). More recent studies of *GDF15*⁵ gene-targeted mice indicating that GDF15 functions as a cardioprotective cytokine during myocardial infarction and heart failure were the first to demonstrate a functional role for GDF15 in vivo (12, 13).

These basic studies have been accompanied by increasing interest in the use of GDF15 as a biomarker for diagnosis, prognosis, and/or risk stratification in different patient populations. Serum GDF15 concentrations have been found to increase during pregnancy (19), and low GDF15 concentrations reportedly are associated with an increased risk of miscarriage (20). Additional studies have shown that patients with pancreatic and colorectal cancer may present with increased circulating GDF15 concentrations (21–23). Moreover, increased circulating GDF15 concentrations have been linked to an enhanced risk of future adverse cardiovascular events in elderly women (24). It is remarkable, therefore, that the preanalytic characteristics of GDF15 and its confounding variables have never been defined for healthy individuals.

In the present study, we established a new sandwich IRMA to measure GDF15 in human serum and plasma. We used this technique to assess the preanalytic characteristics of the analyte and to determine circulating GDF15 concentrations in a cohort of 429 apparently healthy elderly individuals, both to identify potential confounding variables and to establish reference values for future investigations of GDF15 as a biomarker in different patient populations. Given the potential importance of GDF15 in the failing heart (13), we also assessed circulating GDF15 concentrations in 153 patients with chronic heart failure (CHF).

Materials and Methods

MATERIALS

Recombinant human GDF15 (rhGDF15, 957-GD/CF), a polyclonal GDF15 affinity chromatography-purified goat

antihuman GDF15 IgG antibody (AF957), and a monoclonal murine antihuman GDF15 antibody (MAB957) were purchased from R&D Systems. rhTGF- β (T7039) was from Sigma-Aldrich.

GDF15 SANDWICH IRMA

Maxisorp Startubes (Nunc) were coated overnight at 4 °C with 0.5 μ g polyclonal anti-GDF15 antibody in 0.1 mol/L sodium carbonate buffer (pH 9) and then washed twice with phosphate-buffered saline (40 mmol/L sodium phosphate, 150 mmol/L sodium chloride, pH 7.4) containing 1 mL/L Tween 20. Serum and plasma samples (100 μ L) were then diluted 1:1 with assay buffer [30 g/L bovine serum albumin (Sigma-Aldrich), 10 g/L bovine IgG, 10 mL/L goat serum, 1 g/L sodium azide, 1 mol/L sodium chloride, and 40 mmol/L sodium phosphate buffer, pH 7.4], added to the tubes, and incubated for 16 h at 4 °C. Polyclonal anti-GDF15 antibody (20 μ g) was iodinated with 25 MBq ¹²⁵I (Hartmann) with Iodogen (Perbio Science) as previously described (25). Unbound ¹²⁵I was removed by desalting on a 10-mL Sephadex G-25 column (Pharmacia). After removal of the serum or plasma samples, the tubes were washed twice, and 200 μ L of assay buffer containing ~ 7.4 kBq ¹²⁵I-labeled polyclonal anti-GDF15 antibody (tracer) was added to each of the tubes, which were then incubated for 4 h at room temperature. After 3 final washing steps, bound radioactivity was quantified in a gamma counter (LKB Wallac 1261). All measurements were performed in duplicate. In each experiment, a calibration curve was generated with various rhGDF15 dilutions (an rhGDF15 stock solution was stored as aliquots at -70 °C) and used to calculate GDF15 concentrations in individual samples. Pooled control samples (also stored in aliquots at -70 °C) were analyzed with each single run.

In an initial experiment, assay linearity was assessed with serial dilutions of 5 serum samples containing high GDF15 concentrations. These samples were obtained from 2 pregnant women and 3 CHF patients. Independently of the reason for the increased GDF15 serum concentrations, the measured GDF15 concentrations were comparable to the expected concentrations (none of the samples showed a deviation $>15\%$). The assay was linear from ~ 200 to 50 000 ng/L. Moreover, assays of pools of 5 serum samples with low GDF15 concentrations (650–3984 ng/L) with 5 samples with higher concentrations (2506–12 582 ng/L) in 5 different combinations yielded measured concentrations with means well within the range of the expected concentrations (97%–108%).

We used the polyclonal anti-GDF15 antibody for capture and detection in all experiments in the present study, with the exception of 1 validation experiment. In this experiment, we compared the polyclonal anti-GDF15 antibody and a newly available monoclonal anti-GDF15 antibody with respect to antigen capture in serum and citrated plasma (containing 10 mmol/L trisodium citrate) to further evaluate the specificity of the assay.

⁵ Human gene: *GDF15*, growth-differentiation factor 15.

SIZE-EXCLUSION CHROMATOGRAPHY

We equilibrated Sephadex G-100 with Tris buffer (20 mmol/L Tris base, 150 mmol/L sodium chloride, 1 g/L sodium azide, pH 7.0) for 2 h at 80 °C, filled a 408-mL column (Pharmacia) with the swollen dextran beads, and equilibrated the column with this Tris buffer containing 5 g/L bovine serum albumin. We loaded serum and citrated plasma samples (1 mL) from 2 patients with CHF onto the column and collected the eluate in 95 60-drop fractions. We then determined the GDF15 concentration in each fraction by IRMA and used blue dextran (M_r , 2 000 000; Pharmacia) to determine the column's void volume. Bovine serum albumin (M_r , 66 000), rhGDF15 (M_r , 28 000), and amylin (M_r , 3900; Sigma-Aldrich) were used as molecular mass markers.

BLOOD SAMPLES

To establish the GDF15 IRMA and to assess the preanalytic performance of GDF15, we obtained serum samples from apparently healthy medical students [$n = 8$ (6 males); ages, 23–26 years] and their apparently healthy parents [$n = 10$ (5 males); ages, 45–62 years], from CHF patients [$n = 24$ (20 males); ages, 56–73 years], and from women in the 3rd trimester of pregnancy ($n = 4$; ages, 25–29 years). All individuals provided written informed consent, and the ethics committee of Hannover Medical School approved the study.

SWEDISH WOMEN AND MEN AND ISCHEMIC HEART DISEASE (SWISCH) COHORT

GDF15 concentrations were determined in citrated plasma samples from 429 apparently healthy elderly individuals included in the population-based SWISCH study (26). This cohort consisted of 288 men (67.1%) and 141 women (32.9%; median age, 65 years; 25th–75th percentiles, 59–71 years). Individuals with an abnormal resting 12-lead electrocardiogram, cardiovascular medication, established cardiovascular disease, or other chronic disease or acute illness were excluded from the SWISCH study. All participants were required to demonstrate nonpathologic creatinine, blood glucose, and hemoglobin concentrations and normal leukocyte and platelet counts. Citrated plasma samples were stored at -70 °C. We assessed renal function by measuring the cystatin C concentration, inflammatory activity by the C-reactive protein (CRP) concentration, and myocardial wall stress by the N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration. Cystatin C was measured with a latex-enhanced reagent (N Latex Cystatin C) on a BN ProSpec analyzer (Dade Behring). CRP was measured with a chemiluminescent enzyme-labeled immunometric assay (Immulite CRP; Diagnostic Products Corporation) with a detection limit of 0.1 mg/L. NT-proBNP was determined by immunoassay with an Elecsys 2010 (Roche Diagnostics) with a detection limit of 20 ng/L. All individuals provided written informed consent, and the ethics

committees of all participating centers approved the study.

HEART FAILURE POPULATION

GDF15 concentrations were determined in serum samples obtained from 153 patients with compensated (i.e., non-edematous) CHF who were recruited from the outpatient arrhythmia clinic at Hannover Medical School. All of these patients had received an implantable cardioverter-defibrillator for primary or secondary prevention of sudden cardiac death. The CHF diagnosis was based on symptoms, clinical signs, and echocardiographic results according to current practice guidelines (27). This cohort consisted of 129 men (84.3%) and 34 women (15.7%; median age, 68 years; 25th–75th percentiles, 61–73 years). Fifteen, 93, and 38 patients presented with symptoms of New York Heart Association classes I, II, and III, respectively. Because only a few patients had class IV symptoms ($n = 7$), we combined class III and class IV patients. The median left ventricular ejection fraction was 35% (25th–75th percentiles, 25%–46%). Patients were treated with diuretics (48%), angiotensin-converting enzyme inhibitors (77%), β -blockers (78%), and spironolactone (26%). Serum samples were obtained at the time of implantable cardioverter-defibrillator implantation or during follow-up visits and were stored at -70 °C.

STATISTICAL ANALYSIS

Data are presented as the percentage, median (25th–75th percentiles), or mean (SD), as indicated. The CV, calculated as the $(SD/mean) \times 100\%$, was used as a measure of assay imprecision. We used the χ^2 test to evaluate differences in proportions, the Mann–Whitney U -test to analyze differences between the medians of 2 groups, the Kruskal–Wallis test to test for the equality of medians among distinct groups, and ANOVA to test for the differences of means among distinct groups. We used the Spearman rank correlation to identify variables associated with GDF15.

Results

TECHNICAL CHARACTERISTICS OF THE GDF15 IRMA

Detection limit and precision. The detection limit of the assay, calculated as the mean plus 3 SDs for 10 replicate measurements of the zero standard (calibrator free of analyte), was 20 ng/L. The within-run (intraassay) imprecision, determined by measuring 13 serum samples in 8–15 parallel measurements, ranged from 2.8% to 10.6% for samples containing 248–22 480 ng/L GDF15. Total (interassay) imprecision was determined by measuring 16 serum samples in 8–20 assay runs on different days, by 2 different operators, and with different lots of tubes, tracer, and calibrator. The interassay imprecision ranged from 4.0% to 12.2% for samples containing 232–39 370 ng/L GDF15.

Specificity. Size-exclusion chromatographic results for a serum sample obtained from a CHF patient revealed a single peak corresponding in molecular mass to the mature dimeric GDF15 protein (Fig. 1). We obtained virtually identical results with a sample of citrated plasma from another CHF patient (data not shown). To test for potential cross-reactivity with TGF- β , we added increasing amounts of rhTGF- β to 6 serum or citrated plasma samples obtained from apparently healthy individuals, CHF patients, and pregnant women. We observed no cross-reactivity up to a tested concentration of 0.5 mg/L. To further validate the assay, we measured GDF15 concentrations in 77 serum or citrated plasma samples obtained from apparently healthy individuals and from CHF patients by means of 2 parallel IRMA experiments in which we used either the polyclonal or the monoclonal anti-GDF15 antibody for capture and the polyclonal anti-GDF15 antibody for detection. The 2 methods yielded virtually identical results ($\rho = 0.992$; $P < 0.001$; Fig. 2).

Interference studies. To assess whether unrelated biological substances interfere with the GDF15 IRMA, we added several potentially interfering substances to 8 serum or citrated plasma samples, as recommended by the Clinical and Laboratory Standards Institute. ANOVA indicated that the assay was not appreciably influenced by bilirubin (up to a tested concentration of 320 $\mu\text{mol/L}$), hemoglobin (up to 3.2 g/L), albumin (up to 40 g/L), or heparin (up to 400 000 U/L). The GDF15 values obtained for samples with and without added interfering substances differed by $<23\%$ in all cases.

PREANALYTIC PERFORMANCE OF GDF15

We analyzed GDF15 concentrations in parallel in serum, citrated plasma, and EDTA-plasma samples obtained from the same 5 individuals. After correction for sample dilution by the added anticoagulants, the GDF15 concentrations obtained for citrated plasma [99% (9%)] and EDTA-treated plasma [95% (8%)] did not differ signifi-

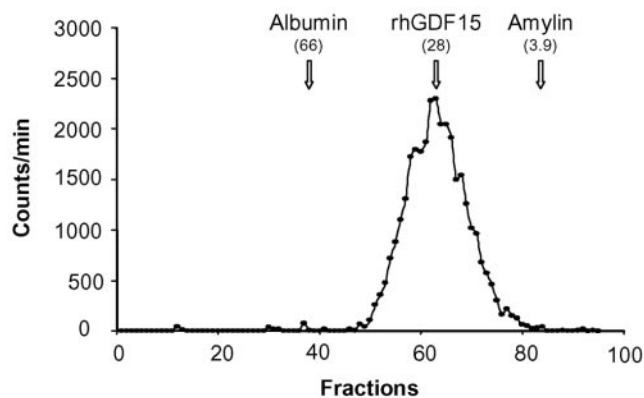


Fig. 1. Size-exclusion chromatography of a serum sample analyzed for GDF15 concentration. Numbers in parentheses indicate relative molecular masses ($\times 1000$).

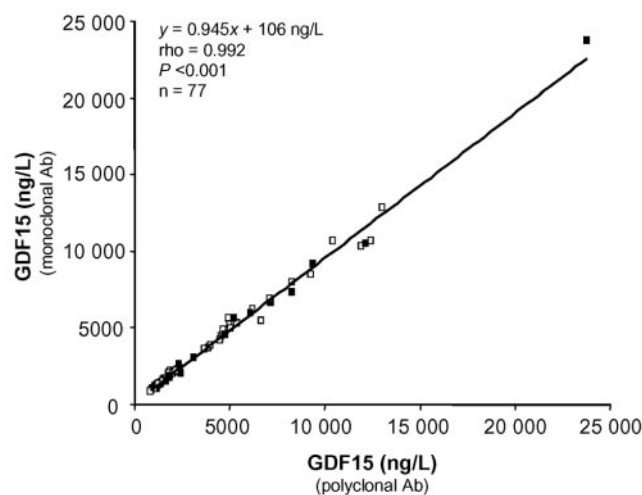


Fig. 2. Correlation of GDF15 concentrations measured with the polyclonal and monoclonal anti-GDF15 antibodies for capture in 2 IRMAs for 77 serum (open squares) and citrated plasma (filled squares) samples.

Ab, antibody.

cantly from the values obtained for serum (100%). Samples of serum or whole blood (containing either citrate or no anticoagulant) from 3 healthy individuals and 2 CHF patients (GDF15 concentration range, 443–41 154 ng/L) stored up to 48 h at room temperature did not produce a discernible loss of GDF15 immunoreactivity (97%–111% vs 100% at baseline). Moreover, 4 cycles of freezing (20 h at -70°C) and thawing (4 h at room temperature) induced no discernible loss of GDF15 immunoreactivity [105% (6%) vs 100% at baseline] in tests of 5 serum samples.

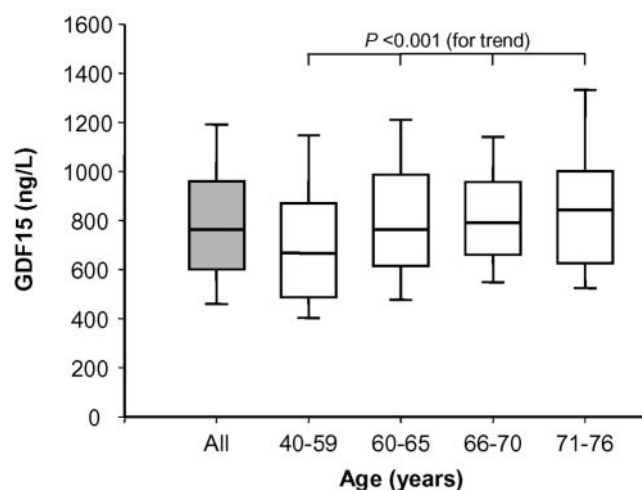


Fig. 3. Circulating GDF15 concentrations in 429 apparently healthy elderly individuals from the SWISCH cohort.

Data are presented as box (25th percentile, median, and 75th percentile) and whisker (10th and 90th percentiles) plots.

Table 1. Relationship between GDF15 quartiles and clinical and biochemical characteristics.^a

	GDF15 quartiles					P ^b
	Total (n = 429)	1st quartile (184–602 ng/L; n = 108)	2nd quartile (603–763 ng/L; n = 107)	3rd quartile (764–959 ng/L; n = 107)	4th quartile (960–2241 ng/L; n = 107)	
Age, years	65 (59–71)	62 (56–69)	65 (59–70)	67 (61–71)	67 (62–72)	<0.001
Male sex, %	67.1	69.4	70.1	63.6	65.4	0.694
BMI, ^c kg/m ²	25.3 (23.1–27.3)	24.9 (22.9–26.6)	25.4 (23.6–27.7)	25.4 (22.9–27.5)	25.6 (23.7–27.4)	0.266
Current smoking, %	14.5	9.3	15.0	17.8	15.9	0.321
Systolic BP, mmHg	140 (130–150)	140 (130–150)	140 (125–150)	140 (130–154)	140 (130–155)	0.175
Diastolic BP, mmHg	80 (75–85)	80 (75–85)	80 (80–84)	80 (76–90)	80 (80–90)	0.420
Cystatin C, mg/L ^d	0.84 (0.76–0.93)	0.78 (0.73–0.88)	0.82 (0.75–0.93)	0.85 (0.79–0.93)	0.89 (0.81–1.00)	<0.001
CRP, mg/L ^e	1.40 (0.79–2.40)	1.20 (0.69–1.83)	1.50 (0.84–2.50)	1.50 (0.78–2.63)	1.60 (0.89–2.85)	0.016
NT-proBNP, ng/L ^f	74 (46–113)	65 (41–106)	79 (49–116)	76 (50–105)	79 (46–118)	0.210

^a Data are presented as the percentage or the median (25th–75th percentiles).

^b χ^2 test for sex and smoking variables; Kruskal–Wallis test for all other variables.

^c BMI, body mass index; BP, blood pressure.

^d Data are available for 100, 95, 99, and 96 individuals in the 1st, 2nd, 3rd, and 4th quartiles, respectively.

^e Data are available for 105, 106, 107, and 105 individuals in the 1st, 2nd, 3rd, and 4th quartiles, respectively.

^f Data are available for 103, 102, 99, and 88 individuals in the 1st, 2nd, 3rd, and 4th quartiles, respectively.

GDF15 CONCENTRATIONS IN APPARENTLY HEALTHY ELDERLY INDIVIDUALS

We used the newly developed IRMA to measure GDF15 concentrations in a cohort of 429 apparently healthy elderly individuals consisting of 288 men (67.1%) and 141 women (32.9%) with a median age of 65 years (25th–75th percentiles, 59–71 years). GDF15 was detected in all samples. The median GDF15 concentration was 762 ng/L, and 334, 460, 600, 959, 1188, and 1532 ng/L marked the 2.5th, 10th, 25th, 75th, 90th, and 97.5th percentiles, respectively (Fig. 3). Individuals older than 65 years had slightly higher median GDF15 concentrations than younger persons: 804 ng/L (25th–75th percentiles, 636–970 ng/L) vs 714 ng/L (25th–75th percentiles, 527–881 ng/L; $P = 0.005$). There was no significant sex difference: 780 ng/L (25th–75th percentiles, 610–967 ng/L) for females vs 749 ng/L (25th–75th percentiles, 588–957 ng/L) for males ($P = 0.507$). As indicated in Table 1 and Fig. 4, increasing GDF15 quartile was associated with age ($P < 0.001$), renal dysfunction (cystatin C, $P < 0.001$), and inflammatory activity (CRP, $P = 0.016$), but there was no significant correlation with sex ($P = 0.694$), body mass index ($P = 0.266$), current smoking ($P = 0.321$), systolic blood pressure ($P = 0.175$), diastolic blood pressure ($P = 0.420$), or NT-proBNP concentration ($P = 0.210$).

GDF15 CONCENTRATIONS IN CHF PATIENTS

We next determined GDF15 concentrations in a cohort of 153 CHF patients consisting of 129 men (84.3%) and 34 women (15.7%) with a median age of 68 years (25th–75th percentiles, 61–73 years). The patient cohort was somewhat older than the healthy control group ($P = 0.002$ vs SWISCH) and included more men ($P = 0.001$ vs SWISCH). GDF15 was detected in all patients. The median GDF15 concentration was 2705 ng/L, and 1054, 1431, 1883, 3994, 6811, and 18 370 ng/L marked the 2.5th, 10th, 25th, 75th, 90th, and 97.5th percentiles, respectively. GDF15 concentrations were significantly higher in heart failure patients than in healthy control individuals ($P < 0.001$ vs SWISCH) and were closely related to New York Heart Association functional class (Fig. 5).

Discussion

We have developed a new sandwich IRMA for the measurement of human GDF15, assessed the preanalytic characteristics of GDF15, evaluated circulating GDF15 concentrations with respect to demographic and biochemical variables in a carefully characterized cohort of apparently healthy elderly individuals, and demonstrated that circulating GDF15 concentrations are increased in CHF patients. This GDF15 IRMA has a detection limit of 20 ng/L and allows reliable analyte quantification over a wide concentration range (~200–50 000 ng/L). No individual in the present study presented with a GDF15 concentration outside this range. The assay has an adequate intraassay and interassay imprecision across the range of values measured in apparently healthy individ-

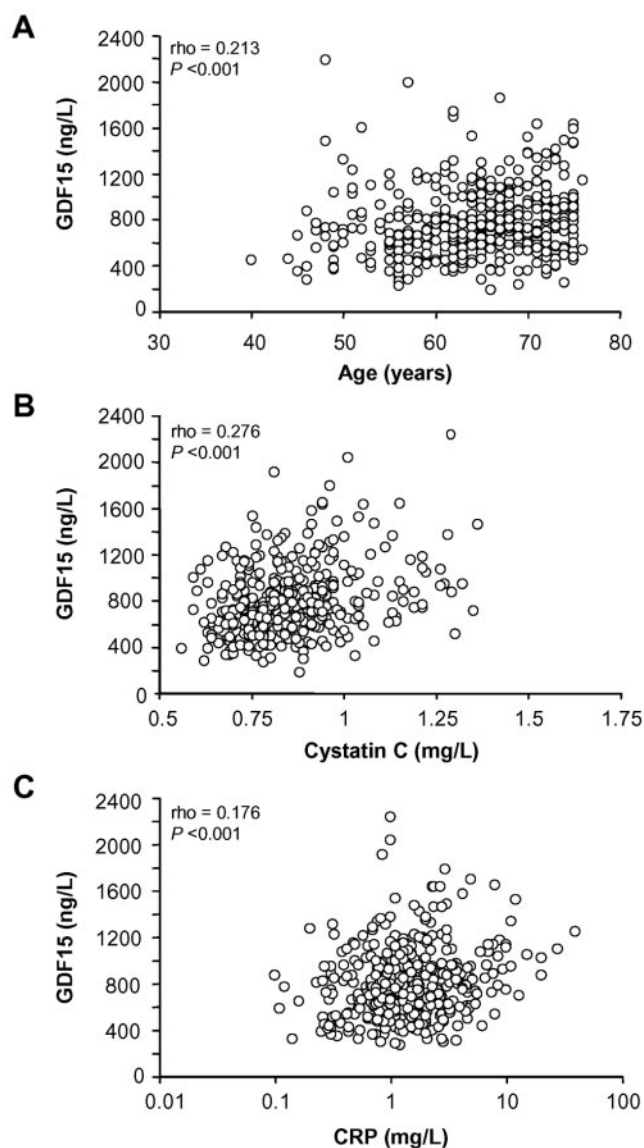


Fig. 4. Correlation between GDF15 and age (A), cystatin C (B), and CRP (C) in apparently healthy elderly individuals from the SWISCH cohort. Data were analyzed with the Spearman rank correlation test.

uals, pregnant women, and patients with cancer or CHF. Dilution studies demonstrated excellent assay linearity. The assay is not appreciably influenced by unrelated biological substances, such as bilirubin, hemoglobin, albumin, and heparin, and shows no cross-reactivity with human TGF- β . Size-exclusion chromatography of a serum sample and a plasma sample from 2 CHF patients produced a single peak with an M_r of $\sim 28\,000$, corresponding to the mass of the mature GDF15 dimer (1). Considering that the polyclonal anti-GDF15 antibody detects both the precursor protein and the mature GDF15 (12), the single peak obtained by size-exclusion chromatography indicates that mostly the mature GDF15 protein is released into the circulation, an observation consistent with previous reports (1, 6, 7, 19). Further supporting the specificity

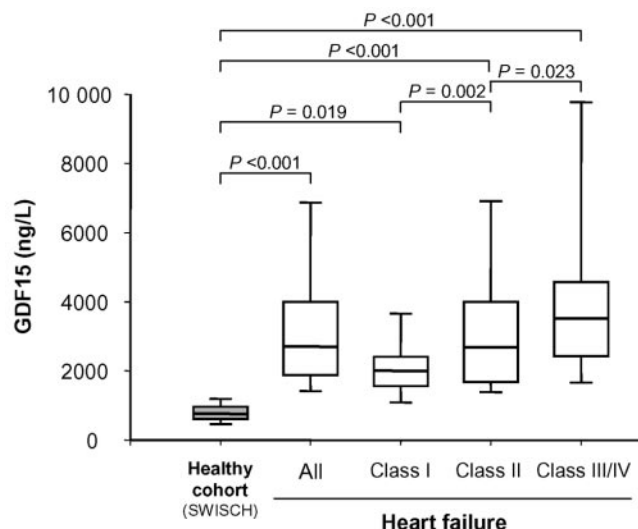


Fig. 5. Circulating GDF15 concentrations in 153 CHF patients and 429 apparently healthy elderly individuals from the SWISCH cohort. Data are presented as box (25th percentile, median, and 75th percentile) and whisker (10th and 90th percentiles) plots.

of the assay is that parallel measurements of patient samples with either the polyclonal or the monoclonal GDF15 antibody for antigen capture yielded virtually identical results. We obtained very similar results in additional studies that compared the performance of the polyclonal and monoclonal antibodies with respect to detection limits, intraassay and interassay precision, specificity, assay linearity, and interference by unrelated biological substances (data not shown).

To our knowledge, only 1 other assay for human GDF15 has been described in the literature. This sandwich ELISA, which is based on a monoclonal antibody that is not commercially available, has been used to measure GDF15 concentrations during pregnancy, in women from the Women's Health Study, and in cancer patients (19–22, 24). By contrast, the assay described in the present report uses only commercially available reagents, which should enable other groups to establish the test and measure GDF15 concentrations in distinct patient populations.

The GDF15 protein was stable in serum and whole blood at room temperature for at least 48 h and was resistant to 4 freeze-thaw cycles. Importantly, the choice of anticoagulant matrix had no influence on analyte measurement. These preanalytic characteristics of human GDF15 may be advantageous in future studies that examine GDF15 concentrations in different disease settings.

We found a slight positive correlation between GDF15 concentration and age in apparently healthy elderly individuals. Increased GDF15 concentrations in plasma have recently been observed to predict future adverse cardiovascular events in the elderly women included in the Women's Health Study, suggesting that GDF15 may be a marker of underlying coronary or cerebrovascular disease

(24). Individuals with manifest cardiovascular disease were excluded from the SWISCH cohort (26); however, because the prevalence of asymptomatic cardiovascular disease increases with age (28), it is possible that some of the individuals with GDF15 concentrations in the upper end of the range may have had asymptomatic cardiovascular disease. Indeed, higher GDF15 concentrations appeared to identify a subgroup of apparently healthy elderly individuals who may have an increased cardiovascular risk because of their increased age and higher cystatin C and CRP concentrations. Future studies might explore the prognostic importance of GDF15 in such individuals. Similarly, occult tumors may have been present in some of these individuals, because increased circulating concentrations of GDF15 have been reported in patients with pancreatic and colorectal cancer (21–23).

Although nonpathologic creatinine values were required for inclusion in the SWISCH study (26), the cystatin C assay, a more sensitive measure of glomerular function that is independent of age, sex, and skeletal muscle mass (29), revealed that renal function was slightly impaired in some individuals [defined as a cystatin C concentration >0.96 mg/L (30)]. Individuals with cystatin C concentrations >0.96 mg/L had $\sim 23\%$ higher GDF15 concentrations than those with nonpathologic cystatin C concentrations, suggesting that GDF15 is cleared from the circulation at least in part through the kidneys and/or that GDF15 synthesis increases in renal disease. GDF15 concentrations were also associated with CRP, a marker of inflammatory activity (31). Consistent with this observation are results of cell culture studies suggesting that GDF15 may be involved in inflammatory reactions by inhibiting macrophage activation (1). Because individuals with manifest infections were excluded from the SWISCH study, the CRP concentrations in this cohort of apparently healthy individuals may reflect asymptomatic inflammation, e.g., an association with atherosclerosis (31). Considering the possibility that individuals from the SWISCH cohort with GDF15 concentrations at the upper end of the spectrum may have occult (cardiovascular) disease, we propose to use 1200 ng/L, the rounded 90th percentile in the SWISCH study, as the upper limit of the reference interval in elderly individuals.

CHF patients had appreciably increased circulating GDF15 concentrations that were closely related to disease severity as determined by the New York Heart Association class. Although the patients in this group were slightly older and included more males than the group of apparently healthy elderly control individuals, these differences cannot explain the 3.6-fold increase in circulating GDF15 concentrations, considering that GDF15 concentrations were increased only slightly with age and were not related to sex in the control group. Increased cardiac GDF15 concentrations have been observed in mice with heart failure (13). Considering that GDF15 is not stored but is rapidly secreted from most cell types, including cardiomyocytes (6, 12), it is possible that increased GDF15

synthesis in the heart partly accounts for the increase in circulating GDF15 concentrations in patients with heart failure. When GDF15 is being considered as a biomarker in heart failure, it must be noted that GDF15 is not a cardiac-specific factor. For example, concentrations as high as 10 000 ng/L have been observed in patients with pancreatic tumors (22). Future studies of larger patient populations could explore the value of GDF15 for prognosis and risk stratification in heart failure patients in the context of clinical variables and other biomarkers.

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