Reference ranges for GDF-15, and risk factors associated with GDF-15, in a large general population cohort

Short title: GDF-15 reference range

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ABSTRACT

Objectives

Growth differentiation factor (GDF)-15 is attracting interest as a biomarker in several areas of medicine. We aimed to evaluate the reference range for GDF-15 in a general population, and to explore demographics, classical cardiovascular disease risk factors, and other cardiac biomarkers associated with GDF-15.

Methods

GDF-15 was measured in serum from 19,462 individuals in the Generation Scotland Scottish Family Health Study. Associations of cardiometabolic risk factors with GDF-15 were tested using adjusted linear regression. Among 18,507 participants with no heart disease, heart failure, or stroke, and not pregnant, reference ranges (median and 97.5th centiles) were derived by decade age bands and sex.

Results

Among males in the reference range population, median [97.5th centile] GDF-15 concentration at age <30 years was 537 [1135]pg/mL, rising to 931 [2492]pg/mL at 50-59 years, and 2152 [5972]pg/mL at ≥80 years. In females, median GDF-15 at age <30 years was 628 [2195]pg/mL, 881 [2323]pg/mL at 50-59 years, and 1847 [6830]pg/mL at ≥80 years. Among those known to be pregnant, median GDF-15 was 19,311pg/mL. After adjustment, GDF-15 was higher in participants with adverse cardiovascular risk factors, including current smoking (+26.1%), those with previous heart disease (+12.7%), stroke (+17.1%), heart failure (+25.3%), and particularly diabetes (+60.2%). GDF-15 had positive associations with cardiac biomarkers cardiac troponin I, cardiac troponin T, and N-terminal pro B-type natriuretic peptide (NT-proBNP).

Conclusions

These data define reference ranges for GDF-15 for comparison in future studies, and identify potentially confounding risk factors and mediators to be considered in interpreting GDF-15 concentrations.

Keywords: biochemical markers; guidelines; reference ranges

List of abbreviations:

GDF	growth differentiation factor
TGF	transforming growth factor
GFRAL	GDNF-family receptor α-like
GS:SFHS	Generation Scotland Scottish Family Health Study
SIMD	Scottish Index of Multiple Deprivation
SMR01	Scottish Morbidity Record
SMR02	Scottish Maternity Record
LOD	limit of detection
NT-proBNP	N-terminal pro B-type natriuretic peptide
cTnI	cardiac troponin I
cTnT	cardiac troponin T
IQI	inter-quartile interval
pTGFB	placental transforming growth factor-beta
PLAB	placental bone morphogenetic protein

Introduction

Growth differentiation factor (GDF)-15, also named macrophage inhibitory cytokine-1, placental transforming growth factor-beta, and placental bone morphogenetic protein is a member of the transforming growth factor (TGF)-β superfamily, and is expressed in low concentrations in many organs [1]. As a stress-induced cytokine, its expression is upregulated because of injury to many organs including in the heart [2,3], lung [4], colon [5], kidney [6], liver [7], pancreas [8], and is expressed in adipose tissue [9]. As such elevated circulating levels of GDF-15 are associated with more advanced disease and poor prognosis in many acute and chronic conditions including heart disease, malignancies, and critical care settings [10–13].

GDF-15 signals via the brainstem restricted GFRAL (GDNF-family receptor α-like) receptor and is thought to supress food uptake and induce cachexia in some conditions [14], as well as mediating at least some of the effects of drugs like metformin and colchicine [15,16]. As a biomarker, higher circulating concentrations of GDF-15 are associated with elevated risk of a range of adverse outcomes in patients with cardiovascular disease, and with cardiovascular disease risk in the general population [17–20]. A clinical assay suitable for in vitro diagnostic use is available for GDF-15, and although its measurement is not currently formally recommended in clinical guidelines for cardiovascular disease, it has recently been granted FDA approval as a companion diagnostic for exploratory cachexia treatment (using Ponsegromab, an anti GDF-15 monoclonal antibody) in some cancer patients [21].

There are currently sparse data from large general population studies exploring the age and sex-stratified reference ranges for GDF-15. Given an evolving role for the GDF-15 biomarker in many areas of clinical medicine including future trials, we sought to establish reference ranges for GDF-15 in a large general population cohort study, and to determine socio-demographics and risk factors associated with GDF-15 concentration.

Materials and methods

Generation Scotland Scottish Family Health Study (GS:SFHS)

The recruitment and design of the GS:SFHS has been reported in detail previously [22–24]. During 2006-2010 potential participants (aged 35–65 years) were identified at random from collaborating general medical practices in Scotland, and invited to participate. Participants were also asked to identify ≥one first-degree relative aged ≥18 years who would be able to participate. A total of 21,476 participants aged between 18 and 98 years attended a research clinic in different urban areas of Scotland. At the clinic, participants had physical and clinical characteristics (including systolic blood pressure (SBP) and body mass index (BMI)) measured according to a standardised protocol and had a questionnaire administered (https://www.ed.ac.uk/generation-scotland/using-resources/scottish-family-health-study). Scottish Index of Multiple Deprivation (SIMD) scores are national composite measures of socioeconomic deprivation and were derived from participant postcodes, with higher scores indicating greater socioeconomic deprivation [25]. Past medical history, including a diagnosis of diabetes mellitus (type 1 or type 2) and prior heart disease, stroke, and cancer was recorded using a self-reported questionnaire. Classification of heart failure status at recruitment was ascertained by

linked data from the Scottish Morbidity Record (SMR01) to identify patients who had been hospitalised for heart failure at any time before their baseline assessment (using International Classification of Disease (ICD)-10 codes I50, I42.0, I42.6, I42.7, I42.9, I11.0). Pregnancy at baseline was identified through linkage to the Scottish Maternity Record (SMR02).

Fasting blood samples were taken, according to a standard operating procedure, and serum samples were separated. Biochemistry measures including total cholesterol, high-density lipoprotein (HDL) cholesterol, and creatinine was measured at the time of collection and additional serum aliquots were stored at -80^{o}C for future biochemical analyses. Estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI equation [26].

Measurement of biomarkers

GDF-15 measurements were undertaken during a single (second) thaw of stored serum aliquots. GDF-15 was measured on a cobas e411 analyser (Roche Diagnostics, Basel, Switzerland) using the manufacturer's reagents and quality control material. Coefficient of variation for GDF-15 was 3.8% for the low control (at 1556pg/mL) and 3.4% for the high control (at 7804pg/mL). The limit of detection (LoD) of the GDF-15 assay is set to 400pg/mL by the manufacturer, and the upper limit of the measuring range was 20,000pg/mL. Samples below the limit of detection were reported as 200pg/mL for continuous analysis and samples above the measuring range as 25,000pg/mL for continuous analysis.

Statistical analysis

Participants with missing data for GDF-15, NT-proBNP, or either cardiac troponin I (cTnI) or cardiac troponin T (cTnT), were excluded from all analyses. By clustered family group, the intra-class correlation coefficient for GDF-15 was 0.06 (95%CI 0.05, 0.08), indicating minimal family clustering; this was therefore not considered a factor in further analyses.

This study was of cross-sectional design. Sex-stratified GDF-15 reference ranges comprising medians, 95th, 97.5th and 99th centiles along with associated bias-corrected 90% confidence intervals (or percentile 90% confidence intervals when no estimate was generated for bias-corrected confidence intervals due to small sample size) were determined by bootstrapping 5000 samples in each age and sex-specific strata. Reference ranges were specifically modelled in participants with no heart disease, heart failure, or stroke, and who were not known to be pregnant (model 1). In a sensitivity analysis, reference ranges were derived after additional exclusions for participants with diabetes, cancer, eGFR<60ml/min/1.73 m^2, NT-proBNP≥400pg/mL (a rule in threshold for heart failure), or cTnI ≥26.2pg/mL, or cTnT≥14pg/mL (thresholds for rule in of myocardial infarction) (model 2). Quantile regression using fractional polynomials was used to further model the relationship between age and the median and 97.5th centile of GDF-15 using model 1.

Associations of GDF-15 (by tertiles of the distribution) with socio-demographics and classical cardiovascular disease risk factors were investigated in the whole cohort. Risk factors were expressed as frequencies and percentages for categorical variables, and for continuous variables were expressed as medians (interquartile interval) when skewed, or as mean (standard deviation) when normally distributed.

The association of GDF-15 with other cardiac biomarkers was illustrated using simple Pearson correlation on z-scores from log-transformed biomarker concentrations. Associations of classical cardiometabolic risk factors with log-transformed GDF-15 were modelled using linear regression with robust standard errors. For these linear regression models, missing data for classical risk factors (1134 missing observations for SIMD score was most frequently missing, no missing observations for age or sex) were imputed by multiple chained imputations over ten datasets. Effect estimates were exponentiated to give the percentage effect on the geometric mean biomarker level. The first model adjusted for age, sex, heart disease, heart failure, stroke, heart failure, diabetes, cancer, and pregnancy. The second model allowed for an interaction of each cardiometabolic risk factor of interest with sex, and included and age-sex interaction in every model. The third model allowed for a categorical age-interaction (ages grouped as ≤50 years, 50-59 years, 60-69 years and ≥70 plus years to avoid unstable estimates in categories with small numbers) and allowed for an age-sex interaction in every model. A final model tested specifically for a BMI-smoking interaction on the basis that these risk factors have a complex relationship. All statistics were performed using STATA version 17.0.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and ethical approval was obtained from the National Health Service Tayside Committee on Medical Research Ethics (REC Reference Number: 05/S1401/89).

Results

Population characteristics

Of the 21,476 GS:SFHS participants 19,462 (90.6%) provided a serum sample with sufficient volume for measurement of GDF-15, cTnI, cTnT and NT-proBNP. Mean age was 47.1 years (standard deviation (sd) 15.0 years), and 8108 participants (41.7%) were male. In the whole cohort, median GDF-15 was 822pg/mL (inter-quartile interval (IQI) 616, 1141pg/mL) and GDF-15 was below the limit of detection in 672 participants (3.5%). Among 876 participants with previous heart disease or stroke, median GDF-15 was 1306pg/mL (inter-quartile interval (IQI) 958, 1987pg/mL). Among 91 participants with baseline heart failure, median GDF-15 was 1776pg/mL (inter-quartile interval (IQI) 1122, 2920pg/mL). Among 58 participants known to be pregnant, median GDF-15 was 19,311pg/mL (inter-quartile interval (IQI) 1033, 25,000pg/mL).

Reference ranges for GDF-15

Among 18,507 participants with no heart disease, stroke, previous heart failure hospitalisation, and not known to be pregnant (model 1), the median overall GDF-15 was 808pg/mL (inter-quartile interval (IQI) 608, 1103pg/mL), median GDF-15 in females was 816 (IQI 623-1103pg/mL), and median GDF-15 in males was 793 (IQI 588-1108pg/mL) (Figure 1). In males, median [97.5th centile] GDF-15 concentration at age <30 years was 537 [1135]pg/mL, rising to 931 [2492]pg/mL at 50-59 years, and 2152 [5972]pg/mL at ≥80 years (Table 1). In females, median GDF-15 at age <30 years was 628 [2195]pg/mL, 881 [2323]pg/mL at 50-59 years, and 1847 [6830]pg/mL at ≥80 years (Model 1, Table 1). In females in the <30 years and 30-39 year age group there were some outliers that drove up the 99th centile

(12,745pg/mL) much higher than the 99th centile in males (1820pg/mL) (Model 1, Table 1). Further exclusions of participants with diabetes, cancer, or elevated cardiac biomarkers (including elevated GDF-15) reduced medians and 97.5th centiles of GDF-15 without ameliorating the overall trend of older age being associated with higher GDF-15 (Model 2, Table 1).

Continuous models of the median and 97.5th centile of GDF-15 (using model 1) showed similar trends, with similar median levels in the sexes, and a slow rise in observed GDF-15 levels up to age 50 years, and then a more rapid rise in both sexes beyond the age of 50 (Figure 2). There were generally more high outlying results among younger females and older males, as reflected in the higher 97.5th centile estimates (Figure 2).

Associations of GDF-15 with cardiovascular risk factors

In the whole cohort of n=19,462 participants, the participants in the upper third of the distribution for GDF-15 were older, had higher BMI, were more likely a current smoker, lower eGFR, had a higher SIMD score indicating greater socioeconomic deprivation, and were more likely to have heart disease or stroke, heart failure, diabetes, or to be pregnant (Table 2). Participants in the upper third of the distribution for GDF-15 were also more likely to use blood pressure or cholesterol medications, and still had generally higher total cholesterol (Table 2) and systolic blood pressure. There was also a strong positive association between GDF-15 with cTnI, cTnT, and NT-proBNP (Table 2). The correlation between GDF-15 and cTnI, cTnT, and NT-proBNP was r=0.24, 0.23, and r=0.31, respectively.

In an adjusted model, age was positively associated with GDF-15 in both sexes (compared to 40-49 year olds, GDF-15 levels approximately doubled in 70-79 year olds), although the association of age with GDF-15 was stronger in males (Table 3). There were strong positive associations with heart disease, stroke, heart failure, cancer, and particularly a strong positive association with diabetes (+60.5%) that was consistent in both sexes (Table 3). Generally these associations with existing disease were strongest in participants aged 50-59 years. GDF-15 was associated with other adverse cardiovascular risk factors, being higher in current smokers and in people with more socioeconomically deprived scores and was inversely associated with eGFR. The association of GDF-15 with eGFR was generally stronger in older age groups (Table 3). BMI was positively associated with GDF-15 in both sexes, although the association was slightly stronger in males (p for sex-interaction 0.003) and weaker in participants age ≥70 years. Total cholesterol was weakly positively associated with GDF-15 in females (p for sex-interaction 0.001), and in younger age groups. GDF-15 was positively associated with cardiac biomarkers in both sexes, particularly NT-proBNP, although the association with troponin I was slightly stronger in females (p<0.001). The association of GDF-15 with cardiac biomarkers was stronger in participants age ≥70 years.

Considering potential interactions between BMI and smoking, every 1kg/m^2 increase in BMI was associated with a 1.0% (95%CI 0.9, 1.2%) higher GDF-15 in non-smokers, although there was no association among smokers (a 0.1% increase (95%CI -0.3, 0.5%)) (p-for interaction <0.001).

Discussion

These data highlight the plethora of physiological and pathophysiological processes that increase circulating GDF-15. Diseases associated with increased GDF-15 include heart disease, heart failure, stroke, cancer, and particularly diabetes, as well as biomarkers of cardiovascular disease such as high sensitivity troponin and NT-proBNP. Once these factors are accounted for, some of the elevation in GDF-15 observed in older people is partially ameliorated. In addition, the highest GDF-15 levels are seen in pregnant females, including some with presumed unidentified early pregnancy. The reference ranges for GDF-15 in this large general population study, stratified by age and sex, will help to contextualise absolute concentrations reported in many future studies which measure this biomarker.

Aside from cardiovascular disease risk prediction, there has been an explosion of interest in GDF-15 in several different clinical conditions. GDF-15 has been recently included in the ABC-bleeding risk score for patients with atrial fibrillation [27]. Recent observations that GDF-15 may be involved in energy balance [28] and may be the key molecule driving weight loss by the drug metformin [15], and the anti-inflammatory effect of colchicine [16], have also hiked interest in the biomarker. The reference ranges we report here for GDF-15 are broadly consistent with, other data. One previous study of 533 healthy adults reported an upper reference limit for GDF-15 of 866pg/mL, reporting no sex differences [29], and another small study a reference interval of 399-1335pg/mL [30]. An earlier study of GDF-15, using an in-house GDF-15 immunoassay in 429 apparently healthy individuals, reported a median GD-15 concentration of 762 ng/L (25th-75th percentiles, 600-959 ng/L) [31]. Our present data from GS:SFHS expand on published data considerably to provide reference intervals by age and sex, illustrating much higher expected levels in older

people. Importantly, our observation that GDF-15 is sometimes very elevated (above the limit of detection) in healthy young females under the age of 40 is consistent with an association of GDF-15 with pregnancy, as seen by the median GDF-15 (>19,000 pg/mL) in females known to be pregnant. GDF-15 has been known by the name placental transforming growth factor-beta (pTGFB) and placental bone morphogenetic protein (PLAB). High levels of GDF-15 gene and protein expression have been observed in the human placenta, follicular fluid, and oocytes, [32,33] serum levels increase rapidly in early pregnancy [34]. There is no reason to believe elevated GDF-15 expression is a result of, or cause of, pathophysiological processes in the mother or the embryo although it has been observed GDF-15 is also associated with hyperemesis gravidarum [35]. The present study has insufficient data to investigate this issue further.

GDF-15 is strongly positively associated with smoking, BMI (among non-smokers), and diabetes in this study, consistent with other work [36,37]. For instance, in the Malmö Diet and Cancer-Cardiovascular Cohort there was a positive association of GDF-15 with incident diabetes over 19 years [38] and directionally similar results were reported in the Whitehall II study [39]. In this sense, an analogy can be made between GDF-15 and the heart failure biomarker NT-proBNP, which we show is moderately strongly correlated with GDF-15. Elevated natriuretic peptides are biomarkers of adverse pathophysiology (volume overload leading to heart failure) but themselves exert beneficial natriuretic diuretic and metabolic effects that partially mitigate the processes that give rise to their expression. Similarly, GDF-15 concentrations are elevated by a wide range of pathophysiological processes and tissue damage, and may similarly exert a range of effects in metabolic and

inflammatory pathways as part of a systemic response to diverse diseases as well as to ageing in general.

Strengths of this study include the use of a general population, as well as the large size and the wide age range, which allows stratified analysis of the reference ranges. Data were available to allow estimation of important correlations of GDF-15 with other emerging cardiac biomarkers high sensitivity troponin and NT-proBNP. GDF-15 was measured using automated assays available to clinical biochemistry departments. Weaknesses include the cross-sectional design of the study; causal inferences should be made with caution. GDF-15 was measured in frozen serum samples on a second thaw, and we were not able to directly investigate the impact of this storage on GDF-15 concentrations, although previous data indicates that GDF-15 is robust to several freeze-thaw cycles [31,40]. Data on use of specific drugs, such as metformin, were not available from participant questionnaires. Data were available to identify pregnancy in some females, but there is also likely to be misclassification of pregnancy leading to some pregnant participants being included in the reference range estimates. The 97.5th centile and other thresholds we report are observations taken from a specific general population and cannot be taken in isolation to be indicative of underlying pathology. These are intended to be used as reference normal ranges for comparison in general population studies; further work would be required to validate their use in clinical practice.

In conclusion, these data are consistent with multiorgan expression of GDF-15 as a stress hormone. Due to an emerging body of research and clinical interest in GDF-15 across the life-course, absolute levels of GDF-15 require contextualisation. These

data reliably define expected levels GDF-15 for reference in clinical and epidemiological studies, and identify potentially confounding risk factors that should be considered in interpreting GDF-15 concentrations.

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Author contributions

P.W. and N.S. conceived and designed the study. A.C. conducted data acquisition. P.W. carried out the statistical analysis. P.W. and N.S. wrote the original manuscript. All authors contributed to the interpretation of the data and critical revision of the manuscript for important intellectual content and approved the final draft. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

PW reports grant income from Roche Diagnostics, AstraZeneca, Boehringer Ingelheim, and Novartis, outside the submitted work. REM has received speaker fees from Illumina and is an advisor to the Epigenetic Clock Development Foundation. NLM has received research grants to the University of Edinburgh from Abbott Diagnostics and Siemens Healthineers that are not related to the current work and has acted as a consultant for Abbott Diagnostics, Siemens Healthineers, Roche, and LumiraDx. SO has provided remunerated consultancy services to Pfizer, AstraZeneca, Novo-Nordisk and ERX Pharmaceuticals. NS has consulted for Afimmune, Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Hanmi Pharmaceuticals, Merck Sharp & Dohme, Novartis, Novo Nordisk, Pfizer, and Sanofi; and received grant support paid to his University from AstraZeneca, Boehringer

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Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and ethical approval was obtained from Research Ethics Committees in Scotland along with the necessary NHS R&D approval.

Data availability

The datasets generated during and/or analysed during the current study are available from the GS access committee https://www.ed.ac.uk/generation-scotland/for-researchers/access on reasonable request.

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Table 1 GS:SFHS reference ranges for GDF-15 by age categories and sex

GDF-15, pg/mL										
	Model 1					Model 2				
	n	50th centile	95th centile	97.5th centile	99th centile	n	50th centile	95th centile	97.5th centile	99th centile
Male										
<30 years	1,412	537 (528, 547)	968 (926, 1030)	1135 (1058, 1195)	1820 (1410, 3334)	1,349	534 (526, 544)	942 (895, 992)	1094 (1052, 1172)	1492 (1248, 1866)
30-39 years	1,220	644 (628, 655)	1193 (1111, 1285)	1442 (1352, 1543)	1995 (1714, 2458)	1,174	641 (626, 652)	1163 (1096, 1245)	1400 (1308, 1483)	1820 (1555, 2437)
40-49 years	1,498	747 (731, 764)	1528 (1441, 1614)	1892 (1734, 2042)	2578 (2193, 2964)	1,378	739 (723, 755)	1448 (1379, 1535)	1740 (1613, 1920)	2346 (2051, 2907)
50-59 years	1,958	931 (915, 948)	2023 (1951, 2132)	2492 (2367, 2639)	3655 (3262, 4256)	1,613	911 (894, 927)	1862 (1790, 1946)	2194 (2065, 2339)	2773 (2475, 3249)
60-69 years	1,240	1171 (1152, 1200)	2867 (2670, 3124)	3837 (3453, 4585)	5872 (4722, 7224)	842	1107 (1087, 1137)	2229 (2118, 2485)	2704 (2514, 2834)	3486 (2860, 4226)
70-79 years	216	1549 (1482, 1635)	3441 (2997, 4132)	4602 (3506, 6470)	7633 (4156, 10353)	81	1363 (1299, 1450)	2651 (2297, 3060)	3065 (2614, 3726)	3428 (2766, 3726)
≥80 years	48	2152 (1946, 2456)	5624 (4649, 6123)	5972 (5546, 6123)	-	8	1944 (1641, 2174)	-	-	-
Female										
<30 years	1,673	628 (616, 641)	1474 (1397, 1571)	2195 (1812, 2643)	12745 (4471, 25000)	1,625	625 (614, 637)	1401 (1347, 1462)	1738 (1580, 1911)	2679 (2193, 3258)
30-39 years	1,753	656 (646, 666)	1412 (1332, 1493)	1950 (1710, 2332)	10561 (3692, 19876)	1,661	649 (640, 660)	1306 (1264, 1344)	1541 (1457, 1682)	2009 (1830, 2361)
40-49 years	2,436	755 (743, 766)	1505 (1447, 1543)	1804 (1666, 1996)	2608 (2323, 3031)	2,229	748 (738, 760)	1419 (1379, 1461)	1595 (1543, 1669)	2057 (1792, 2252)
50-59 years	2,821	881 (869, 892)	1835 (1749, 1916)	2323 (2195, 2508)	3387 (2979, 3951)	2,417	861 (850, 874)	1621 (1564, 1680)	1920 (1850, 2018)	2525 (2205, 2910)
60-69 years	1,738	1063 (1046, 1086)	2307 (2185, 2441)	2832 (2607, 3210)	4517 (3571, 5668)	1,342	1027 (1004, 1048)	1981 (1884, 2096)	2326 (2193, 2456)	2745 (2482, 3396)
70-79 years	385	1445 (1392, 1505)	3097 (2848, 3641)	3805 (3316, 4418)	5368 (4225, 9173)	201	1349 (1290, 1415)	2540 (2259, 2997)	3066 (2561, 3744)	3738 (3209, 4225)
≥80 years	109	1847 (1653, 2112)	4396 (3695, 6119)	6830 (4537, 16357)	12340 (7327, 16357)	28	1407 (1235, 1545)	2986 (2658, 3111)	-	-

Model 1: Estimates from 18,507 participants with no heart disease, heart failure, or stroke, and not known to be pregnant.

Model 2: Estimates from 15,948 participants with no heart disease, heart failure, or stroke, not known to be pregnant, with no diabetes, or previous cancer, eGFR≥60ml/min/1.73 m^2, NT-proBNP <400pg/mL, cTnI<26.2pg/mL, cTnT<14pg/mL, and GDF15<10,000pg/mL.

Estimates are for 50^{th}, 95^{th}, 97.5^{th} and 99^{th} centiles (90% confidence intervals for each estimate). "-" indicates estimates omitted due to low n.

GS:SFHS, Generation Scotland Scottish Family Health Study; GDF, growth differentiation factor; eGFR, estimated glomerular filtration rate

Table 2 Population characteristics in 19,462 GS:SFHS participants, stratified by tertiles of GDF-15.

	Overall	Tertile 1 (≤679.5pg/mL)	Tertile 2 (679.6-1008pg/mL)	Tertile 3 (≥1009pg/mL)
	N=19,462	n=6488	n=6487	n=6487
Age	47.06 (14.96)	37.07 (12.32)	47.78 (12.38)	56.34 (13.43)
Male sex	8108 (41.7%)	2838 (43.7%)	2531 (39.0%)	2739 (42.2%)
Body mass index, kg/m^2 (N missing=179)	26.66 (5.16)	25.68 (4.81)	26.64 (4.87)	27.65 (5.59)
Systolic blood pressure, mmHg (N missing=77)	131.34 (17.79)	126.12 (15.17)	131.13 (17.09)	136.78 (19.25)
Total cholesterol, mmol/L (N missing=104)	5.10 (1.08)	4.85 (1.01)	5.26 (1.05)	5.19 (1.13)
HDL-cholesterol, mmol/L (N missing=142)	1.46 (0.41)	1.46 (0.38)	1.48 (0.41)	1.44 (0.43)
SIMD score, units divided by 10 (N missing=1133)	1.70 (1.45)	1.64 (1.37)	1.61 (1.41)	1.85 (1.57)
eGFR, ml/min/1.73 m^2ml/min/1.73 m2 (N missing=68)	95.11 (17.38)	103.86 (15.19)	94.62 (14.71)	86.84 (17.71)
Current smoker (N missing=625)	3054 (16.2%)	809 (12.9%)	883 (14.0%)	1362 (21.8%)
Heart disease	693 (3.6%)	54 (0.8%)	141 (2.2%)	498 (7.7%)
Stroke	250 (1.3%)	22 (0.3%)	47 (0.7%)	181 (2.8%)

Heart failure	91 (0.5%)	6 (0.1%)	6 (0.1%)	79 (1.2%)
Diabetes	562 (2.9%)	41 (0.6%)	86 (1.3%)	435 (6.7%)
Known pregnancy	58 (0.3%)	9 (0.1%)	5 (0.1%)	44 (0.7%)
Previous cancer	1521 (7.8%)	206 (3.2%)	454 (7.0%)	861 (13.3%)
Use of cholesterol lowering medications	1282 (6.6%)	106 (1.6%)	275 (4.2%)	901 (13.9%)
Use of blood pressure lowering medications	1574 (8.1%)	136 (2.1%)	380 (5.9%)	1058 (16.3%)
cTnI, pg/mL	1.90 (0.60, 3.10)	1.50 (0.60, 2.30)	1.90 (1.20, 2.90)	2.50 (1.50, 4.00)
cTnT, pg/mL	3.30 (1.50, 6.03)	1.50 (1.50, 4.78)	3.01 (1.50, 5.38)	4.57 (1.50, 8.14)
NT-proBNP, pg/mL	52 (27, 96)	39 (20, 68)	50 (27, 90)	73 (39, 138)

Values are n (%), mean (sd), or median (IQI). Data represent data from n=19,462 except where number missing (N miss) is indicated in the row.

GS:SFHS, Generation Scotland Scottish Family Health Study; GDF, growth differentiation factor; HDL, high-density lipoprotein; SIMD, Scottish Index of Multiple Deprivation; eGFR, estimated glomerular filtration rate; cTnI, cardiac troponin I; cTnT, cardiac troponin T; NT-proBNP, N-terminal pro B-type natriuretic peptide

Table 3 Adjusted association of cardiovascular risk factors with GDF-15 (n=19,462).

	Overall	Sex interaction			Age interaction				
	Adjusted association	**Adjusted association in feamles**	**Adjusted association in mmales**	**P for sex interaction**	**Adjusted association in age <50**	**Adjusted association in age 50-59**	**Adjusted association in age 60-69**	**Adjusted association in age ≥70**	**P for age interaction**
Age category				<0.001					
18-29 years	-23.4% (-25.3, -21.5)	-15.6% (-18.5, -12.5)	-32.0% (-34.2, -29.7)		-	-	-	-	-
30-39 years	-14.1% (-16.1, -12.1)	-11.3% (-14.2, -8.4)	-18.0% (-20.7, -15.3)		-	-	-	-	-
40-49 years	Ref	Ref	Ref		-	-	-	-	-
50-59 years	21.2% (19.0, 23.3)	18.0% (15.4, 20.6)	25.9% (22.4, 29.4)		-	-	-	-	-
60-69 years	47.6% (44.7, 50.6)	41.9% (38.3, 45.6)	55.9% (51.1, 60.9)		-	-	-	-	-
70-79 years	89.8% (83.3, 96.5)	82.6% (74.9, 90.7)	103.6% (92.5, 115.3)		-	-	-	-	-
80+ years	158.9% (141.1, 178.0)	145.7% (124.1, 169.2)	188.8% (159.8, 221.1)		-	-	-	-	-
Male sex	-1.5% (-2.9, -0.2)	-	-20.0% (-23.1, -16.7)		-26.6% (-30.2, -22.8)	-13.1% (-16.8, -9.2)	-10.5% (-12.3, -8.7)	-9.1% (-15.4, -2.4)	<0.001
BMI, per kg/m^2	0.6% (0.4, 0.7)	0.4% (0.3, 0.6)	0.9% (0.6, 1.1)	0.008	0.8% (0.6, 1.0)	0.7% (0.5, 1.0)	1.3% (1.0, 1.6)	-0.4% (-1.1, 0.3)	<0.001
SBP, per 5mmHg	0.1% (-0.1, 0.3)	0.4% (0.1, 0.7)	0.2% (-0.1, 0.5)	0.439	1.4% (1.0, 1.8)	0.2% (-0.1, 0.6)	0.2% (-0.2, 0.6)	-0.2% (-0.9, 0.6)	<0.001
Total cholesterol, per mmol/L	0.4% (-0.4, 1.2)	2.0% (0.9, 3.1)	-0.7% (-1.8, 0.4)	0.001	9.4% (8.1, 10.6)	-2.4% (-3.6, -1.2)	-3.7% (-5.1, -2.2)	-3.0% (-5.6, -0.4)	<0.001
HDL cholesterol, per 0.1 mmol/L	-1.1% (-1.3, -0.9)	-0.9% (-1.2, -0.7)	-1.2% (-1.5, -0.9)	0.208	-0.5% (-0.8, -0.2)	-1.4% (-1.8, -1.1)	-1.3% (-1.7, -0.9)	-1.6% (-2.3, -1.0)	<0.001
SIMD score, per 10 units	3.7% (3.2, 4.2)	3.6% (2.9, 4.3)	4.0% (3.2, 4.8)	0.443	2.9% (2.1, 3.7)	5.0% (4.0, 6.1)	4.4% (3.2, 5.6)	3.9% (1.8, 6.0)	0.011
eGFR, per 5mls/min/1.72m^2	-2.1% (-2.4, -1.8)	-1.9% (-2.3, -1.5)	-2.2% (-2.7, -1.8)	0.284	-2.4% (-2.8, -2.0)	-2.4% (-3.0, -1.7)	-3.9% (-4.6, -3.3)	-6.6% (-7.5, -5.8)	<0.001
Current smoker	26.1% (23.7, 28.6)	25.4% (22.1, 28.7)	26.8% (23.3, 30.4)	0.566	22.1% (18.9, 25.4)	36.2% (31.5, 41.1)	26.9% (20.4, 33.8)	19.7% (6.6, 34.3)	<0.001
Heart disease	12.7% (8.5, 17.1)	9.4% (3.3, 15.8)	11.8% (6.3, 17.7)	0.571	5.3% (-8.4, 21.0)	19.3% (11.8, 27.2)	6.9% (0.5, 13.7)	14.9% (5.9, 24.8)	0.074
Stroke	17.1%	15.2%	18.1%	0.711	10.4%	39.8%	8.0%	17.7%	0.035

	(9.6, 25.0)	(6.3, 24.8)	(6.5, 30.9)		(-7.3, 31.4)	(21.7, 60.5)	(-3.7, 21.3)	(5.4, 31.4)	
Heart failure	25.3% (9.3, 43.8)	41.6% (11.4, 80.1)	14.3% (-2.4, 33.9)	0.140	30.6% (-39.9, 183.8)	28.6% (-4.7, 73.4)	5.1% (-11.3, 24.6)	53.4% (24.3, 89.3)	0.052
Diabetes	60.2% (52.0, 68.8)	65.3% (52.7, 78.9)	53.1% (42.7, 64.1)	0.153	56.8% (39.8, 75.9)	76.9% (61.6, 93.7)	58.0% (43.8, 73.5)	41.3% (22.8, 62.7)	0.051
Use of cholesterol medications	10.4% (7.0, 13.9)	14.5% (9.7, 19.5)	5.3% (0.8, 10.0)	0.006	34.5% (20.7, 50.0)	17.4% (11.3, 23.8)	7.0% (2.0, 12.2)	1.5% (-5.4, 8.9)	<0.001
Use of BP medications	12.8% (9.9, 15.8)	14.8% (10.9, 18.8)	9.9% (5.7, 14.2)	0.096	28.5% (18.8, 39.0)	16.3% (11.3, 21.5)	10.6% (6.1, 15.1)	7.7% (0.5, 15.3)	0.002
cTnI, per log pg/mL	5.0% (4.0, 5.9)	6.8% (5.4, 8.2)	3.6% (2.3, 5.0)	0.001	6.6% (5.2, 8.0)	4.8% (3.1, 6.5)	7.1% (4.6, 9.6)	20.5% (15.5, 25.8)	<0.001
cTnT, per log pg/mL	3.4% (2.3, 4.5)	4.2% (2.6, 5.7)	2.3% (0.9, 3.8)	0.095	-0.1% (-1.7, 1.5)	4.9% (2.8, 6.9)	8.0% (5.8, 10.2)	27.9% (22.6, 33.5)	<0.001
NT-proBNP, per log pg/mL	7.7% (6.8, 8.6)	7.4% (6.1, 8.8)	6.8% (5.6, 8.0)	0.488	7.3% (5.9, 8.6)	6.4% (4.6, 8.3)	8.9% (7.0, 10.9)	21.5% (18.1, 25.1)	<0.001

A positive percentage indicates a relative increase in GDF-15 for a corresponding change in the risk factor, while a negative percentage indicates a relative reduction in GDF-15.

Overall model is adjusted for age, sex, heart disease, heart failure, stroke, heart failure, diabetes, cancer, and pregnancy. Model separately by sex additionally allow for an interaction of the variable of interest with sex, with an age-sex interaction also included in every model. Model separately by age category additionally includes an age-sex interaction, with an age-sex interaction also included in every model.

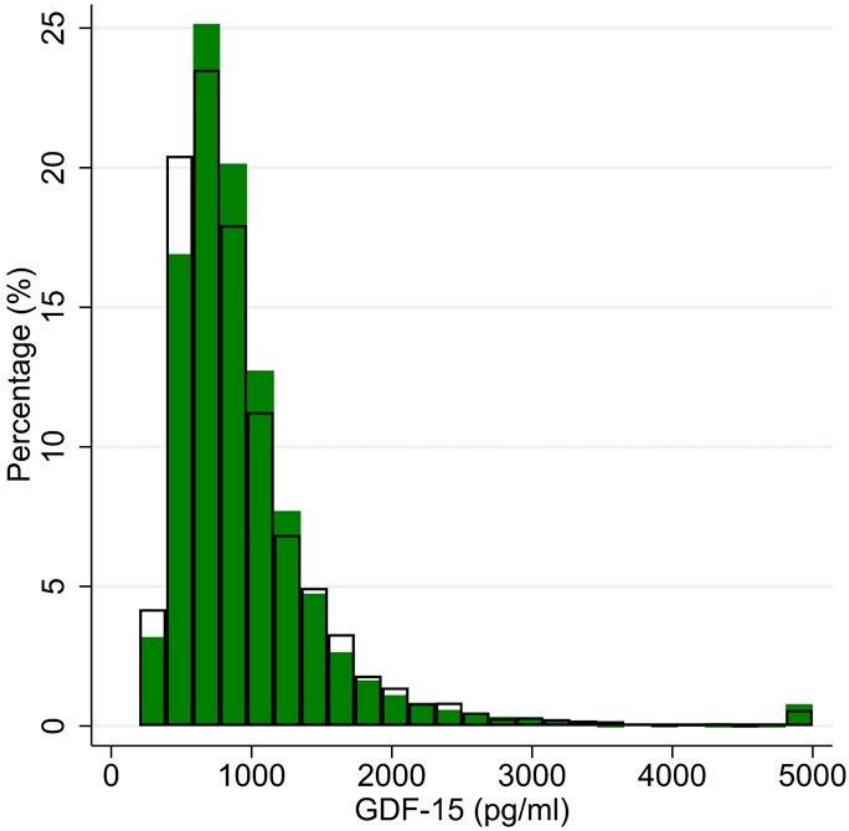
GDF, growth differentiation factor; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; SIMD, Scottish Index of Multiple Deprivation; eGFR, estimated glomerular filtration rate; BP, blood pressure; cTnI, cardiac troponin I; cTnT, cardiac troponin T; NT-proBNP, N-terminal pro B-type natriuretic peptide

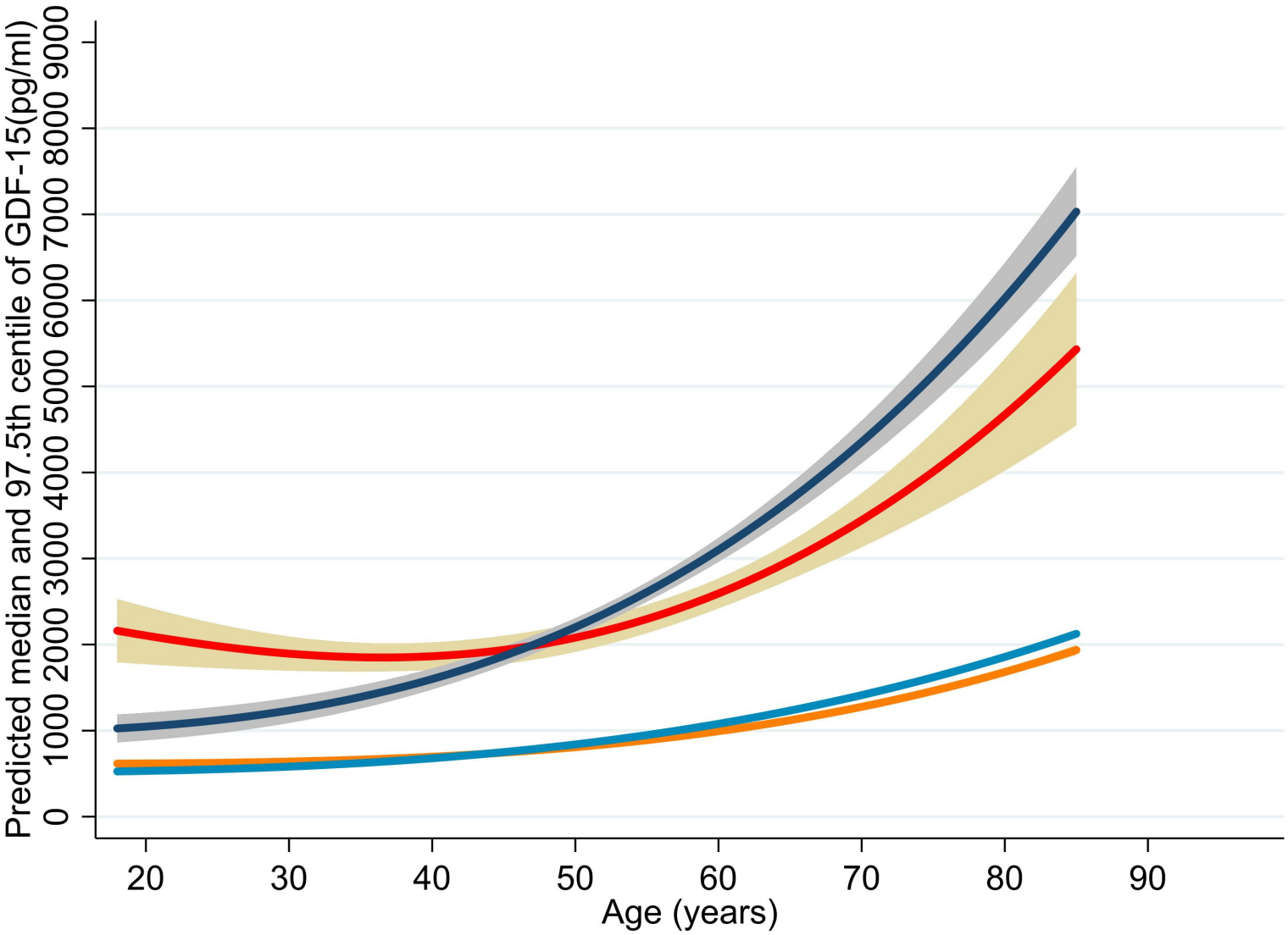
Figure Legends

Figure 1 Histogram of GDF-15 distribution, separately in males (clear bars) and females (green bars), in 18,507 participants without heart disease, heart failure, or stroke, and not known to be pregnant.

Figure 2 Association between age and the 50th centile (males light blue, females orange) and the 97.5th centile (males dark blue, females red) of GDF-15 in a continuous model.

Coloured areas are 95% CI. Modelled in 18,507 participants without heart disease, heart failure, or stroke, and not known to be pregnant.


Percentage (%)
0
5
10
15
20
25
0
1000
2000
3000
4000
5000
GDF-15 (pg/ml)


Predicted median and 97.5th centile of GDF-15(pg/ml)
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
20
30
40
50
60
70
80
90
Age (years)