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Growth differentiation factor 15 (GDF-15) as a biomarker of cardiovascular risk in chronic musculoskeletal pain

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Abstract

Background: It is unknown whether growth differentiation factor 15 (GDF-15) is associated with chronic musculoskeletal pain (CMP) and whether or not its association with incident cardiovascular disease (CVD) changes according to CMP status.

Methods: 1,957 randomly-selected adults aged ≥65 years without prior CVD were followed up between 2015-2023. CMP was classified according to its intensity, frequency, and interference with daily activities. The association between GDF-15 levels and CMP was assessed using linear models with progressive inclusion of potential confounders, whereas the association between GDF-15 and CVD risk was evaluated with Cox-proportional hazard models with similar adjustment and interaction terms between GDF-15 and CMP. The incremental predictive performance of GDF-15 over standard predictors was evaluated using discrimination and risk reclassification metrics.

Results: GDF-15 concentrations were 6.90% (95%CI:2.56;11.25) higher in individuals with CMP, and up to 8.89% (4.07;15.71) and 15.79% (8.43;23.16) higher in those with ≥3 CMP locations and interfering pain. These increased levels were influenced by a higher prevalence of cardiometabolic risk factors, functional impairments, depressive symptoms, and greater levels of inflammation in individuals with CMP. In fully-adjusted models, a two-fold increase in GDF-15 was associated with a with a 1.49 increased risk (95%CI: 1.08; 2.05) of a CVD event in individuals with CMP, but not among those without CMP [1.02 (0.77; 1.35)]; p-interaction 0.041. Adding GDF-15 to models including the Framingham Risk Score improved predictive performance among individuals with CMP.

Conclusions: We provide evidence that GDF-15 could serve as a biomarker to assess CMP, as well as to predict CVD incidence in individuals with CMP.

Keywords: Biomarkers; pain; cardiovascular disease (CVD).

INTRODUCTION

Chronic musculoskeletal pain (CMP) is a major public health problem. According to the fifth wave of the Survey of Health, Ageing and Retirement in Europe (SHARE), in 2013 its overall prevalence among community-dwelling Europeans aged ≥50 was around 36%. In this subgroup of the population, CMP often coexists with cardiovascular disease (CVD)Haga clic o pulse aquí para escribir texto.[1] although the exact mechanisms that link the two conditions are uncertain. Adults with CMP show significant reductions in physical activity and impairments in mental health, sleep and diet quality, all known risk factors of CVD.[2] Moreover, the use of certain drugs to treat pain, such as non-steroidal anti-inflammatory medications, may also increase the risk of CVD.[3,4] Chronic pain also induces systemic inflammation,[5] which may promote vascular damage and contribute to the development and progression of atherosclerosis. Finally, overactivation of the sympathetic nervous system in chronic pain may induce elevations in blood pressure and heart rate,[6,7] and contribute to atherosclerosis by inducing platelet activation.[8]

Risk assessment in individuals lacking a prior cardiovascular event remains a challenge. Prior research has investigated whether single or combined cardiac biomarker approaches can enhance risk prediction beyond classical models based on classical risk factors for primary prevention.[9–12] In particular, N-terminal pro-brain natriuretic peptide (NT-ProBNP) and high sensitivity Troponin T (hs-TNT), which are well recognized as clinical biomarkers for targeting preventive measures in patients with coronary artery disease and heart failure, respectively, show promising results as predictors for incident cardiovascular events in subjects without previous episodes.[10–12] On the other hand, growth differentiation factor 15 (GDF-15), a member of the transforming growth factor B superfamily, has been proposed as a non-specific biomarker to improve CVD risk prediction in primary prevention. Its levels increase in response to cellular stressors, such

as hypoxia, inflammation, or oxidative stress, playing a tissue protective role through up- and down-regulation of several signaling pathways.[13,14] GDF-15 has shown to improve CVD risk prediction algorithms in patients with type 2 diabetes[15] and atherosclerotic CVD,[16] and to be a prognostic biomarker of mortality in patients with heart failure and/or acute coronary syndrome.[17,18] Furthermore, since it also reflects the subclinical chronic inflammation typical of old age, and is a powerful predictor of all-cause mortality in this population subgroup, it has been suggested as a potential target for pharmacological and lifestyle interventions aimed at mitigating the burden of age-related chronic diseases and functional decline.[19]

The evidence for a relationship between CMP and GDF-15 is scarce, and their biological mechanisms are unknown. In a cross-sectional study of 1078 individuals aged 17 to 80 years belonging to 99 families from Israel, researchers found elevated plasma GDF-15 levels in those with low back pain disability.[20] In another study, carried out in 46 patients with rheumatoid arthritis and 36 demographically matched controls, elevated levels of GDF-15 were associated with the disease.[21] Also, in a study with 221 migraine patients and 124 healthy controls, the first showed increased serum GDF-15 compared to the latter, and levels were correlated with increased migraine-related burden and disability.[22]

The aim of the present study is threefold: 1) to evaluate the association between circulating GDF-15 and CMP in older adults with no previous history of CVD; 2) to assess whether GDF-15 predicts CVD differently in individuals with and without CMP; and 3) to evaluate whether the predictive ability of GDF-15 for incident CVD in individuals with CMP might be better than that of cardiovascular risk equations and other well-stablished cardiovascular biomarkers.

METHODS

Study design.

A total of 3,274 participants aged 65 years or older were selected through sex and district stratified random sampling of the Community of Madrid health card registry (ClinicalTrials.gov NCT03541135).[23] Between December 2015 and June 2017, these participants were interviewed and examined during home visits, ensuring comprehensive data collection on sociodemographic characteristics, health behaviors, morbidities, and functional status.

The study was approved by the Clinical Research Ethics Committee of the Hospital Universitario "La Paz" of Madrid (Protocol #HULP-PI 1793), and participants gave written informed consent.

Baseline data collection

Self-reported information was collected on age; sex (male or female); education (<high school, high school, and >high school); smoking (never, former, and current smoker); and alcohol intake (never, former, and current drinker). Physical activity was assessed with the questionnaire from the EPIC-Spain cohort[24] while sedentary behavior was estimated by time spent watching television using the Nurse´s Health Study[25] questionnaire validated in Spain Adherence to the Mediterranean diet was measured with the Mediterranean Diet Adherence Screener (MEDAS) index. In home interviews, participants' body weight and body height were measured and the body mass index (BMI) was calculated as weight in kg divided by squared height in m; while blood pressure was determined 3 times at 1–2-minute intervals with standardized procedures and the mean of the last 2 readings of blood pressure used for analysis. Participants also reported the use of antihypertensive, lipid-lowering and antidiabetic drugs, which was verified against drug packages during the home visits. 12-hour fasting blood creatinine, glucose, total

cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured with colorimetric enzymatic methods using an Atellica Solution® analyzer (Siemens Healthineers). Glomerular filtration rate (GFR) was estimated from plasma creatinine values using the CKD-EPI equation, and type 2 diabetes was defined as a participant-reported medical diagnosis, fasting glucose ≥126 mg/dl or use of antidiabetic medication.

Regarding physical functional limitations, lower-extremity performance was assessed with the Short Physical Performance Battery (SPPB)[26] and participants with a score ≤9 were deemed to have impaired performance. Hand grip strength was measured with a Jamar dynamometer in the dominant hand, and those in the lowest sex- and BMI-adjusted quintile of the maximum value of two consecutive measurements were considered to have low strength. Finally, depression symptoms were assessed with the 10-item Geriatric Depression Scale (GDS-10).[27]

GDF-15

During the home interviews, fasting blood samples were obtained in rapid serum tubes with a thrombin-based coagulation activator and polymer gel (Becton Dickinson). The tubes were centrifuged at 3000 rpm for 10 minutes and the serum was divided into aliquots and stored at -80ºC at the Department of Preventive Medicine and Public Health of the Universidad Autónoma de Madrid until final analysis (up to 4 years). GDF-15, hs-TnT, NT-ProBNP, and interleukin 6 (IL-6) were measured in serum at the Department of Laboratory Medicine of the “Hospital Universitario La Paz" by Elecsys® electrochemiluminescence immunoassay using a cobas® 6000 analyzer (Roche Diagnostics).[28]

Pain

Using 10 questions from the European Chronic Pain Survey,[29] CMP was defined as pain lasting 6 weeks or longer at specific body sites (head/neck, back, bones/joints, lower limbs, upper limbs) and classified according to its intensity, frequency, interference with daily activities, and number of sites.[30] To define pain intensity as mild, moderate or severe, we used a numeric rating scale from 1 (no pain) to 10 (worst pain imaginable) to categorize pain according to the following ranges: 1-3, 4-7, 8-10, respectively. Frequent pain was defined as that occurring ≥ 2 times/week, while pain-related interference was considered when pain moderately or completely hindered participation in daily activities. Finally, multisite CMP was defined as pain in three or more locations.

Cardiovascular disease and mortality follow-up

Incident cardiovascular events were identified through the electronic health records of primary health care centers from January 1, 1980 to January 30, 2023. Using the International Classification of Primary Care-2 codes, CVD was defined as a composite of ischemic heart disease (K74), myocardial infarction (K75), congestive heart failure (K77), stroke (K90), and atherosclerosis and peripheral vascular disease (K92).

The number of deaths in the cohort from baseline to January 30, 2023, was ascertained using the Spanish National Death Index, which has demonstrated a specificity of probability matching close to 100%. The follow-up period was calculated from the date of the baseline examination until the occurrence of the first cardiovascular event, death, or the censored date (January 31, 2023), whichever occurred first. The median follow-up time for participants who did not experience a cardiovascular event was 6.21 years (range: 0.49-7.16 years).

Statistical analyses

Prevalent CVD cases (i.e., those diagnosed before the study entry date) were excluded from the analyses (n=601). In addition, 84 participants with a self-reported medical diagnosis of ischemic heart disease, myocardial infarction, heart failure, or stroke prior to the study start date; 76 with a diagnosis of cancer-since several types of cancer have been associated with elevated circulating GDF15 levels; 416 with no GDF-15 or pain measures; and 140 who lacked data on potential confounders were excluded, resulting in a final sample of 1,957 participants.

Differences in sociodemographic variables, lifestyle behaviors, cardiometabolic risk factors, functional impairments, depression symptoms, and inflammatory and cardiovascular damage biomarkers according to pain characteristics were assessed by ANOVA and chi-square tests. The association between CMP and log-transformed GDF-15 was evaluated using different linear regression models. In the basic model (model 1), only sociodemographic factors were entered as covariates. We then fitted models adding information on potential confounders including lifestyle behaviors (model 2), cardiometabolic risk factors (model 3), physical performance variables (model 4), depressive symptoms (model 5), biomarkers of inflammation (log-transformed IL-6 and neutrophil to lymphocyte ratio, model 6) or biomarkers of cardiovascular damage (hs-TnT and NT-ProBNP, model 7). To assess the combined contribution of these factors to the studied association, we also fitted a final model with simultaneous adjustment for all the aforementioned confounders (model 8). Mean percentage differences in GDF-15 concentrations were calculated by subtracting 1 from the exponentiated β coefficients in the regression models and multiplying the result by 100.

To examine the potential association between baseline GDF-15 levels and CVD incidence, Cox-proportional hazard models were fitted with age as the time scale and individual starting follow-up times (age at baseline) treated as staggered entries, and

adjustment variables similar to models 1 to 8. The assumption of hazards proportionality was evaluated based on the smoothed association between age and scaled Schoenfeld residuals, with no major departures from proportionality.

The performance of cardiovascular risk prediction models incorporating GDF-15 against the established Framingham Risk Score in individuals with and without CMP was assessed using two discrimination metrics, the Area Under the Curve (AUC) and the Harrell`s C-index, and two measures of risk reclassification, the Net Reclassification Improvement (NRI) and the Integrated Discrimination Index (NDI). To mitigate overfitting to the training data, 10-fold cross-validation was executed, with the respective metrics averaged across folds. Subsequently, bootstrap resampling was applied to the cross-validated AUC, allowing for the derivation of statistical inferences and the computation of 95% bias-corrected bootstrap confidence intervals, as implemented in the STATA command CVAUROC.[31]

RESULTS

At baseline, the mean age of study participants was 71.3 (SD:4.3) years, 54.8% were women, and 32.0 % suffered from CMP. Among the latter, 79.4%, 32.4%, and 26.4% reported frequent, severe, and interfering pain, respectively; and the mean number of pain locations was 2.7 (SD: 1.6). The likelihood of frequent, severe, and interfering pain was higher among women, participants with lower education, never smokers, never and former drinkers, more sedentary participants and those having mobility problems, those with worse physical performance, as well as participants with a diagnosis of osteoarthritis, arthritis or depression. Oldest adults, those with diabetes and less physically active, more often experienced interfering pain (**Table 1**).

Crude geometric means and their geometric standard deviations for serum levels of GDF-15 were 1174.5 pg/mL (1.59) and 1232.3 pg/mL (1.64) for those with and without CMP, respectively. After adjusting for age, sex and educational level and compared to participants who did not experience CMP (model 1 in **Table 2**), GDF-15 concentrations were 6.90% (95%CI: 2.56; 11.25) higher in those with pain. Further adjustment for lifestyle risk factors (model 2) hardly changed the results; whereas adjustment for cardiometabolic risk factors (model 3), functional impairments (model 4), depressive symptoms (model 5), biomarkers of inflammation (model 6) and subclinical cardiovascular damage (model 7), produced, respectively, a 27.5%, 22.2%, 27.6%, 29.7, and 31.7% reduction in the magnitude of the association. The simultaneous adjustment for all the aforementioned confounders (model 8) resulted in a 63.0% percentage reduction in the effect estimate and a loss of statistical significance [2.55%; (-1.15; 6.25)].

During follow-up, 222 cases of incident CVD were diagnosed. **Table 3** presents the results of the models on baseline GDF-15 concentrations and CVD risk. GDF-15 concentrations were associated with the risk of CVD events in all subgroups of individuals with CMP, independently of baseline levels of cardiometabolic risk factors, functional impairments, depressive symptoms or biomarkers of subclinical cardiovascular damage. In models adjusted for all potential confounders (model 8), a two-fold increase in GDF-15 was associated with a 1.49 increased risk (95%CI: 1.08; 2.05) of a CVD event in individuals with CMP, but not among those without CMP [1.02 (0.77; 1.35)]; p-interaction 0.041. When CMP characteristics were taken into account, the strongest associations (Hazard Ratios [95%CI]) were observed among those with interfering [1.98 (1.19; 3.30)], intense [1.83 (1.09; 3.06)], multisite [1.75 (1.13; 2.71)], and persistent [1.67 (1.17; 2.39)] CMP.

Figures 1 and **2** illustrate Receiver Operating Characteristic (ROC) curves and their corresponding areas under the curve (AUC) for predicting CVD risk. The analyses integrate the Framingham Risk Score, GDF-15, and their combined predictive performance. Additionally, **Supplementary Table 1** presents a comparative analysis of discrimination metrics, including AUC and Harrell's C-index, alongside risk reclassification measures (NRI and IDI). Overall, the findings suggest that GDF-15 enhances model discrimination and reclassification among individuals with CMP, but not among those without CMP.

Supplementary Table 2 presents the mean percentage changes in hs-TnT and NT-ProBNP concentrations in relation to the presence of CMP. Upon adjustment for age, sex, and educational level, individuals with CMP displayed a 7.06% increase in hs-TnT concentrations compared to those without CMP (95%CI: 3.49;10.63). However, no statistically significant differences were observed in NT-ProBNP levels between the groups (mean difference: 3.16; 95%CI:-2.65; 9.97). Incorporating depression symptoms and inflammatory biomarkers into hs-TnT models (**Supplementary Tables 2 and 3)** yielded smaller changes in the magnitude of the associations compared to those observed for GDF-15 (i.e. 7.5 vs 27.5% and 15.7 vs 29.7%, respectively, for dichotomous models), suggesting that these factors may have a more pronounced relationship with GDF-15 compared to hs-TnT.

Contrary to what was found for GDF-15, the association of hs-TnT with incident CVD showed no modification by CMP, and lost significance in individuals with CMP when adjusting for lifestyle and cardiometabolic risk factors **(Supplementary Table 4).** The AUC was similar for hs-TNT and ProBNP in individuals without CMP (**Figure 3**) , but higher for GDF-15 than hs-TnT and Pro-BNP in individuals with CMP, especially when other variables (i.e., sociodemographic and lifestyle-related factors) that are often missing

in the clinical setting were not taken into account (semicrude cross-validated AUC: 0.64 (95%CI: 0.57-0.70) for GDF-15 vs 0.53(95%CI: 0.45-0.60) and 0.54 (95%CI: 0.47-0.62) for pro-BNP and hs-TnT, respectively; p-value for comparison of the ROC curves =0.012) (**Figure 4**).

DISCUSSION

In this study of community-dwelling older adults without CVD, higher levels of GDF-15 were associated with worse pain experience, higher frequency, greater intensity, and higher number of pain locations in individuals with musculoskeletal pain. These associations were influenced by a higher prevalence of cardiovascular risk factors, poorer physical performance, increased levels of depression, and elevated inflammation among individuals with CMP. The incorporation of GDF-15 into predictive CVR models enhanced prediction accuracy in individuals with CMP; furthermore GDF-15 demonstrated superior predictive capability for cardiovascular events in individuals with CMP compared to both hs-TnT and pro-BNP.

Inflammation is a pivotal factor in the development and progression of CVD.[32] It coexists with most cardiovascular risk factors (e.g. smoking, high blood pressure, hyperlipemia) and is common in atherosclerosis, playing a key role in the development of endothelial cell dysfunction, plaque formation and progression, and ultimately plaque rupture. GDF-15, linked to aging and age-related conditions,[33] acts as a key immunoregulatory protein connecting inflammation to immunosuppression, potentially perpetuating immune suppression in chronic aging-induced inflammation.[34] GDF-15 is upregulated in atherosclerosis induced by proinflammatory cytokines, such as tumor necrosis factor (TNF)-alpha,[35] impairing endothelium-dependent vasodilation and promoting plaque progression and angiogenesis via NF-κB signaling.[36] Its deficiency inhibits

atherosclerosis progression by modulating the IL-6 dependent inflammatory response to vascular injury.[37] GDF-15 also facilitates LDL oxidization,[35] induces myocardial fibrosis,[38] and rises after cardiac ischemia, potentially serving as a proxy for silent cardiac ischemia in patients with frequent, disabling and intense CMP.

GDF-15 emerges as a promising biomarker for chronic pain and its related conditions. In this line of research, previous studies indicate its association with the severity of lumbar chronic pain[20] and migraine-related disability.[22] Moreover, circulating levels of GDF-15 correlate with bone erosion in spondylarthritis,[39] activity scores in rheumatoid arthritis,[40] and a higher probability of arthralgias in patients with Behçet´s disease.[41] Therapeutically, GDF-15 has been proposed as a potential target for managing osteoarthritis, with experimental evidence showing that it can accelerate chondrocyte senescence.[42] In particular, research in arthritic rats has revealed the involvement of GDF-15 in the regulation of nociceptive transmission,[43] evidenced by its ability to modulate nociceptive response thresholds, reduce the excitability of small-diameter dorsal root ganglia neurons, and regulate Nav1.8 channels to change dorsal root ganglion nociceptive neuronal excitability. In addition to its impact on skeletal and neural pathways, a plausible connection exists between GDF-15 concentrations and CMP through muscular disease. In mouse models, increasing serum and muscle levels of GDF-15 lead to reduced skeletal muscle mass and function,[44] while epidemiological studies in ageing populations show an inverse association between blood levels of GDF-15 and both muscle mass and strength,[44,45] along with associations with frailty,[46] slower gait speed, and lower physical performance,[47] —all recognized risk factors for CMP.[30]

This study has several strengths. First, it is the largest to evaluate the association between GDF-15 concentrations and CMP. Second, it studies an extensive list of health behaviors,

clinical variables, and biomarkers that could contribute to the observed association. In this regard, an interesting finding is that the association between GDF-15 and CMP was partially confounded by the presence of functional limitations and depressive symptoms, which are highly prevalent in individuals with CMP; indeed, there is increasing evidence that GDF-15 may negatively impact functionality[48] and increase the risk of depression[49,50] through mechanisms other than inflammation. Third, we showed that GDF-15 better predicted the risk of a CVD event in individuals with CMP than in those without when no sociodemographic and lifestyle-related variables are available. This has important clinical implications, as it was not the case for hs-TNT, considered in clinical practice as the "gold standard" to diagnose myocardial damage. Among the study limitations, the cross-sectional design of some analyses precludes identification of whether CMP, alongside an increased prevalence of conditions associated with CVD risk—such as cardiovascular risk factors, compromised physical performance, heightened levels of depression or inflammation—causes elevated levels of GDF-15, or whether heightened GDF-15 levels serve as a catalyst for some of these conditions. This study presents a complex scenario, likely involving bidirectional relationships. The absence of a validation cohort poses a significant risk of overfitting, which we sought to mitigate by implementing internal cross-validation to produce a more realistic estimate of predictive performance. Also, misclassification of CVD outcomes remains a concern, despite confidence in primary healthcare electronic records´ reliability for diagnosing myocardial infarction, stroke, or congestive heart failure.[51–54] Variations in healthcare-seeking behavior and incomplete documentation could lead to underreporting or misclassification of CVD outcomes. While individuals with chronic pain may have better-recorded diagnoses due to more frequent primary care visits, the association between the Framingham equation, biomarkers like TnT or proBNP, and cardiovascular risk was

similar in both groups, suggesting minimal differential bias in the association with GDF-15. Finally, we lacked information on polymorphisms of the genes encoding GDF-15, which may be associated with individual differences in CVD risk.[56] Finally, similar to other ageing cohorts, we cannot rule out the presence of healthy bias.

In conclusion, although more evidence is needed, our results provide new evidence that GDF-15 could be a candidate biomarker for assessing CMP and monitoring pain interventions, and suggest that it may be a good predictor of CVD events in individuals with CMP.

Data Sharing Statement

Data are available from corresponding author on a reasonable request.

Conflict of interest statement

The authors have no conflict of interest to declare

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Table 1: Socioeconomic, lifestyle-related and clinical factors of study participants according to pain characteristics (n=1,957).

	No pain	Frequency (times/wk)		Intensity (VAS)		Nº locations		Interference activities	
		<2	≥2	1-7	8-10	1-2	>2	No	Yes
	n=1,331	n=129	n=497	n=423	n=203	n=333	N=292	n=461	n=165
Age	71.4;4.3	70.7;3.5	71.5;5	71.0;4.1	71.9;4.5	70.8;3.9	71.9;5.0	71.0;4.2	72.1;4.5
Sex, female	48.7	55.0	71.0	63.1	77.3	58.6	78.1	64.2	77.6
Education									
<secondary	62.1	60.5	68.2	64.3	69.5	64.0	69.9	63.8	74.5
>secondary	19.5	20.2	12.1	14.9	11.3	15.6	11.3	15.6	8.48
Smoking									
Never	51.3	51.2	61.2	56.5	64.5	57.1	61.6	57.3	64.2
Former	38.5	38.8	31.2	35.0	28.1	34.5	30.5	34.5	27.9
Current	10.2	10.1	7.65	8.51	7.39	8.41	7.88	8.24	7.88
Alcohol drinking									
Never	17.4	11.6	24.7	18.9	28.6	18.3	26.4	21.5	23.6

Moderate	72.1	78.3	62.8	69.7	58.1	70.6	60.6	67.9	60.6
Heavy	5.86	3.88	4.23	3.78	4.93	4.50	3.77	4.56	3.03
Former	4.73	6.20	8.25	7.57	8.37	6.61	9.25	6.07	12.7
METs-h/wk,		72.8;							
leisure-time pa	68.4;37	35.7	68.5;36	71.3;36.0	65.4;35.1	70.8;36.3	67.6;35.1	72.5;35	60.5;37
TV time; h/wk	21.7;10	20.0;9.82	23.7;12	22.3;10.7	24.1;12.5	21.9;10.8	24;11.9	22.1;10	25.1;13
Adherence to									
Med Diet	7.24;1.6	7.09;1.6	7.1;1.6	7.14;1.6	7.08;1.6	7.24;1.7	6.99;1.5	7.18:1.6	6.95:1.6
BMI; m/kg^2	27.5;3.9	28.5;5.0	28.6;5	28.2;4.7	28.6;5.0	27.8;4.51	28.9;5.11	28.1;4.7	29.0:5.1
Total-Chol	196;30.0	197;35.0	196;34	197;33.3	196;35.5	195;31.8	199;36.2	197; 34	195;35
HDL-Chol	55.0;15	55.4;13.6	55.5;14	55.0;14.2	56.0;13.4	54.4;13.7	56.4;14.1	55.3;14	55.5;14
Triglycerides	111;56.9	118;52.5	118;52	119;57.1	112;44.7	114;56.7	120;49.5	116;51	118;59
SBP	135;18.3	133;17.7	134;17	134;15.6	132;19.3	134;16.7	133;17.1	134;17	133;17
eGFR	82.0;12	80.7;12.7	80.5;14	80.6;13.5	80.4;13.4	80.5;12.5	80.6;14.5	81.1;13	79.0;14
Diabetes	18.0	18.6	19.1	17.7	21.7	18.9	19.2	16.9	24.8

Osteoarthrosis	33.0	41.1	65.4	57.7	66.0	46.8	76.0	57.0	69.7
Arthritis	3.9	6.2	15.3	11.8	16.7	7.81	19.9	10.2	22.4
Depression	2.8	3.10	10.9	6.38	15.3	6.61	12.30	6.51	17.0
Mobility limitations	24.3	29.5	64.6	52.2	68.0	44.10	72.60	51.4	73.9
Low grip	15.1	10.9	23.8	19.1	25.4	14.20	29.20	20.0	24.4
Low SPPB	21.2	24.0	34.1	29.5	37.2	25.1	40.1	28.3	42.5
Il-6	2.99;4.6	3.47;4.89	3.72;6.56	3.69;7.13	3.63;3.87	3.70;7.63	3.64;4.20	3.61;6.96	3.83;3.62
NLR	2.09;1.13	2.04;0.91	2.08;1.01	2.09;1.02	2.04;0.93	2.12;0.93	2.02;1.05	2.09;1.01	2.03;0.93

Baseline characteristics of study participants according to pain characteristics. Values are expressed as percentages for categorical variables and as means (standard deviations) for continuous variables. Abbreviations: BMI = Body mass index; Chol=cholesterol; eGFR = Estimated glomerular filtration rate; h= Hour; MED=Mediterranean; METs=Metabolic equivalents of tasks; NLR=Neutrophil-lymphocyte ratio; PA=Physical activity; SBP=Systolic Blood Pressure; SPPB=Short Physical Performance Battery; wk= Week

Table 2: Mean percentage change-MPC- (95% confidence intervals) in GDF-15 concentrations according to chronic musculoskeletal pain presence and their characteristics in older adults with no history of cardiovascular disease or cancer (n=1,957)

Model (M)	M1	M2	M3	M4	M5	M6	M7	M8
Pain (yes/no)	6.90*	6.76*	5.00*	5.37*	5.00*	4.85*	4.71*	2.55
	(2.56; 11.25)	(2.47; 11.04)	(1.25; 8.76)	(1.02; 9.72)	(0.57; 9.44)	(0.55; 9.15)	(0.50; 8.93)	(-1.15; 6.25)
Frequency								
No pain	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1,00
<2 times/wk	3.32	3.92	1.71	3.26	2.95	1.62	3.10	0.04
	(-4.81; 11.44)	(-4.09; 11.94)	(-5.28; 8.69)	(-4.79; 11.32)	(-5.16; 11.05)	(-6.39; 9.63)	(-4.76; 10.96)	(-6.80; 6.87)
≥2 times/wk	7.90*	7.55*	5.93*	5.97*	5.61*	5.74*	5.16*	3.26*
	(3.17; 12.62)	(2.88; 12.22)	(1.84; 10.02)	(1.22; 10.73)	(0.75; 10.46)	(1.07; 10.42)	(0.57; 9.75)	(-0.77; 7.30)
Intensity (VAS)								
No pain	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mild/moderate	6.17*	6.37*	4.37*	5.29*	4.96	4.32	4.10	2.00
(1-7)	(1.22;11.13)	(1.49; 11.25)	(0.10; 8.65)	(0.34; 10.23)	(-0.02; 9.94)	(-0.58; 9.21)	(-0.70; 8.90)	(-2.20;6.21)
Severe (8-10)	8.50*	7.60*	6.38*	5.55	5.10	6.00	6.06	3.76
	(1.76;15.25)	(0.93; 14.27)	(0.56; 12.20)	(-1.24; 12.34)	(-1.84; 12.04)	(-0.66; 12.66)	(-0.47; 12.60)	(-1.96;9.48)
Nº locations								
No pain	1.00	1.00	1.00	1.00*	1.00	1.00	1.00	1.00
1-2	4.56	5.16	1.67	4.33	3.59	2.77	3.60	0.51
	(-0.86; 9.98)	(-0.18; 10.50)	(-2.99; 6.34)	(-1.06; 9.72)	(-1.84; 9.01)	(-2.57; 8.12)	(-1.64; 8.84)	(-4.06;5.08)
≥3	8.89*	8.80*	9.25*	6.77*	6.96*	7.51*	6.33*	5.32
	(4.07; 15.71)	(3.04; 14.56)	(4.21; 14.28)	(0.88; 12.67)	(0.96; 12.95)	(1.75; 13.26)	(0.67; 11.99)	(0.32; 10.31)
Interference								
No pain	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Non-interfering	4.56 (-0.80; 8.79)	4.46 (-0.28; 9.19)	3.28 (-0.85; 7.42)	2.98 (-1.81; 7.77)	2.99 (-1.83; 7.81)	2.43 (-2.31; 7.17)	2.23 (-2.42; 6.88)	1.54 (-2.52; 5.60)
Interfering	15.79* (8.43; 23.16)	13.81* (6.50; 21.13)	10.24* (3.87; 16.61)	12.92* (5.47; 20.38)	11.85* (4.21; 19.49)	12.27* (4.98; 19.56)	12.32* (5.18; 19.46)	5.71 (-0.60; 12.02)

Model 1: Adjustment for sex, age and educational level.

Model 2: Model 1 + further adjustment for lifestyle risk factors [i.e. tobacco smoke (never and former smoker); alcohol consumption (never, moderate, heavy and former drinker); recreational physical activity (METs-hour/week) ; television-viewing time (hours/week) and diet quality (Mediterranean Diet Adherence Score)]

Model 3: Model 1 + further adjustment for cardiometabolic biological risk factors [i.e. body mass index (kg/m^2), diabetes, lipid-lowering treatment, total cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL), blood pressure lowering treatment, systolic blood pressure (mmHg), estimated glomerular filtration rate ($mL/min/1.73m^2$).

Model 4: Model 1 + further adjustment for functional impairments (low SPPB, low grip strength)

Model 5: Model 1 + further adjustment for number of depressive symptoms.

Model 6: Model 1 + further adjustment for biomarkers of inflammation (i.e. IL6, neutrophil to lymphocyte ratio)

Model 7: Model 1 + further adjustment for other biomarkers of cardiovascular damage (hs -TnT- and NTproBNP).

Model 8: Model 1+ further adjustment for covariates included in models 1 to 7

*p <0.05

Table 3: Hazard Ratios (95% confidence intervals) for cardiovascular disease risk according to baseline GDF-15 levels. Results are expressed per doubling increase in GDF-15.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Overall	1.54*	1.49*	1.46*	1.51*	1.50*	1.46*	1.36*	1.19
	(1.29; 1.85)	(1.24; 1.80)	(1.17; 1.83)	(1.25; 1.82)	(1.25; 1.80)	(1.22; 1.76)	(1.12;1.65)	(0.93, 1.51)
No pain	1.27*	1.24	1.21	1.25	1.25	1.20	1.18	1.02
	(1.00;1.62)	(0.97; 1.58)	(0.92; 1.59)	(0.98; 1.60)	(0.98; 1.59)	(0.94; 1.53)	(0.92; 1.50)	(0.77; 1.35)
Pain	1.98*	1.88*	1.90*	1.94*	1.94*	1.92*	1.67*	1.49*
	(1.52; 2.57)	(1.44; 2.46)	(1.40; 2.57)	(1.48; 2.55)	(1.48; 2.53)	(1.48; 2.51)	(1.26; 2.21)	(1.08; 2.05)
p-int.	0.014	0.021	0.014	0.017	0.015	0.000	0.049	0.041
Frequency (times/wk)								
Non-persistent (<2)	1.41	1.38	1.38	1.40	1.29	1.30	1.08	1.09
	(0.80; 2.45)	(0.80; 2.41)	(0.76; 2.51)	(0.80; 2.45)	(0.79; 2.42)	(0.74; 2.27)	(0.62; 1.86)	(0.61; 1.94)

Persistent (≥2)		2.23*	2.10*	2.11*	2.18*	2.18*	2.22*	1.95*	1.67*
		(1.64; 3.02)	(1.54; 2.85)	(1.50; 2.96)	(1.59; 2.98)	(1.60; 2.96)	(1.62; 3.03)	(1.42; 2.67)	(1.17; 2.39)
	p-int.	0.016	0.027	0.019	0.020	0.017	0.001	0.002	0.041
Intensity (VAS)									
Non-intense (1-7)		1.35	1.33	1.37	1.40	1.36	1.40	1.15	1.35
		(0.89; 2.04)	(0.88; 2.01)	(0.90; 2.09)	(0.92; 2.12)	(0.90; 2.06)	(0.92; 2.13)	(0.76; 1.73)	(0.88; 2.07)
Intense (8-10)		1.97*	1.88*	2.97*	1.84*	1.97*	2.01*	1.97*	1.83*
		(1.21; 3.22)	(1.15; 3.07)	(1.20; 32)	(1.12; 3.05)	(1.20; 3.23)	(1.22; 3.31)	(1.21; 3.21)	(1.09 ; 3.06)
	p-int.	0.019	0.032	0.021	0.036	0.021	0.015	0.025	0.050
Nº locations									
1-2 locations		1.71*	1.64*	1.61*	1.77*	1.69*	1.68*	1.54*	1.31
		(1.16; 2.53)	(1.11; 2.43)	(1.06; 2.44)	(1.19; 2.61)	(1.14; 2.50)	(1.13; 2.50)	(1.03; 2.30)	(0.86; 2.01)
≥3 locations		2.32*	2.20*	2.29*	2.18*	2.27*	2.23*	1.83*	1.75*
		(1.59; 3.38)	(1.50; 3.22)	(1.50; 3.50)	(1.47; 3.22)	(1.55; 3.33)	(1.52; 3.26)	(1.24; 2.68)	(1.13; 2.71)
	p-int.	0.024	0.035	0.020	0.039	0.025	0.018	0.117	0.066

Interference									
Non-interfering		1.60*	1.55*	1.53*	1.64*	1.59*	1.55*	1.35	1.30
		(1.14; 2.25)	(1.10; 2.18)	(1.05; 2.23)	(1.16; 2.31)	(1.13; 2.23)	(1.09; 2.18)	(0.95; 1.92)	(0.89; 1.91)
Interfering		2.96*	2.73*	2.80*	2.70*	2.92*	2.94*	2.51*	1.98*
		(1.88; 4.66)	(1.73; 4.31)	(1.74; 4.52)	(1.69; 4.32)	(1.84; 54.63)	(1.86; 4.67)	(1.57; 4.00)	(1.19; 3.30)
	p-int.	0.005	0.010	0.006	0.014	0.005	0.003	0.016	0.043

p-int: P value for interaction obtained from Wald tests for interaction terms

Model 1: adjustment for sex, age and educational level.

Model 2: model 1 + further adjustment for lifestyle risk factors [i.e. tobacco smoke (never and former smoker); alcohol consumption (never, moderate, heavy and former drinker); recreational physical activity (METs-hour/week); television-viewing time (hours/week) and diet quality (Mediterranean Diet Adherence Score)]

Model 3: model 1 + further adjustment for cardiometabolic biological risk factors [i.e. body mass index (kg/m^2), diabetes, lipid-lowering treatment, total cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL), blood pressure lowering treatment, systolic blood pressure (mmHg), estimated glomerular filtration rate ($mL/min/1.73m^2$).

Model 4: model 1 + further adjustment for functional impairments (low SPPB, low grip strength)

Model 5: model 1 + further adjustment for number of depressive symptoms.

Model 6: model 1 + further adjustment for biomarkers of inflammation (i.e. IL6, neutrophil to lymphocyte ratio)

Model 7: model 1 + further adjustment for biomarkers of cardiovascular damage

Model 8: model 1 + further adjustment for covariates included in models 1 to 7

*p <0.05

Figure 1: Receiver Operating Characteristic (ROC) curves and their associated areas under the curve (AUC) for predicting CVD risk in models using the Framingham Risk Score, GDF-15, or both parameters in individuals with no pain.

Figure 2: Receiver Operating Characteristic (ROC) curves and their associated areas under the curve (AUC) for predicting CVD risk in models using the Framingham Risk Score, GDF-15, or both parameters in individuals with pain.

Figure 3: Receiver Operating Characteristic (ROC) curves and their associated areas under the curve (AUC) for predicting CVD risk in models using hsTNT, proBNP, GDF-15, or all biomarkers in individuals with no pain.

Figure 4: Receiver Operating Characteristic (ROC) curves and their associated areas under the curve (AUC) for predicting CVD risk in models using hsTNT, proBNP, GDF-15, or all biomarkers in individuals with pain.