Research Article

Elevated Plasma Growth and Differentiation Factor 15 Is Associated With Slower Gait Speed and Lower Physical Performance in Healthy Community-Dwelling Adults

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Abstract

Background: Growth and differentiation factor 15 (GDF-15) has been associated with obesity, muscle wasting, and cachexia. The receptor for GDF-15 was recently identified in the brainstem and regulates food intake and metabolism. The relationship of plasma GDF-15 with the age-associated decline of muscle mass and strength, gait speed, and physical performance in adults has not been well characterized.

Methods: Plasma GDF-15, grip strength, 6-m gait speed, 400-m walking test time, lower extremity physical performance score, appendicular lean mass, and fat mass were measured in 194 healthy adult participants, aged 22–93 years, of the Baltimore Longitudinal Study of Aging.

Results: Plasma GDF-15 concentrations increased with age ($p < .001$) and were higher in whites compared with blacks and Asians ($p = .04$). Adults with higher plasma GDF-15 had slower 6-m gait speed, longer 400-m walking time, and lower physical performance score in multivariable analyses adjusting for age and race. Plasma GDF-15 was not associated with grip strength, appendicular lean mass, or fat mass.

Conclusions: Elevated plasma GDF-15 is associated with slower gait speed, higher 400-m walking time, and lower physical performance in very healthy community-dwelling adults. The relationship between plasma GDF-15 and sarcopenia-related outcomes may be stronger in the population not selected to be healthy, and this hypothesis should be tested in a representative population.

Keywords: Aging, Growth and differentiation factor 15, Physical performance, Sarcopenia, Skeletal muscle.

About one-third of women and one-half of men aged at least 60 years in the United States are estimated to have sarcopenia (1), defined as the loss of skeletal muscle mass, strength, and performance with aging (2). A decrease in muscle cross-sectional area, loss of muscle fibers, and muscle fiber atrophy occur with advancing age. Humans lose approximately 20%–40% of both skeletal muscle mass and strength from 20 to 80 years of age (3,4). Low skeletal muscle mass is associated with low strength (5), lower extremity physical performance (6), functional impairment (7), falls (8), and physical disability (7,9). Skeletal muscle strength is predictive of disability

(10) and mortality (11). There is currently no consensus operational definition of sarcopenia (2). However, sarcopenia is often defined by using a threshold of low grip strength or muscle mass, sometimes in combination with slow walking speed (12–15). The pathogenesis of sarcopenia still remains poorly understood.

Growth and differentiation factor 15 (GDF-15), a divergent member of the transforming growth factor- β superfamily, has recently been implicated in muscle wasting, cachexia, mitochondrial diseases, obesity, and energy balance (16,17). In healthy individuals, GDF-15 is expressed in low amounts in liver, lung, kidney, and a

wide variety of other tissues (16,18). GDF-15 is secreted as a 40-kDa propeptide that is cleaved in the endoplasmic reticulum to produce a mature active 25-kDa homodimer in the circulation (19,20). The normal range of plasma GDF-15 concentrations in healthy, non-pregnant humans is 200–1200 pg/mL (18). GDF-15 is upregulated in many different tissues in response to cellular stress (16). Various stimuli such as hypoxia, inflammation, oxidative stress, and injury can activate p53, hypoxia-inducible factor-1 α , nuclear factor- κ B, and other transcription factors to upregulate GDF-15 expression (16,18).

The receptor for GDF-15, glial cell line–derived neurotrophic factor family- α -like (GFRAL) (21–24), is located in a discrete region of the brainstem, the area postrema, and nucleus tractus solitarius. The area postrema is a specialized brain region with a semi-permeable blood–brain barrier that allows systemic signals to reach the central nervous system (18). Stimulation of GFRAL by GDF-15 leads to a reduction in appetite, food intake, and body weight in mouse models (21–24). Circulating GDF-15 can act via the GFRAL receptor to impair appetite and reduce food intake, which could play an important role in the anorexia of aging and its hypothesized relationship to sarcopenia (25,26). GDF-15 may also directly affect other tissues, as it has been implicated in other signaling pathways such as Smad through transcriptional regulation, AKT1, and Erk1/2, through mechanisms that are currently being elucidated (27–29). The recent finding that GDF-15 is one of the circulating proteins that most strongly correlate with chronological age (30) suggests the hypothesis that biological activities of GDF-15 may be relevant in the development of the aging phenotypes such as the decline in muscle mass and strength and decline in mobility performance that is observed in many aging individuals. Understanding whether GDF-15 is related to these outcomes independent of confounders is a first step to understand whether biological pathways modulated by this molecule should be considered for disability prevention in older individuals.

We hypothesized that plasma GDF-15 was associated with sarcopenia-related outcomes in adults. To address this hypothesis, we examined the relationship between circulating GDF-15 and muscle mass and strength, gait speed, and physical performance in healthy adults.

Methods

The participants were 194 adults in the Baltimore Longitudinal Study of Aging (BLSA), a prospective open cohort study of community-dwelling volunteers, largely from the Baltimore and Washington area, as described in detail elsewhere (31). Participants were assessed at an in-patient study clinic, the National Institute on Aging Clinical Research Unit, MedStar Harbor Hospital in Baltimore, Maryland, for follow-up visits every 1–4 years, with more frequent follow-up for older participants. They underwent 2.5 days of medical, physiological, and psychological examinations. The 194 participants, age range 22–93 years, were selected as subsample from the larger BLSA study population. The participants were seen between 2015 and 2016. There were 34 inclusion criteria and 12 exclusion criteria, as detailed elsewhere (31). The most salient inclusion criteria are summarized: age at least 20 years; weight more than 110 lbs; body mass index less than 30 kg/m²; nonsmokers; not pregnant; able to walk 400 m without assistance or symptoms; no cognitive impairment; no history of chronic diseases (except controlled hypertension); no significant hormonal dysfunction; no history of genetic diseases, cancer, or infectious diseases, including active syphilis, gonorrhea, or

tuberculosis; normal fasting glucose; normal complete blood count; creatinine more than 1.6 mg/dL; negative serology for HIV, hepatitis B, and C, and negative urine drug screen. The study protocol was approved by the institutional review boards of the National Institute of Environmental Health Science (National Institutes of Health, NC) and the Johns Hopkins School of Medicine. The study protocol was conducted in accordance with the 1964 Declaration of Helsinki. At every visit, after the scope, procedures and related risk were explained to participants; they consented to participate in the study and signed an informed consent document.

Information about lifestyle factors such as smoking and years of education were assessed by self-report. Body mass index and blood pressure were assessed during a standard medical examination. Blood tests were performed at a Clinical Laboratory Improvement Amendments certified clinical laboratory at MedStar Harbor Hospital. Complete blood count was measured using an XE-2100 hematology analyzer (Sysmex, Kobe, Japan). Plasma glucose concentration was measured by the glucose oxidase method (Beckman Coulter, Fullerton, CA).

Grip strength was measured by a Smedley hand dynamometer, which was calibrated to body weight and adjusted for hand comfort and fit. Subjects were told to place their arms in a relaxed stationary position. Three grip measurements were taken. The highest strength was recorded for each hand. Gait speed was measured over a 6-m course and the participants were asked to walk at their usual pace. The time to complete the walk was converted into gait speed (m/s). The better performance of two trials was used for the analysis. In the 400-m walking time test, participants were instructed to walk for ten 40-m laps as quickly as possible receiving feedback and encouragement after completion of each lap. All participants completed the test. Lap time was recorded after the completion of each lap using a stopwatch. Lower extremity physical performance was assessed at each visit using the Health ABC Physical Performance Battery (PPB) (32). PPB is based on standing balance, chair stands, 6-m usual gait speed, and narrow walk balance test. Scoring of the PPB ranges continuously from 0 to 4, where a higher score indicates superior performance. Total body dual-energy X-ray absorptiometry was performed using a Prodigy Scanner (General Electric) and analyzed with version 10.51.006 software (General Electric). Appendicular lean mass, the sum of lean tissue in the arms and legs, derived from dual-energy X-ray absorptiometry measurements, was used as an approximation of muscle mass.

Measurement of Plasma GDF-15

Blood was collected from participants who stayed overnight at the National Institute on Aging Clinical Research Unit, MedStar Harbor Hospital in Baltimore, Maryland. Blood samples were drawn from the antecubital vein between 07:00 and 08:00 am after an overnight fast. Participants were not allowed to smoke, engage in physical activity, or take medications before the blood sample was collected. Blood samples were immediately stored at 4°C, centrifuged within 4 hours, then immediately aliquoted and frozen at –80°C. Plasma GDF-15 was measured using the SOMAscan assay (SomaLogic, Boulder, CO), which has been described in detail elsewhere (33,34). This study specifically targeted GDF-15 of all the analytes in the SOMAscan platform because of the extremely strong relationship of GDF-15 with age (30) and the recent discovery of GFRAL (21–24). The data reported are GDF-15 abundance in relative fluorescence units. Data normalization was conducted according to Center for Human Immunology pipeline. First, hybridization

control normalization removes individual sample variance on the basis of signaling differences between microarray and Agilent scanner. Second, median signal normalization removes inter-sample differences within a plate due to technical differences such as pipetting variation. Third, calibration normalization removes variance across assay runs. In addition, interplate normalization process using Center for Human Immunology cite specific calibrator is performed to allow quality control of the normalization across all experiments conducted at the Center for Human Immunology (34). The overall technical variability of the assay is low, with a median intraplate coefficient of variation (CV) in the approximately 3%–4% range. The interplate CV for GDF-15 was 3.4% and 8.3% overall.

Statistical Analysis

The analysis presented here was based on a cross-sectional design. Pearson correlations were used to examine the relationship between plasma GDF-15 concentrations and demographic factors, sarcopenia-related outcomes, and other factors. Continuous variables were reported as mean $\pm$ standard deviation. One way analysis of variance was used to compare plasma GDF-15 by race category. Separate multivariable linear regression models were used to examine the relationship between plasma GDF-15 and sarcopenia-related outcomes. Standardized beta coefficients were calculated for each model. Age and race were included as covariates in the multivariable models, as these variables were significantly associated with plasma GDF-15. Additional models were run with the addition of an interaction term of GDF-15 and sex to determine whether the relationship between GDF-15 and outcomes differed by sex. Scatterplots were used to display the relationship between plasma GDF-15 and selected sarcopenia-related outcomes adjusting for age and race. The level of significance used in this study was p value is of less than .05.

Results

The characteristics of the study participants are shown in Table 1. Pearson correlations of plasma GDF-15 with demographic and other characteristics of the study participants are shown in Table 2. Plasma GDF-15 had significant positive correlations with age and 400-m walking test time. Plasma GDF-15 had significant negative correlations with appendicular lean mass, grip strength, gait speed, and physical performance score. There were no significant associations of plasma GDF-15 with education, body mass index, or fat

mass. There were no significant differences in plasma GDF-15 between men and women ($p > .05$). Plasma GDF-15 concentrations were higher in whites compared with blacks and Asians ($p = .035$; Figure 1). Multivariable linear regression models for the relationship of plasma GDF-15 with sarcopenia-related outcomes are shown in Table 3. Models were adjusted for age and race. Plasma GDF-15 was positively associated with 400-m walking test time ($p = .002$) and negatively associated with gait speed ($p = .03$) and physical performance score ($p = .04$). Plasma GDF-15 was not significantly associated with appendicular lean mass, fat mass, or grip strength ($p > .05$). Scatterplots of the relationship of plasma GDF-15 with gait speed, 400-m walking test time, and Health Aging and Body Composition Study (PPB) score are shown in Figure 2. When an interaction term between GDF-15 and sex was added to the respective multivariable linear regression models, there were no significant interactions, showing that the relationship between GDF-15 and sarcopenia-related outcomes did not significantly differ by sex.

Discussion

This study shows that elevated plasma GDF-15 concentrations are associated with low gait speed and low physical performance in healthy adults. This study adds sarcopenia-related outcomes to the growing number of aging outcomes that have been associated with higher plasma GDF-15 in adults. Elevated plasma GDF-15

Table 2. Pearson Correlations of Plasma Growth and Differentiation Factor 15 With Demographic and Other Characteristics of the Study Participants

Characteristic	Pearson correlation	p
Age, y	0.78	<.001
Education, y	−0.09	.20
Body mass index, kg/m ²	−0.002	.98
Appendicular lean mass, kg	−0.19	<.001
Fat mass, kg	−0.03	.64
Grip strength, kg	−0.32	<.001
Gait speed, m/s	−0.35	<.001
400-m walking test time, s	0.54	<.001
Physical performance score	−0.50	<.001

Table 1. Characteristics of 194 Healthy Study Participants From the Baltimore Longitudinal Study of Aging

Characteristic	Mean (SD) or %
Age, y	59.1 (19.7)
Sex, % female	51.0
Race, %	
White	70.6
Black	22.2
Asian	7.2
Education, y	17.3 (2.5)
Body mass index, kg/m ²	26.4 (4.6)
Appendicular lean mass, kg	47.9 (10.3)
Fat mass, kg	24.9 (9.9)
Grip strength, kg	34.8 (12.1)
Gait speed, m/s	1.26 (0.23)
400-m walking test time, s	253 (47)
Physical performance score	3.18 (0.37)

Figure 1. Plasma growth and differentiation factor 15 (GDF-15) concentrations by race in participants of the Baltimore Longitudinal Study of Aging. Full color version is available within the online issue.

Table 3. Linear Regression Models for the Relationship of Plasma Growth and Differentiation Factor 15 With Sarcopenia-Related Outcomes, Adjusted by Age and Race

Characteristic	Beta	SE	B*	p
Appendicular lean mass, kg	-0.70	2.62	-0.028	.80
Fat mass, kg	-0.23	2.60	-0.015	.90
Grip strength, kg	0.06	2.90	0.002	.98
Gait speed, m/s	-0.12	0.05	-0.24	.03
400-m walking test time, s	34.1	10.7	0.32	.002
Physical performance score	-0.17	0.08	-0.20	.04

*Note: Standardized beta coefficient.

levels are associated with many chronic diseases and predict adverse outcomes in adults with cardiovascular disease (35), diabetes (36), chronic kidney disease (37), and cancer (38). This study corroborates the strong positive linear relationship between age and plasma GDF-15 described in nearly all clinical and epidemiological studies (18,30,35–38). This study also showed no significant difference in plasma GDF-15 concentrations between men and women, a finding that has been reported previously in apparently healthy community-dwelling adults (39,40).

The biological mechanisms by which elevated circulating GDF-15 could negatively affect skeletal muscle health remain is not understood. The recent discovery of the GDF-15 receptor, GFRAL (21–24), provides a potential mechanism for downregulation of food intake in older adults. Anorexia is common in older adults and is considered to play a key role in the pathogenesis of sarcopenia (25,26). The anorexia of aging is attributed to many different factors, including decreases in smell and taste; alterations in gastrointestinal function such as gastric emptying, problems with dentition and swallowing; changes in hormones such as ghrelin, polypharmacy; and impairments in activities of daily living related to eating, cooking, and obtaining food (25,26). From the third to the ninth decade of life, total energy intake decreases by 1,000–1,200 kcal in men and 600–800 kcal in women (41). Although energy expenditure also tends to decrease with age, the decrease in energy intake in high-risk individuals may be greater than expenditure, resulting in weight loss (42). Anorexia, measured using questions related to food intake and appetite, was associated with sarcopenia (43) and slower gait speed, physical performance battery score, and lower grip strength compared to those without anorexia (44). In addition, adults with anorexia were at a higher risk of developing disability more than 2 years of follow-up (44). Future studies are needed to examine the relationship of circulating GDF-15 with the anorexia of aging and sarcopenia-related outcomes.

The strengths of this study include the highly standardized collection of sample and data on sarcopenia-related outcomes in a well-characterized cohort of adults followed in the BLSA. The subsample of participants in the BLSA was selected for this study on the basis of being healthy, representing a wide range of age, and with no chronic diseases, as this study was aimed to characterize plasma GDF-15 in “healthy aging.” The findings from this study cannot necessarily be extrapolated to adults with the usual burden of chronic diseases. In addition, the participants in the BLSA tend to be more educated and of high socioeconomic status than the general population. In this study, there was no association between plasma GDF-15 and fat mass. However, obesity, that is, body mass index more than 30 kg/m², was one the exclusion criteria for selection of adults from the BLSA for the present substudy. This study involved a recent subsample of adults studied from 2015 to 2016 who had

Figure 2. Relationship of plasma growth and differentiation factor 15 (GDF-15) with (A) gait speed, (B) 400-m walking test time, and (C) Health Aging and Body Composition Study physical performance battery (HABC PPB) score in participants of the Baltimore Longitudinal Study of Aging. Linear models adjusted by age and race. Full color version is available within the online issue.

not yet had sufficient time for follow-up visits. Further longitudinal studies are needed in the future to examine the relationship between GDF-15 and change in gait speed and physical performance over time. This study is limited in that the subjects studied were generally healthy and none of the participants fit the diagnostic definitions of sarcopenia proposed in the literature (12–15).

Plasma GDF-15 was associated with 6-m gait speed, 400-m walk time, and physical performance score but not with muscle strength

or mass. A muscle that is energetically challenged after a while shows signs of decline in strength. It is possible that the association with physical function could be mediated by other mechanisms, such as neuromuscular control or modulation of mitochondrial function that becomes evident only above certain threshold of physical expenditure without affecting peak force (45). These and other possible mechanisms should be explored in future studies both animal models and in humans. Studies also show that lean mass as measured by dual-energy X-ray absorptiometry does not predict function (46). GDF-15 has been implicated in cellular signaling pathways (27–29) independent of GFRAL in brainstem. Possible modulation of mitochondrial function by GDF-15 has not been characterized.

There is a growing interest in manipulation of the GDF-15 pathway to reduce obesity or prevent cachexia (16). This study suggests that the GDF-15 pathway plays a role in skeletal muscle health. Studies involving GDF-15 knockout mice and transgenic mice overexpressing GDF-15 have provided insight into the relationship of GDF-15 with metabolism, but there are some notable inconsistencies with human studies. GDF-15 knockout mice have increased food intake, greater weight, and increased adiposity (47). Transgenic mice overexpressing GDF-15 have decreased food intake, lower weight, reduced fat mass, improved glucose tolerance (48), and improved insulin sensitivity (49,50). In contrast, elevated plasma GDF-15 in humans is associated with impaired glucose tolerance and insulin resistance (51). Transgenic mice overexpressing GDF-15 have increased life span compared to wild-type mice (49). In contrast, elevated circulating GDF-15 is an independent predictor of mortality in humans (35). In study populations with a high burden of cardiovascular disease, elevated GDF-15 has been associated with elevated plasma C-reactive protein (51,52).

Further studies are needed to characterize the relationship of circulating GDF-15 with sarcopenia-related outcomes in older adults with the usual burden of chronic diseases, age-related proinflammatory state, and with obesity. Whether elevated plasma GDF-15 is an independent predictor of sarcopenia-related outcomes should be addressed in prospective studies. There is recent interest in targeting the GFRAL receptor and GDF-15 pathway for obesity and cachexia (53). For obesity, enhancing GDF-15/GFRAL signaling could potentially decrease appetite and food intake and lead to weight loss. Conversely, in cachexia, blockage of the same signaling pathway could increase appetite and food intake and lead to weight gain. If future studies corroborate and expand on the relationship of high circulating GDF-15 with sarcopenia-related outcomes described in this study, there would be implications for also targeting GDF-15 as a strategy for prevention or treatment of sarcopenia.

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Conflict of Interest

L.F. serves on the editorial board of JGMS. B.S. is a former SomaLogic, Inc, employee and a company shareholder. The remaining authors declare no conflicts of interest.

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