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Changes in systemic GDF15 across the adult lifespan and their impact on maximal muscle power: the Copenhagen Sarcopenia Study

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Abstract

Background Although growth differentiation factor 15 (GDF15) is known to increase with disease and is associated with low physical performance, the role of GDF15 in normal ageing is still not fully understood. Specifically, the influence of circulating GDF15 on impairments in maximal muscle power (a major contributor to functional limitations) and the underlying components has not been investigated.

Methods Data from 1305 healthy women and men aged 20 to 93 years from The Copenhagen Sarcopenia Study were analysed. Circulating levels of GDF15 and markers of inflammation (tumor necrosis factor-alpha, interleukin-6, and high-sensitivity C-reactive protein) were measured by ELISA (R&D Systems) and multiplex bead-based immunoassays (Bio-Rad). Relative (normalized to body mass), allometric (normalized to height squared), and specific (normalized to leg muscle mass) muscle power were assessed by the Nottingham power rig [leg extension power (LEP)] and the 30 s sit-to-stand (STS) muscle power test. Total body fat, visceral fat, and leg lean mass were assessed by dual energy X-ray absorptiometry. Leg skeletal muscle index was measured as leg lean mass normalized to body height squared.

Results Systemic levels of GDF15 increased progressively as a function of age in women (1.1 ± 0.4 pg·mL^{-1}·year^{-1}) and men (3.3 ± 0.6 pg·mL^{-1}·year^{-1}) (both $P < 0.05$). Notably, GDF15 increased at a faster rate from the age of 65 years in women (11.5 ± 1.2 pg·mL^{-1}·year^{-1}, $P < 0.05$) and 70 years in men (19.3 ± 2.3 pg·mL^{-1}·year^{-1}, $P < 0.05$), resulting in higher GDF15 levels in men compared with women above the age of 65 years ($P < 0.05$). Independently of age and circulatory markers of inflammation, GDF15 was negatively correlated to relative STS power ($P < 0.05$) but not LEP, in both women and men. These findings were mainly explained by negative associations of GDF15 with specific STS power in women and men (both $P < 0.05$).

Conclusions A J-shaped relationship between age and systemic GDF15 was observed, with men at older age showing steeper increases and elevated GDF15 levels compared with women. Importantly, circulating GDF15 was independently and negatively associated with relative STS power, supporting the potential role of GDF15 as a sensitive biomarker of frailty in older people.

Keywords Growth differentiation factor 15; Sit-to-stand; Leg extension power; Sarcopenia; Frailty

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Introduction

Growth differentiation factor 15 (GDF15) is a cytokine released in response to stress or injury[1] that has been identified as an important biomarker for cardiovascular disease, metabolic disease, cancer, cognitive impairment, mitochondrial dysfunction, and cachexia.[1,2] Increases in circulating levels of GDF15 with increasing age have been reported in the literature,[3–6] with higher levels related to increased mortality risk.[2,4,7–10] The relationship between circulating GDF15 and age has been shown to be curvilinear[2,10,11]; however, the specific age at which GDF15 levels starts to accelerate or whether these increases are sex specific still need to be clarified.

Notably, among 1301 circulating proteins measured in a cohort of older people from the In Chianti study, GDF15 proved to be among the strongest predictors of mobility limitations when assessed at 9 years follow-up.[12] Thus, circulating GDF15 is considered a potential core biomarker of frailty in older people.[13,14] Interestingly, a close relationship between GDF15 and another hallmark of ageing, that is, chronic low-grade inflammation has been demonstrated, but the interplay between these biomarkers and how they contribute to muscle dysfunction are not fully elucidated.[3,9,15,16] The influence of GDF15 on functional ability might be due to its effects on the neuromuscular system, because recent studies have shown that elevated GDF15 concentrations were related to muscle wasting in intensive care unit patients,[17] with patients demonstrating muscle weakness during their hospital stay also exhibiting increased plasma and muscle mRNA expression levels of GDF15, respectively, compared with controls.[18] Further, circulating GDF15 levels have been observed to be negatively associated with muscle mass, handgrip strength, and physical performance.[2,5,9,19] However, no knowledge exists about the relationship of circulating GDF15 with relative muscle power and its underlying components. Importantly, low relative muscle power is a stronger predictor of mobility limitations, frailty, and disability among older adults compared with sarcopenia.[20] Furthermore, relative muscle power, assessed as maximal leg extension power (LEP), decreases with age due to changes in allometric (normalized to height squared) muscle power and body mass index (BMI) as observed across the lifespan.[21] Specifically, allometrically scaled muscle power declines with ageing at annual rates of 1–2% between the age of 40 and 60 years to reach steeper annual decline rates of 2–4% above the age of 60 years in both women and men.[21] In addition, BMI has been shown to increase annually 0.3–0.4% from 20 to ~70 years of age, amplifying the annual losses expressed as maximal relative muscle power normalized to body mass.[21] However, the role of GDF15 and the potential relation to muscle power production have not previously been investigated.

Thus, the aim of the present investigation was (i) to assess the potential relationship between circulating GDF15, age, and sex and (ii) to assess the influence of circulating GDF15 on relative muscle power and its underlying components.

Material and methods

Study cohort

The *Copenhagen Sarcopenia Study*[22] is a population-based cross-sectional study conducted between 2013 and 2016, whose participants were recruited from a random sample of 20 000 men and women (aged 20 to 101 years) taking part in the *Copenhagen City Heart Study*.[23] Subjects were invited to participate in the present investigation using the following exclusion criteria: pregnancy, acute or chronic medical illness, surgery within the last 3 months, cancer, medication known to affect body composition (e.g. corticosteroid administration), and any history of compromised ambulation or prolonged immobilization. A total of 1305 subjects (729 women and 576 men; aged 20 to 93 years) accepted to participate in the present investigation (*Table* 1). According to the data reported elsewhere regarding physical activity levels[24] and functional status,[22] the present study participants were considered healthy and physically active. All participants gave their written informed consent. The study was performed in accordance with the Helsinki Declaration and approved by the Ethical Committee of Copenhagen (H-3-2013-124).

Body composition

A stadiometer and scale device were used to record the height and body mass of the participants without shoes and while wearing light clothing. Height (m) was assessed to the nearest 0.1 cm and body mass (kg) to the nearest 0.1 kg. BMI was obtained from the ratio between body mass and height squared ($kg \cdot m^{-2}$). Total body fat, visceral fat, and legs lean mass were assessed by dual energy X-ray absorptiometry (iDXA, GE Lunar, Madison, USA) and analysed using commercially available software (Encore software Version 16.0). Due to the inter-individual variation in these body composition components being highly influenced by body size, leg skeletal muscle index (legs SMI) was calculated as the ratio between leg lean mass and height squared ($kg \cdot m^{-2}$), and total body fat index and visceral fat index were calculated as total body fat and visceral fat normalized to height squared ($kg \cdot m^{-2}$), respectively.

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Table 1 Main characteristics of the study participants per sex and age group

	Young women (n = 172)	Middle-aged women (n = 261)	Older women (n = 296)	Young men (n = 110)	Middle-aged men (n = 235)	Older men (n = 231)
Age (years)	29.9 ± 5.2	52.5 ± 7.3	75.1 ± 7.0	30.0 ± 5.2	52.7 ± 7.3	74.1 ± 6.0
Body mass (kg)	64.4 ± 9.7	69.7 ± 12.0	67.6 ± 12.2	83.0 ± 12.4	85.2 ± 12.0	82.9 ± 14.9
Height (m)	1.68 ± 0.07	1.67 ± 0.06	1.63 ± 0.07	1.83 ± 0.07	1.81 ± 0.07	1.77 ± 0.07
BMI (kg·m^{-2})	22.7 ± 3.1	24.9 ± 4.4	25.5 ± 4.5	24.8 ± 3.4	26.1 ± 3.6	26.5 ± 4.2
Fat index (kg·m^{-2})	6.85 ± 2.43	8.75 ± 3.53	9.92 ± 3.36	5.51 ± 2.52	7.00 ± 2.61	8.03 ± 3.00
Visceral FI (kg·m^{-2})	0.08 ± 0.09	0.23 ± 0.23	0.38 ± 0.25	0.18 ± 0.17	0.43 ± 0.30	0.58 ± 0.35
Inflammation						
TNF-α (pg·mL^{-1})	11.3 ± 5.0	14.6 ± 7.4	16.6 ± 6.9	13.0 ± 6.1	13.7 ± 6.2	18.6 ± 14.0
IL-6 (pg·mL^{-1})	0.4 ± 0.4	0.7 ± 0.4	0.5 ± 0.7	0.4 ± 0.3	0.4 ± 0.4	0.7 ± 2.4
hsCRP (pg·mL^{-1})	1.5 ± 3.6	1.5 ± 2.7	2.4 ± 4.8	1.1 ± 1.7	1.6 ± 3.0	3.0 ± 8.7

BMI, body mass index. FI, fat index; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha. Data are presented as mean ± standard deviation. Subjects were divided into young (20–39 years), middle-aged (40–64 years) and older (≥65 years) men and women.

Maximal muscle power

Leg extension power

Maximal LEP was assessed by the Nottingham power rig (Medical Engineering Unit, University of Nottingham Medical School, Nottingham, UK).[25] This device measures unilateral lower-limb extension power with the participants seated in an upright position, their arms folded across the chest, knees flexed having one foot resting on the floor, and the other foot positioned on the dynamometer pedal connected to a flywheel. After two familiarization trials, the participants were instructed to push the pedal forward as hard and fast as possible. The test was performed separately on each leg, and measurements were repeated for each limb until maximal power output could not be increased further. At least five repetitions were performed with a 30 s resting period between successive attempts. Strong verbal encouragement and visual feedback were provided to all study participants to ensure a maximal volitional effort. The highest LEP value was selected for further analysis.

Sit-to-stand muscle power

The 30 s sit-to-stand (STS) test involves recording the number of STS repetitions performed continuously by the subjects in 30 s. After the cue 'ready, set, go!', participants performed STS repetitions as rapidly as possible on a standardized armless chair (0.45 m seat height) starting from the sitting position with their buttocks touching the chair to full standing position with their arms crossed over the chest. A stopwatch was started simultaneously on the 'go!' cue, and it was stopped when the 30 s time limit was reached. The total number of completed STS manoeuvres during the 30 s period was recorded. Strong standardized verbal encouragement was given throughout the test. The subjects were allowed to try one to two times with an adequate resting period (30–60 s) before the definitive STS test was annotated. As described in details elsewhere,[26–28] STS mean muscle power (W) was calculated as

$$\text{STS power} = \frac{\text{Body mass} \times 0.9 \times g \times [\text{Height} \times 0.5 - \text{Chair height}]}{\left[\frac{\text{Time}}{N \text{ of reps}}\right]} \times 0.5$$

where body mass is expressed in kg, body height and chair height in m, and time in s (30 s in the current setting). Briefly, 0.9 is a coefficient to calculate the proportion of body mass that is lifted during the STS maneuver, 0.5 in the numerator is a coefficient to calculate leg length, and 0.5 in the denominator is a coefficient to calculate the relative duration of the concentric phase in each STS repetition.

Finally, both muscle power measures (LEP and STS power) were expressed relative to body mass (W·kg^{-1}),[20] whereas allometric power (W·m^{-2}) was calculated as the ratio of absolute power and body height squared, and specific power (W·kg^{-1}) was calculated as the ratio between absolute power and leg lean mass (one leg for LEP and two legs for STS power).

Determination of GDF15 and markers of inflammation

Blood samples were collected from the antecubital vein and frozen at −80°C after the corresponding procedures for plasma and serum preparation. Plasma GDF15 was measured using DuoSet ELISA kits (R&D Systems, USA). Briefly, plates were pre-coated with capture antibody according to the manufacturer instructions. Then, 100 μL of pre-cleared plasma was applied per well and supplemented with equal volume of DuoSET kit-supplied reagent dilution buffer. Recombinant standard dilution series (5 to 1280 pg·mL^{-1}) were included. The plates were incubated overnight at 22–24°C. All consecutive washes, subsequent antibody incubation, streptavidin-HRP binding, and colorimetric processing were conducted according to manufacturer instructions. Plates

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were examined with a micro-plate reader (EnSpire Multilabel Reader, Perkin-Elmer, USA) at 450 nm. Calculations were conducted using sigmoidal curve fitting with curve fitted to the results of the standards measurements on each plate. Given the relationship between GDF15 and markers of inflammation,[3,9,15,16] plasma tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and serum high-sensitivity C-reactive protein (hsCRP) were measured to assess the effects of GDF15 independently from low-grade inflammatory status. TNF-α and IL-6 were assessed in plasma using commercially available multiplex magnetic bead-based immunoassay kits (Bio-Rad, USA). Serum levels of hsCRP were assessed using latex-entrenched immune-turbidimetry analyses (Roche Diagnostics, Switzerland).

Statistical analysis

Data are presented as mean ± standard deviation unless otherwise stated. Analyses were performed separately in women and men. The association between age and plasma GDF15 was assessed by regression analyses. Firstly, linear, quadratic, and cubic regression models were compared based on the coefficient of determination (r^2) change in order to determine the most suitable regression model based on changes in *F* values. Then, segmented (interval confined) regression analyses were performed to determine whether and at what boundary age a change in the slope (i.e. altered rate of change) emerged in the relationship between age and GDF15. Using an iterative approach several age points were evaluated (30, 35, 40, 45, 50, 55, 60, 65, 70, 75, and 80 years) at different age intervals (20–45, 20–50, 25–55, 30–60, 35–65, 40–70, 45–75, 50–80, 55–85, 60–90, and 65–95 years, respectively). Subsequently, a single regression model was created considering the age points at which a statistically significant change in regression slope was observed. Linear mixed effects models were used to assess differences by sex and age groups (young: 20.0 to 39.9 years; middle-aged: 40.0 to 64.9 years; and old: ≥65 years), both set as fixed factors, while participants were included as a random factor. The models were calculated using maximum likelihood estimation and the best-fitting covariance structure. Pairwise comparisons were conducted applying Bonferroni's corrections.

For the assessment of the influence of plasma GDF15 levels on relative muscle power and its determining components, a two-step process was followed using linear mixed effect models. First, the unadjusted association (*r* values) of plasma GDF15 with relative muscle power and its constitutive components (i.e. BMI, leg SMI, allometric power, and specific power) was separately assessed (bivariate analysis), and further adjusted for age as well as for age and inflammatory markers (TNF-α, hsCRP, and IL-6). The association between GDF15 and total body fat index and visceral fat index was also evaluated. Secondly, the partial contribution [standardized (Std.) β values] of the basic components of relative muscle power (i.e. BMI, leg SMI, and specific muscle power) to the association between GDF15 and relative muscle power (multivariate analysis) was assessed, and again adjusted for age as well as for age and inflammatory markers. As BMI, total body fat index, and visceral fat index all are measures of obesity, only the one parameter showing the greatest association to GDF15 was included in the final model. All statistical analyses were performed using SPSS v24 (SPSS Inc., Chicago, Illinois), and the level of significance was set at α = 0.05 using two-tailed testing.

Results

Results on LEP, 30 s STS, and GDF15 are presented in *Table* 2 specified for sex and age groups.

Association between plasma GDF15 and age

Our regression analyses showed a quadratic J-shaped relationship between age and GDF15 in women (r^2 = 0.30; F = 147.061; $P < 0.001$) (*Figure* 1A). In addition, segmented regression analyses yielded two main phases of variation in GDF15 throughout the adult lifespan in women (r^2 = 0.31; F = 151.901; $P < 0.001$): GDF15 increased between 20 and 65 years at a rate of 1.1 ± 0.4 pg·mL^{-1}·year^{-1} (P = 0.017) and above 65 years at a faster rate of 11.5 ± 1.2 pg·mL^{-1}·year^{-1} ($P < 0.001$).

Likewise, men demonstrated a cubic J-shaped relationship between age and GDF15 (r^2 = 0.32; F = 83.495; $P < 0.001$) (*Figure* 1B) with segmented regression analyses revealing two phases of variation in GDF15 (r^2 = 0.32; F = 128.694; $P < 0.001$) (*Figure* 1B): GDF15 increased between 20 and 70 years at a rate of 3.3 ± 0.6 pg·mL^{-1}·year^{-1} ($P < 0.001$) while increasing at a steeper rate above the age of 70 years of 19.3 ± 2.3 pg·mL^{-1}·year^{-1} ($P < 0.001$).

There were statistically significant differences by sex in both phases, with men showing higher rates of age-related variation in GDF15 compared to women (both $P < 0.05$) (*Table* 2).

Bivariate association of plasma GDF15 with relative muscle power and its components

Unadjusted *r* values for the different relationships observed between GDF15 and relative muscle power (either LEP or STS power) and its components (i.e. BMI, leg SMI, and allometric and specific power) are reported in *Table* 3, while adjusted *r* values are shown in *Table* 4 for women and *Table* 5 for men.

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Table 2 Lean mass, plasma GDF15, and maximal lower-limb muscle power in young (20–39 years), middle-aged (40–64 years), and older (≥65 years) women and men

	Young women	Middle-aged women	Older women	Young men	Middle-aged men	Older men
Leg SMI ($kg \cdot m^{-2}$)	5.1 ± 0.6	5.2 ± 0.6	4.9 ± 0.6[a,b]	6.3 ± 0.7*	6.2 ± 0.7*	5.8 ± 0.7*[a,b]
GDF15 ($pg \cdot mL^{-1}$)	177.1 ± 95.4	197.5 ± 88.3	323.2 ± 188.8[a,b]	154.5 ± 58.2	223.8 ± 107.1[a]	375.5 ± 242.3*[a,b]
STS power						
Relative ($W \cdot kg^{-1}$)	6.3 ± 1.4	5.1 ± 1.5[a]	3.3 ± 1.1[a,b]	7.4 ± 1.5*	6.2 ± 1.7*[a]	4.3 ± 1.4*[a,b]
Allometric ($W \cdot m^{-2}$)	142.0 ± 36.1	124.6 ± 35.4[a]	82.5 ± 27.5[a,b]	183.8 ± 42.0*	161.0 ± 42.8*[a]	112.4 ± 38.3*[a,b]
Specific ($W \cdot kg^{-1}$)	28.1 ± 5.9	24.2 ± 6.4[a]	16.8 ± 4.9[a,b]	29.3 ± 5.6	26.1 ± 6.6*[a]	19.4 ± 6.0*[a,b]
LEP						
Relative ($W \cdot kg^{-1}$)	3.6 ± 0.8	3.0 ± 0.9[a]	1.9 ± 0.7[a,b]	4.7 ± 0.9*	4.0 ± 1.0*[a]	2.7 ± 0.9*[a,b]
Allometric ($W \cdot m^{-2}$)	82.0 ± 20.4	73.7 ± 20.1[a]	48.1 ± 17.0[a,b]	115.4 ± 24.5*	104.7 ± 26.5*[a]	72.3 ± 24.1*[a,b]
Specific ($W \cdot kg^{-1}$)	32.2 ± 6.8	28.6 ± 7.0[a]	19.6 ± 6.4[a,b]	36.9 ± 6.8*	33.7 ± 7.8*[a]	24.8 ± 7.7*[a,b]

GDF15, growth differentiation factor 15; LEP, leg extension power; SMI, skeletal muscle index; STS, sit-to-stand.
Data are presented as mean ± standard deviation.
*Significant differences compared to women ($P < 0.05$).
[a]Significant differences compared with young.
[b]Significant differences compared with middle-aged.

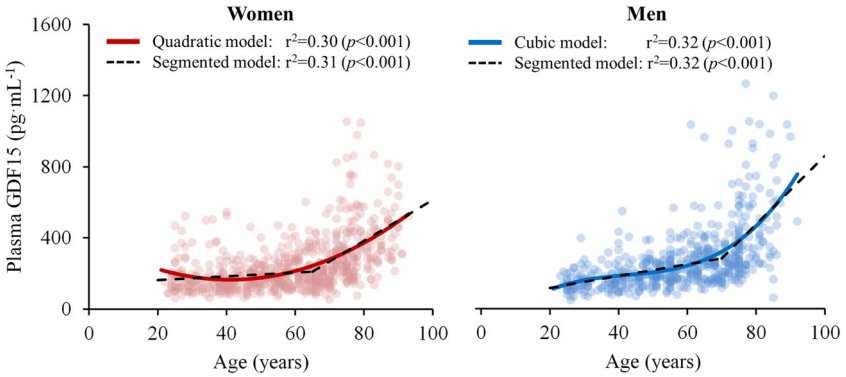

Figure 1 Trajectories of plasma GDF15 with age in women and men. GDF15, growth differentiation factor 15.

Body mass index and legs skeletal muscle index

In women, there was no association of plasma GDF15 with BMI or legs SMI after adjusting for age (both $r = 0.02$ and $P \geq 0.566$) or age and inflammatory markers (both $r \leq 0.06$ and $P \geq 0.133$) (*Table* 4). In contrast, both BMI and legs SMI were significantly associated to plasma GDF15 in men after adjusting for age (both $r = 0.12$ and $P \leq 0.015$) as well as age and markers of inflammation ($r = 0.16$ and 0.15, respectively, both $P = 0.002$) (*Table* 5).

Leg extension power

No association was found in women between plasma GDF15 and relative, allometric, or specific LEP when adjusting for age (all $r \leq 0.06$ and $P \geq 0.095$) or for age and inflammatory markers (all $r \leq 0.05$ and $P \geq 0.196$) (*Table* 4). Similarly, in men, there was no association between plasma GDF15 and relative LEP when adjusting for age ($r = 0.06$ and $P = 0.090$) or for age and inflammatory markers ($r = 0.05$ and $P = 0.181$) (*Table* 5). However, men demonstrated a negative association between plasma GDF15 and allometric and specific LEP after adjusting for age (both $r = 0.08$ to 0.10 and $P \leq 0.045$), and with allometric but not specific LEP after adjusting for age and inflammatory markers ($r = 0.09$, $P = 0.034$).

Sit-to-stand power

Significant associations between plasma GDF15 and relative, allometric, and specific STS power were observed in women after adjusting for either age (all $r = 0.09$ to 0.12 and $P \leq 0.004$) or age and inflammatory levels (all $r = 0.06$ to 0.10 and $P \leq 0.045$) (*Table* 4). Similarly, men exhibited significant associations between GDF15 and relative, allometric, and specific STS power after adjusting for age (all $r = 0.10$ to 014 and $P \leq 0.009$) or age and inflammation status (all $r = 0.07$ to 0.12 and $P \leq 0.048$) (*Table* 5).

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Table 3 Association between systemic levels of GDF15 and measures of body composition and relative muscle power according to sex and age

	Women				Men			
	Young	Middle-aged	Older	All	Young	Middle-aged	Older	All
BMI	0.04	0.11*	−0.04	0.08**	0.13	0.03	−0.14**	−0.01
Fat index	0.03	0.11*	−0.04	0.12**	0.06	0.08	−0.11	0.08
Visceral FI	0.02	0.22**	−0.03	0.20***	0.18*	0.13*	−0.07	0.17***
Leg SMI	0.04	−0.01	−0.04	−0.09**	0.19*	−0.12*	−0.17**	−0.23***
LEP								
Relative	0.01	−0.21**	−0.15**	−0.35***	0.16	−0.10	−0.24***	−0.39***
Allometric	0.02	−0.16**	−0.17**	−0.34***	0.26**	−0.10	−0.26***	−0.38***
Specific	−0.01	−0.17**	−0.17**	−0.35***	0.13	−0.05	−0.24***	−0.36***
STS power								
Relative	0.01	−0.28***	−0.26***	−0.41***	−0.04	−0.18**	−0.26***	−0.42***
Allometric	0.02	−0.25***	−0.27***	−0.39***	0.04	−0.18**	−0.29***	−0.41***
Specific	0.01	−0.25***	−0.29***	−0.40***	−0.08	−0.13**	−0.24***	−0.38***

BMI, body mass index; FI, fat index; LEP, leg extension power; SMI, skeletal muscle index; STS, sit-to-stand.
Unadjusted bivariate association (*r* values).
*$P < 0.10$,
**$P < 0.05$,
***$P < 0.001$.

Table 4 Association between systemic levels of GDF15 and measures of body composition and relative muscle power in women adjusted for age and inflammatory markers

	Adjusted for age			Adjusted for age and inflammatory markers		
	r	$\beta^a \pm 95\%$ CI	*P*	*r*	$\beta^a \pm 95\%$ CI	*P*
BMI	0.02	−0.07 ± 0.24	0.566	0.06	−0.18 ± 0.23	0.133
Fat index	0.04	−0.08 ± 0.19	0.408	0.07	−0.16 ± 0.18	0.076
Visceral FI	0.01	0.00 ± 0.01	0.793	0.04	−0.01 ± 0.01	0.303
Leg SMI	0.02	−0.01 ± 0.04	0.699	0.03	−0.01 ± 0.04	0.532
LEP						
Relative	0.03	−0.02 ± 0.04	0.295	0.01	−0.01 ± 0.04	0.690
Allometric	0.05	−0.73 ± 1.01	0.155	0.04	−0.61 ± 1.02	0.244
Specific	0.06	−0.30 ± 0.36	0.095	0.05	−0.24 ± 0.36	0.196
STS power						
Relative	0.09	−0.10 ± 0.07	0.004	0.06	−0.07 ± 0.07	0.045
Allometric	0.11	−2.92 ± 1.71	<0.001	0.10	−2.58 ± 1.73	0.004
Specific	0.12	−0.55 ± 0.30	<0.001	0.10	−0.46 ± 0.31	0.003

BMI, body mass index; FI, fat index; LEP, leg extension power; SMI, skeletal muscle index; STS, sit-to-stand.
Adjusted bivariate association (*r* values).
[a]Change per each 100 pg·mL^{-1} increase in GDF15.

Table 5 Association between systemic levels of GDF15 and measures of body composition and relative muscle power in men adjusted for age and inflammatory markers

	Adjusted for age			Adjusted for age and inflammatory markers		
	r	$\beta^a \pm 95\%$ CI	*P*	*r*	$\beta^a \pm 95\%$ CI	*P*
BMI	0.12	−0.24 ± 0.19	0.015	0.16	−0.31 ± 0.20	0.002
Fat index	0.10	−0.15 ± 0.14	0.042	0.13	−0.20 ± 0.14	0.005
Visceral FI	0.07	−0.01 ± 0.04	0.104	0.10	−0.02 ± 0.02	0.020
Leg SMI	0.12	−0.05 ± 0.04	0.011	0.15	−0.06 ± 0.04	0.002
LEP						
Relative	0.06	−0.04 ± 0.05	0.090	0.05	−0.03 ± 0.05	0.181
Allometric	0.10	−1.66 ± 1.36	0.017	0.09	−1.50 ± 1.39	0.034
Specific	0.08	−0.41 ± 0.40	0.045	0.07	−0.34 ± 0.41	0.110
STS power						
Relative	0.10	−0.10 ± 0.08	0.009	0.07	−0.08 ± 0.08	0.048
Allometric	0.14	−3.65 ± 2.09	<0.001	0.12	−3.11 ± 2.13	0.004
Specific	0.11	−0.44 ± 0.32	0.008	0.08	−0.34 ± 0.33	0.043

BMI, body mass index; FI, fat index; LEP, leg extension power; SMI, skeletal muscle index; STS, sit-to-stand.
Adjusted bivariate association (*r* values).
[a]Change per each 100 pg·mL^{-1} increase in GDF15.

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Multivariate association between GDF15 and main components of relative muscle power

There was a significant association of BMI, legs SMI, and specific STS power with plasma GDF15 in both women (Std. β = 0.12, −0.13, and −0.37, respectively) and men (Std. β = 0.21, −0.33, and −0.31, respectively) (all $P < 0.001$) (*Table* 6). Specific STS power (but no other parameters) remained significantly associated with plasma GDF15 in both women and men when the model was adjusted for age (Std. β = −0.16 and −0.12; both $P \leq 0.006$). Furthermore, specific STS power was negatively associated to plasma GDF15 in women and men after adjusting for age and inflammatory markers (Std. β = −0.14 and −0.10; both $P \leq 0.034$), while a trend (P = 0.083) was observed for leg SMI in men only (Std. β = −0.11) (*Table* 6).

Discussion

The present study investigated the role of GDF15 in normal ageing, and in particular its relationship to low muscle power, because the latter is one of the main contributors to impaired physical function in old adults. The main findings of the present study were (i) a J-shaped relationship was found to exist between age and GDF15, with men at older age showing steeper increases and elevated GDF15 levels compared with women, and (ii) circulating levels of GDF15 were independently and negatively associated to relative STS power in both men and women.

GDF15 is considered an overall stress-induced cytokine that is released in response to tissue injury[1] and has been shown to increase during progressive ageing.[3–6] However, it is less clear whether there is a specific stage in life in which GDF15 increases at an accelerated rate, and differences between women and men are inconsistently reported.[2,3,29,30] In the present study, no sex differences in plasma GDF15 were observed before the age of 65 years, from which age plasma GDF15 levels remained systematically higher in men compared to women. This observation might explain the disparate conclusions found in the literature. In addition, the sex differences in plasma GDF15 observed at older age in the present study could be explained by a greater rate of increase in GDF15 observed in men compared with women throughout the adult lifespan. In both cases, the increase in circulating GDF15 levels was particularly evident after the sixth decade of life (65 years in women and 70 years in men).

Notably, elevated levels of circulating GDF15 have been associated with several types of chronic disease and conditions, including acute and chronic inflammation,[31] mitochondrial dysfunction,[2] frailty,[32] and all-cause mortality.[4] Furthermore, GDF15 has been inversely associated with physical performance in older people[9] and proven to be an independent predictor of declining physical function.[30] In the current study, plasma GDF15 was negatively associated to relative STS power, which in turn is a strong predictor of physical performance in older people.[12,20] In contrast, no relationship was observed between GDF15 and maximal LEP. The discrepancy between the two tests may well be related to differing biomechanical characteristics of the tests. Thus, LEP expresses maximal unilateral lower-limb power produced during an effort lasting <1 s, while STS power expresses average bilateral lower-limb muscle power exerted during a continuous 30 s effort. In this sense, the role of GDF15 as a mitokine[15] could explain the present observation of a stronger association with mechanical muscle power exerted during more sustained efforts. Notably, among the basic components of relative STS muscle power, specific STS power (i.e. absolute 30 s STS power/leg lean mass) was independently (negatively) associated with circulating GDF15 levels in both women and men, indicating that elevated GDF15 levels are associated with reduced functional muscle quality at old age (≥65 years).

Table 6 Multivariate association between GDF15 and the basic components of relative muscle power

	Women			Men		
	r	Std. β ± 95% CI	*P*	*r*	Std. β ± 95% CI	*P*
Model 1	0.41			0.44		
BMI		0.12 ± 0.11	<0.001		0.21 ± 0.11	<0.001
Leg SMI		−0.13 ± 0.11	<0.001		−0.33 ± 0.11	<0.001
Specific STS power		−0.37 ± 0.07	<0.001		−0.31 ± 0.08	<0.001
Model 2	0.49			0.53		
BMI		−0.03 ± 0.11	0.642		−0.01 ± 0.12	0.901
Leg SMI		0.01 ± 0.11	0.875		−0.09 ± 0.12	0.149
Specific STS power		−0.16 ± 0.09	<0.001		−0.12 ± 0.09	0.006
Model 3	0.51			0.54		
BMI		−0.09 ± 0.11	0.124		0.00 ± 0.12	0.992
Leg SMI		0.05 ± 0.11	0.393		−0.11 ± 0.12	0.083
Specific STS power		−0.14 ± 0.09	0.003		−0.10 ± 0.09	0.034

BMI, body mass index; SMI, skeletal muscle index; STS, sit-to-stand.
Model 1, unadjusted. Model 2, adjusted for age. Model 3, adjusted for age and inflammatory markers (IL-6, TNF-α, and hsCRP).

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These data suggest that the relationship between GDF15 and relative STS power is influenced by the association of GDF15 with specific STS power (i.e. power production per unit of muscle mass) in women and men, while legs SMI might also have a relevant (albeit not significant) role in the relationship of GDF15 with relative STS power in men. The different results observed in women and men might be due to differences in hormone concentrations and changes with ageing. The greater age-related increase in GDF15 and higher GDF15 levels at older age noted in men vs. women may explain the stronger association of GDF15 with skeletal muscle mass (leg lean mass) presently observed in men. This may indicate that greater levels of circulating GDF15 are necessary to observe a relationship with muscle mass compared with muscle function. In any case, these sex-specific findings deserve a more thorough investigation in future studies.

Previous studies have tried to elucidate the mechanisms by which GDF15 could play a role in skeletal muscle metabolism and function. Both plasma and muscle mRNA expression of GDF15 were found to be higher in intensive care unit patients that developed muscle weakness, which was related to the inhibition of microRNAs involved in muscle proliferation, differentiation, and recovery.[18] A possible mechanism linking the increased levels of GDF15 with impaired neuromuscular function with increasing age has recently been proposed.[33] Increased Akt-independent activation of mTORC1 with ageing has been shown to up-regulate GDF15 gene expression in humans through the activation of the transcription factor STAT3.[33] Concomitantly, GDF15 led to increased caspase 3 activity, while up-regulating autophagic marker LC3 and inducing increases in protein ubiquitination and oxidation.[33] Of note, this process produced muscle atrophy, loss of type II fibres (especially important for muscle power production), mitochondrial dysfunction, and reductions in maximal isometric muscle force production and exercise capacity.[33] Importantly, in a transgenic mouse model, these Akt-independent mTORC1-induced degenerative effects were partially reversed by silencing of GDF15.[33]

Nevertheless, the identification of a peripheral receptor of GDF15 is needed to better understand its peripheral action on skeletal muscle mass and neuromuscular function. The GDNF family alpha-like (GFRAL) receptor has been identified as a target for GDF15 action in the central nervous system, participating in the negative regulation of feeding behaviour in mice.[34,35] Interestingly, treatment with a therapeutic antagonistic monoclonal antibody for GDF15-GFRAL reversed cancer cachexia in mice, which was translated to improved function.[36] However, the evidence on the physiological effects of GDF15 in mice and humans is contradictory. For example, transgenic mice overexpressing GDF15 have increased lifespan compared with wild-type mice, while elevated circulating GDF15 is an independent predictor of all-cause mortality in humans.[10] In addition, studies conducted in mice have demonstrated a positive role of GDF15 in the maintenance of spinal cord motor neurons, preventing the loss of motor axons and reductions in physical performance.[37] In contrast, circulating GDF15 is negatively associated with maximal muscle power and physical performance in humans (present data). Regarding these contradictory observations, it is possible that transient peaks in GDF15 may be beneficial (e.g. after a single bout of high-intensity exercise[38]), while chronically elevated systemic levels are detrimental to skeletal muscle homeostasis and neuromuscular function.

Among the limitations of the current study, this was designed as a cross-sectional investigation, and so no direct cause-effect relationships between GDF15 and maximal lower-limb muscle power could be established. In addition, despite including healthy individuals only, it could not be completely ruled out that some subjects might have had undiagnosed medical conditions, which potentially could have affected the present results, especially in the oldest age groups.[39] Furthermore, muscle power during the 30 s STS test was not measured directly, but estimated using an equation, which on the other hand, has been adequately validated in previous studies against gold standard instruments.[26–28]

In conclusion, systemic GDF15 was observed to increase progressively as a function of age, with a steeper rate of rise after the sixth decade of life. Further, GDF15 levels increased more rapidly in men compared with women, leading to elevated GDF15 levels in older men compared with older women. Importantly, circulating GDF15 was independently and negatively associated with relative lower-limb muscle power produced during maximal functional efforts (30 s STS), but not during very brief ($<$1 s) maximal muscle actions (Nottingham power rig). This association was mainly due to a negative relationship between GDF15 and specific muscle power (power normalized to leg lean mass) in both women and men. The present findings along with previous evidence reported in the literature support that GDF15 may serve a future role as a biomarker of frailty in older people.

Conflict of interest

The authors declare that have no conflict of interest.

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Ethical guidelines statement

All authors comply with the Ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[40] This study was approved by the Ethical Committee of Copenhagen (H-3-2013-124) and was performed in accordance with the ethical standards laid down in the 1965 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study.

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