Endocrine (2019) 63:112–119
https://doi.org/10.1007/s12020-018-1763-5

ORIGINAL ARTICLE

Adult individuals with congenital, untreated, severe isolated growth hormone deficiency have satisfactory muscular function

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Received: 4 August 2018 / Accepted: 11 September 2018 / Published online: 24 September 2018

Abstract

Purpose While growth hormone (GH) and the insulin-like growth factor type I (IGF-I) are known to exert synergistic actions on muscle anabolism, the consequences of prolonged GH deficiency (GHD) on muscle function have not been well defined. We have previously described a large cohort of subjects with isolated GHD (IGHD) caused by a mutation in the GH-releasing hormone receptor gene, with low serum levels of GH and IGF-I. The aim of this study was to assess muscular function in these IGHD subjects.

Methods A total of 31 GH-naïve IGHD (16 males) and 40 control (20 males) subjects, matched by age and degree of daily physical activity, were enrolled. Fat free mass was measured by bioelectrical impedance; muscle strength by dynamometry of handgrip, trunk extension, and knee extension; myoelectric activity and muscle fatigue by fractal dimension; conduction velocity in vastus medialis, rectus femoris, and vastus lateralis muscles by surface electromyography.

Results The IGHD group showed higher knee extension strength both when corrected for weight and fat free mass, and higher handgrip and trunk extension strength corrected by fat free mass. They also exhibit higher conduction velocity of the muscles vastus medialis, rectus femoris, and vastus lateralis, but lower free fat mass and myoelectric activity of the vastus medialis, rectus femoris and vastus lateralis. There were no differences between the two groups in fractal dimension in all studied muscles.

Conclusion Individuals with untreated IGHD have better muscle strength parameters adjusted for weight and fat free mass than controls. They also exhibit greater peripheral resistance to fatigue, demonstrating satisfactory muscle function.

KeyWords GH; IGF-I · GHRH receptor · Dynamometry · Muscle function

Introduction

Mass and muscle function are intrinsically related. However, muscle function is not only related to muscle mass, but it rather reflects the interaction of several systems, including nervous, muscular and biomechanical factors [1]. On other hand, muscular function proves to be of utmost importance for the development of the skeleton, mediating bone health throughout the life. Such interaction occurs through muscle contraction, which is responsible for mechanical stimuli on

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bone tissue, thus promoting bone mass and bone resistance [2, 3].

In addition to its critical role on longitudinal growth, growth hormone (GH) has important metabolic functions such as stimulating lipolysis, gluconeogenesis, and bone and muscular anabolism. GH increases muscle anabolism mostly indirectly, through the circulating and locally produced insulin-like growth factor type I (IGF-I), a lipotropic, hypoglycemic, and anabolic peptide [4, 5], and to a lesser extent, directly via its receptor (GHR) [6, 7]. The anabolic effects involve synergistic properties of endocrine, paracrine, and autocrine GH/IGF-I on bone and muscles [8–10].

A strong association between low serum IGF-I and low muscle mass was described in the little mouse, a murine model of isolated GH deficiency (GHD) (IGHD) due to an inactivating mutation in the GH releasing hormone receptor (GHRHR) gene [11], and in mice with GH resistance due to GHR gene knockout (GHRKO) [12, 13]. However, the reduced muscle mass does not seem to have a negative impact on muscle function in these animals [14, 15].

Humans with GHD (often in the context of panhypopituitarism) have decreased muscle mass and increased fat mass [16, 17], apparently without negative impact on muscle function [18]. On the other hand, GH replacement therapy (GHRT) in individuals with GHD and athletes results in increased muscle mass, without marked changes in strength [19–21].

In Itabaianinha county, northeastern Brazil, we have described a large cohort of individuals with severe IGHD due to a homozygous mutation (c57 + 1 G → A) in the GHRHR gene (*GHRHR*, OMIM n. 612781) [22], resulting low serum GH and IGF-I throughout life [23]. These individuals have proportionate severe short stature, reduced fat free mass, and increased fat mass [24], but normal bone mineral content and resistance to fractures [25, 26]. It is possible that their healthy bone phenotype reflects adequate muscle function, helping them in the adaptation to the environment. They perform abundant daily physical activities in rural or pottery work, with normal quality of life and longevity [27, 28]. This cohort is an ideal model to study the consequence of lifetime untreated GHD on muscle function.

The aim of this study was to assess muscle function of subjects with lifelong untreated IGHD from the Itabaianinha kindred.

Subjects and methods

Subjects

In a cross-sectional study, adult GH-naïve IGHD subjects and controls paired by age, sex and level of physical activity were recruited by advertising in the local Dwarfs Association building and by word of mouth among the inhabitants of Itabaianinha County. Inclusion criteria for IGHD group were age 18 years or above and homozygosity for the c57 + 1 G → A *GHRHR* mutation for the IGHD and for the wild type allele for controls [22]. The IGHD status was previously confirmed in this cohort by abnormal GH responsiveness (with peak values less than 1 ng/ml) to clonidine, GHRH, and insulin-induced hypoglycemia, associated to very low, often undetectable IGF-I levels [22, 23]. Exclusion criteria were previous GHRT, functional limitations, neuromuscular, and orthopedic disorders. From the 105 (some deceased) IGHD subjects, initially described [22], 54 were eligible for this study [28]. From these, nine had received GHRT and 14 were not in Itabaianinha during the time of the study. At the end, 31 subjects with IGHD and 40 local controls were enrolled. A two-step protocol was performed between December 2017 and February 2018. In the first step, we collected anthropometric data and measured fat free mass and fat mass, and in the second one, we performed specific functional tests: muscle strength (handgrip, trunk extension, and knee extension), myoelectric activity and fatigue (assessed in the vastus medialis, rectus femoris, and vastus lateralis muscles).

The Federal University of Sergipe Institutional Review Board approved these studies, and all subjects gave written informed consent.

Anthropometric data and level of physical activity

Height was assessed by a vertical stadiometer (MD®, Brazil), and weight by a digital scale (DIGI-HEALTH Serene®, Brazil). Body mass index (BMI) was calculated by the formula weight (kg)/height $(cm)^2$ [29].

The International Physical Activity Questionnaire (IPAQ) short form was used to assess the level of physical activity in the last 7 days (days per week and time per day). The assessed types of activity were walking; moderate-intensity activities (that take moderate physical effort and make you breathe somewhat harder than normal, like carrying light loads, bicycling at a regular pace, or doubles tennis); and vigorous-intensity activities (activities that take hard physical effort and make you breathe much harder than normal, like heavy lifting, digging, aerobics, or fast bicycling). The activity level was coded in high = 3 (at least an hour more moderate-intensity activity over and above the basal level of activity, or half an hour of vigorous-intensity activity over and above basal levels daily); moderate = 2 (half an hour of at least moderate-intensity on most days); and low = 1 (not meeting any of the criteria for either of the previous categories) [30].

Fat free mass and fat mass

We used a tetrapolar bioelectrical impedance device with 50 KHz (Model 450, Biodynamics, LTDA, and São Paulo, Brazil) to assess the absolute (Kg) and relative (%) values of fat free mass and fat mass. Subjects were in supine position with active adhesive electrodes placed on the right wrist and right ankle. The subjects were asked to fast for 12 h (ad libitum water intake was allowed up to 1 h before testing) [31], and to avoid high intensity physical activities for 24 h before the test [32, 33].

Muscle strength (handgrip, trunk extension and knee extension)

A dynamometer (Miotec®, Porto Alegre, Brazil) was used to assess muscle strength. First, subjects were familiarized with the tests and then performed three maximal isometric contractions (5 s of contraction and 15 s of resting between repetitions); the mean of the repetitions was calculated [34]. The software (Miograph, Miotec®, Porto Alegre, Brazil) was used for visual feedback under verbal command by the investigator. The same well-trained investigator (A.L A-G) performed all the tests.

Handgrip strength was performed according to the American Society of Hand Therapists guideline [35]. Trunk extension strength was assessed with subjects in a standing position with 10 degrees in semi-flexed knee [36]. Knee extension strength was assessed by using an adjustable dynamometer chair equipped with a load cell (Aeph, São Paulo, Brazil). Participants were instructed to sit with the hips and knees at 90 and 60 degrees, respectively [37, 38].

Myoelectric activity

Myoelectric potential of the vastus medialis, rectus femoris, and vastus lateralis (dominant limb) was assessed by the eight-channels surface electromyographer (New Miotool, Miotec®, Porto Alegre, Brazil). The Ag/AgCl bipolar electrodes, with an inter-electrode distance of 20 mm (Double Trace, Shanghai Litu Medical Appliances Co., Ltd. China), were placed according to current recommendations of surface EMG for non-invasive assessment of muscles (SENIAM) [39]. The skin was shaved and cleaned with an alcohol swap before fixing the electrodes. Subjects sat on an adapted chair with the hip and knee flexed at 90, and 60 degrees, respectively (greater angle of isometric torque extension for knee) [40, 41]. A non-elastic strap to avoid wrong movements during the test stabilized the individuals. We used a high-and low-pass filter of 20 and 500 HZ, respectively to remove potential artifacts.

Prior to the experiments, all subjects were asked to elicit maximal voluntary isometric contraction (knee extension) for signal normalization. During the experiment, subjects performed three maximum voluntary isometric contraction for 5 s, with 120 s resting between them, as published [40]. For data analysis and visual feedback we used a software (Miograph, Miotec®, Porto Alegre, Brazil), and Root Mean Square were calculated and normalized to their respective value at 100% of maximum voluntary isometric contraction [42]. The first and the last second were discarded to avoid bias.

Muscle fatigue

The software BioTrainner (Miotec®, Porto Alegre, Brazil) was used to asses muscle fatigue of vastus medialis, vastus lateralis, and rectus femoris. Subjects performed a sub-maximal isometric contraction (60% of maximum) for 60 s or until exhaustion. Studies show that isometric contractions (60% of maximum) estimate the mutual interaction of both peripheral and central fatigue [43]. The same subject's position for myoelectric activity assessment was adopted. Muscle fatigue was assessed by conduction velocity that suggests peripheral fatigue and fractal dimension. Fractal dimension is an index based on the assumption that the normal interference in surface electromyographic pattern has fractal properties. It is a mathematical index, which measures the ratio of the change in detail to the change in scale. Fractal dimension is an indicator of progressive motor unit synchronization, that estimates central fatigue [43, 44] by analyzing the median frequency and amplitude of the electromyographic signal. Higher conduction velocity indicates lower peripheral fatigability, and higher fractal dimension indicates higher central fatigability.

Statistical analysis

Statistical analysis was performed using the statistical software SPSS/PC 8.0 (SPSS, Inc., Chicago, IL). Values for continuous variables are expressed as the mean ± standard deviation and frequency to the qualitative variables. Student *t* test as used for comparison of the two groups. Pearson's correlation coefficient was used to analyze correlations between fat free mass and age to the studied variables in the IGHD group. Significance was established by a p value lower than 0.05.

Results

Table 1 shows the anthropometric and physical activity data. Age, sex, BMI, and IPAQ level were not different between groups. As expected, height, weight, and fat free mass were significantly lower in the IGHD group. A 20.68% of the IGHD showed high physical activity, 27.58%

Table 1 Anthropometric and physical activity assessed by the international physical activity questionnaire (IPAQ) in isolated GH deficiency (IGHD) and controls

	IGHD (31)	Controls (40)	*p* value
Sex (males)	16	20	1.000
Age (years)	46.5 ± 12.4	43.2 ± 11.2	0.992
Weight (Kg)	38.0 ± 6.9	66.6 ± 10.7	< 0.001
Height (cm)	125.5 ± 7.6	166.0 ± 10.3	< 0.001
Body mass index (Kg/m^2)	23.4 ± 3.4	24.1 ± 2.8	0.354
Fat free mass (%)	63.5 ± 13.1	77.0 ± 8.4	< 0.001
Fat free mass (Kg)	25.6 ± 7.2	53.8 ± 12.1	< 0.001
Fat mass (%)	36.5 ± 13.2	23.17 ± 8.5	< 0.001
Fat mass (Kg)	13.4 ± 3.7	17.1 ± 6.0	0.003
Physical activity level	1.45 ± 0.5	1.51 ± 0.5	0.616

Data are expressed as mean ± standard deviation. The scoring system and the domains of IPAQ questionnaire should be detailed in the methods section

Table 2 Muscle strength in isolated GH deficiency (IGHD) and controls

	IGHD (31)	Controls (40)	*p* value
Handgrip strength (kgf)	20.9 ± 9.5	30.8 ± 8.7	< 0.001
Trunk extension strength (kgf)	25.3 ± 9.0	39.2 ± 6.1	< 0.001
Knee extension strength (kgf)	19.2 ± 8.3	19.5 ± 10.2	0.920
Handgrip strength/bw (kgf/kg)	0.55 ± 0.24	0.46 ± 0.10	0.060
Trunk extension strength/bw (kgf/kg)	0.68 ± 0.28	0.60 ± 0.12	0.130
Knee extension strength/bw (kgf/kg)	0.51 ± 0.22	0.28 ± 0.13	< 0.001
Handgrip strength/fat free mass (kgf/kg)	0.88 ± 0.52	0.57 ± 0.11	0.003
Trunk extension strength/fat free mass (kgf/kg)	1.08 ± 0.51	0.75 ± 0.17	0.002
Knee extension strength/fat free mass (kgf/kg)	0.79 ± 0.38	0.35 ± 0.15	< 0.001

Data are expressed as mean ± standard deviation

bw body weight, *kgf* kilogram force

moderate and 51.72% low activity. Among the controls 25.64% showed high, 30.76% moderate, and 43.58% low level of physical activity.

Table 2 shows the muscle strength data. Handgrip strength and trunk extension strength were lower in IGHD group. Knee extension strength was not different between the two groups. When strength was corrected by weight, the difference of handgrip and trunk extension disappeared, and knee extension strength was actually higher in subjects with IGHD. When strength parameters were corrected by fat free mass, IGHD subjects showed higher handgrip, and both trunk and knee extension. Table 3 shows the myoelectric activity and muscle fatigue data. IGHD subjects showed

Table 3 Myoelectric activity and muscle fatigue (IGHD) and controls

	IGHD (31)	Controls (40)	*p* value
Myoeletrical activity vm (%)	87.2 ± 16.5	95.4 ± 12.4	0.002
Myoeletrical activity rf (%)	79.1 ± 17.5	93.9 ± 14.0	< 0.001
Myoeletrical activity vl (%)	81.5 ± 16.3	97.0 ± 13.1	< 0.001
Fractal dimension vm (%)	49.2 ± 8.6	45.8 ± 9.1	0.119
Fractal dimension rf (%)	42.9 ± 6.8	42.5 ± 6.6	0.786
Fractal dimension vl (%)	52.5 ± 5.8	50.1 ± 8.8	0.186
Conduction velocity vm (Hz)	110.6 ± 10.8	100.1 ± 12.1	0.002
Conduction velocity rf (Hz)	117.8 ± 16.1	99.1 ± 17.0	0.002
Conduction velocity vl (Hz)	115.9 ± 14.2	109.9 ± 13.4	0.050
Contraction times (s)	57.0 ± 5.2	57.5 ± 4.6	0.683

Data in percentage refer to maximal values in each variable; central fatigue was assessed by the fractal dimension (%) and peripheral fatigue by the conduction velocity (Hz) in the muscles vastus medialis (vm), rectus femoris (rf), and vastus lateralis (vl). Higher conduction velocity indicates lower fatigability, and higher fractal dimension indicates higher fatigability

lower myoelectric activity of vastus medialis, vastus lateralis and rectus femoris. There was no difference on fractal dimension (signal amplitude %), which indicates similar central fatigability. However, conduction velocity (median frequency Hz) in IGHD was higher than control indicating peripheral lower fatigability in IGHD group. Furthermore, 32.2% of IGHD and 28.2% controls had exhaustion, not keeping muscle contraction for 60 s. The IGHD group showed a positive correlation between knee extension strength and fat free mass ($r = -0.41$, $p = 0.021$), and two negative correlations: one between trunk extension strength and age ($r = -0.47$, $p = 0.007$), and one between myoelectric activity in vastus lateralis and age ($r = -0.38$, $p = 0.035$).

Discussion

The role of GH and IGF-I action on muscle anabolism is well recognized, but the impact of lack of these hormones on muscle function is controversial. Here, we report that, in general, individuals with a severe congenital lifelong and untreated IGHD had better muscle strength parameters adjusted for weight and fat free mass than controls matched by gender, age, and degree of physical activity. They also exhibit greater peripheral resistance to fatigue, demonstrating satisfactory overall muscle function. In the present paper, we confirmed previous data on body composition in this IGHD cohort, namely the marked reduction of fat free mass and the increase in percentage of fat mass [45]. Therefore, although these individuals have reduced fat free mass, this does not seem to interfere in the production of strength. Several factors may justify these findings, as

neuromuscular efficiency, biomechanical issues, type of muscle fibers may surpass the amount of muscle mass in terms of muscle function [1].

We have previously published that mineral bone density of these IGHD subjects is essentially normal, with maintenance of bone quality and actually decreased risk of spine fractures in old age [25, 26]. Based on the mechanosthatic theory on the interaction between bone and muscular tissues [2], we believe that this satisfactory quality of bone healthy mirrors the normal muscular function showed in the present study.

Due to the low prevalence of severe IGHD and its common treatment in developed countries, there are not many studies to compare with ours. However, a small study in seven adults with idiopathic childhood onset IGHD (previously treated during childhood), despite having low fat free mass, did not reveal alterations in muscle strength parameters [18]. Studies of GHD in hypopituitarism often report marked reduction in muscle mass, with mild reduction in muscle function [16, 46].

Data about GH replacement therapy (GHRT) are controversial. A multi-center randomized, double-blind clinical trial of GH treatment in 166 adult GHD patients found that GH replacement improved body composition, with a reduction in fat mass and an increase in muscle mass, but with no detectable effect on performance to exercise and quality of life [47]. Another study including 17 hypopituitary subjects showed that a three-month GHRT normalized IGF-I levels, reduced fat mass, and had a significant effect on mitochondrial function, but exercise capacity was unchanged [48]. On the other hand, ten years of GHRT in twenty-four hypopituitary patients above 60 years of age, with multiple anterior pituitary deficiencies resulted in a transient increase in isometric knee flexor strength [49]. Finally, a meta-analysis involving 268 adult GHD patients showed evidence that GHRT improves exercise performance [50]. These important observations refer to patients with acquired hypopituitarism, different under many aspects from our lifelong congenital IGHD subjects. Our data convincingly show that individuals living with very low levels of GH and IGF-I have adequate muscle function and exercise capacity. However, the impact of GHRT on muscle function in this cohort is unknown.

Even in non-GHD athletes, a recent meta-analysis shows that the use of GH results in changes in body composition, with an increase in lean mass and a decrease in fat mass, but without an obvious increase in muscle strength and aerobic capacity [21]. Therefore, it seems that the effects of GH on muscle are more relevant for mass than for function.

Another important factor that influences muscular function is the practice of physical exercise, specifically resistance training by the recruitment of motor units [51]. There is a stronger relationship between muscle strength and physical exercise than between muscle strength and mass [51, 52]. Therefore, it is important to highlight that in our study there was no difference in the level of physical activity between the IGHD and the control group.

Interestingly, we found that IGHD subjects have higher knee extension strength when corrected for weight or fat free mass. Knee extensors have great relevance, as they are responsible for locomotion and daily activities, and their function is a predictor of fragility and mortality [53]. Handgrip and trunk extension strength corrected by bodyweight were not different between groups, but there was a trend of increase in handgrip strength in IGHD ($p = 0.06$). While a recent Dutch study done in older non-GHD subjects indicated that lower IGF-I levels are associated with lower handgrip strength and worse physical performance [54], this is not confirmed by our data. Trunk dynamometer could not be adjusted to the severe short stature, and this probably underestimated the values in the IGHD group. Even so, handgrip and trunk extension strength corrected by fat free mass were higher in IGHD than in controls. Correlation analysis indicates that other factors may be more relevant for muscle function than lean mass. Similarly, age (which in this model corresponds to the duration of GHD) does not seem impact significantly muscle function.

Although IGHD individuals had greater corrected knee extension strength, they had lower myoelectric activity. Some authors reported a correlation between strength and myoelectric activity; however this relationship is not observed in the totality of the force range, nor for all muscles [55]. Measurement of strength by dynamometry reflects the overall contraction of the muscle group, whereas surface electromyography results in localized muscular electrical activity. Another factor that may have influenced the test is the increase fat mass in IGHD subjects, which may have influenced the uptake of signals, because fat promotes greater resistance and impedance to signals [56].

Adults with acquired GHD commonly complain of excessive fatigue that seems to be associated more with impaired aerobic than muscular performance [17]. “Central” fatigue is a form of fatigue originating in the central nervous system and it is estimated by fractal dimension. Conversely, “peripheral” fatigue is directly related to the muscle tissue (reduced blood flow, accumulation of metabolites, or mitochondrial oxidative function) and is estimated by the velocity conduction [57]. A previous study revealed that patients with GHD (probably acquired), have reduced quality of life associated with fatigue of predominant central component [57]. Maybe the muscle pattern in this condition reflects associated conditions.

In contrast to the Itabaianinha IGHD subjects, individuals with Laron dwarfism (due to GH resistance) often complain of weakness and fatigue, and seem to have impaired physical performance, assessed by a computerized

myometric system [58, 59]. These data apparently discordant with the increased physical performance of our IGHD subjects suggest that in our subjects low but detectable GH levels guarantee some direct muscular effect, not possible due to the absolute lack of GH action in Laron patients. Interestingly, contrarily to the human disease, mice with generalized GHR gene ablation (GHRKO) have better muscular function compared with normal mice, assessed by the Rotarod test [14]. Therefore, the GHRKO mouse has a muscular function similar to our IGHD subjects. In addition, mice with generalized GHRH gene ablation also exhibit increased locomotor activity suggesting appropriate muscular function [60]. As a whole, these data suggest a dissociation between muscle mass and function in several models of GH resistance or deficiency.

In conclusion, individuals with congenital severe and lifetime IGHD had better muscle strength parameters adjusted for weight and fat free mass than controls. They also exhibit greater peripheral resistance to fatigue, demonstrating satisfactory overall muscle function.

Acknowledgements The authors thank the Associação do Crescimento Físico e Humano de Itabaianinha, for assistance.

Funding This work was not supported by public or private funds.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that there is no conflict of interest.

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