Lifelong exercise, but not short-term high intensity interval training (HIIT), increases GDF11, a marker of successful ageing: A preliminary investigation

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ABSTRACT

Lifelong exercise is associated with regulation of skeletal mass and function, reductions in frailty, and successful ageing. Yet, the influence of exercise on myostatin and myostatin-interacting factors is relatively under examined in older males. Therefore, we investigated whether serum total myostatin, free myostatin, follistatin, and growth and differentiation factor 11 (GDF11) were altered following high intensity interval training (HIIT) in a group of 13 lifelong sedentary (SED; 64 [6] years) and 11 lifelong exercising (LEX; 62 [6] years) older males. SED follistatin was moderately greater than LEX pre-HIIT (Cohen's d = 0.66), and was largely greater post-HIIT (Cohen's d = 1.22). The HIIT-induced increase in follistatin was large in SED (Cohen's d = 0.82) and absent in LEX (Cohen's d = 0.03). GDF11 was higher in LEX pre- (Cohen's d = 0.49), and post- (Cohen's d = 0.63) HIIT compared to SED. HIIT resulted in no change to GDF11 in LEX or SED (Cohen's d = 0.00-0.03). Peak power output and GDF11 correlated (r = 0.603), independent of grouping. Differences in GDF11 with lifelong exercise training, paired with the correlation between GDF11 and peak power output, suggest GDF11 may be a relevant myostatin-interacting peptide to successful ageing in humans, and strategies to maintain this need to be further explored.

KEYWORDS

Ageing · Exercise · Follistatin · GDF11 · HIIT · Myostatin

RUNNING TITLE

GDF11 is increased in successful ageing

49 INTRODUCTION

50 Myostatin (originally growth and differentiation factor 8 [GDF8]) is a pro-catabolic, anti-
51 anabolic peptide hormone that is a central regulator of skeletal muscle mass (Elliott et al.,
52 2012). Secreted by skeletal muscle, myostatin is found in an active unbound (free) form, or
53 bound to its own pro-peptide, or separate peptides such as follistatin, or follistatin-related
54 gene (FLRG; Amthor et al., 2004, Gilson et al., 2009, Hill et al., 2002), each inhibiting its
55 biological function. Myostatin has both paracrine and endocrine effects (Zimmers et al.,
56 2002), although it is the endocrine function which appears key for regulation of muscle mass,
57 due to an observed inverse correlation with muscle mass in humans (Gonzalez-Cadavid et al.,
58 1998). Moreover, inhibition of this endocrine function results in muscle hypertrophy in mice
59 (Whittemore et al., 2003).

60

61 Ageing is associated with a progressive loss of muscle mass and associated function (Metter
62 et al., 2002) The rate of loss of muscle mass and function with ageing is noted to differ
63 between individuals, which gave rise to ‘usual’ and ‘successful’ ageing hypothesis (Rowe
64 and Kahn, 1987). A more recent definition of successful ageing being “optimisation of life
65 expectancy while minimising physical and mental deterioration and disability” (Bowling and
66 Dieppe, 2005), a trait that is often seen in life-long masters athletes (Pollock et al., 2015).
67 Whilst the role of myostatin in regulation of muscle mass is well described, there are few
68 data, and no prospective studies to contextualise the influence of myostatin within the ‘cycle
69 of frailty’ that precedes sarcopenia. From the few cross-sectional studies, one observed ~50%
70 higher plasma myostatin in older sedentary (~63-75 years of age) compared with younger
71 healthy (~20-35 years of age) men (Yarasheski et al., 2002). However, this was not replicated
72 in a study of men aged ~22, ~69, and ~76 years of age, regardless of sarcopenic severity
73 (Ratkevicius et al., 2011). Recently, we have observed an inverse association between age

and plasma myostatin in a large group (n = 88) of healthy individuals aged 18-72 years of age (Elliott et al., 2016). Considering the current incomplete understanding concerning the role of myostatin and myostatin-interacting peptides in the ageing process, the pool of evidence needs to be extended.

Growth and differentiation factor 11 (GDF11) is a peptide with similar sequence homology as myostatin, and it is possible that both peptides share similar signalling pathways and biological influence within skeletal muscle. Unlike myostatin however, the expression of GDF11 is not limited to skeletal muscle tissue (Lee and McPherron, 1999, Walker et al., 2016). There also appears to be an indicated role for GDF11 in the ageing process; higher circulating GDF11 in middle-aged mice has been positively associated with longevity and exposure of aged mice to a youthful systemic environment led to restoration of skeletal muscle and hepatic cellular function (Zhou et al., 2016). Similarly, the ageing muscle phenotype is partially offset by provision of recombinant GDF11, as demonstrated by increased grip strength and running endurance in mice (Sinha et al., 2014).

Whilst it remains to be seen whether these findings can be consistently replicated, or indeed translated to the human model of ageing, only a small number of studies that have examined the effects of exercise training on serum myostatin and associated mRNA expression, whilst GDF11 remains unexamined in the human exercise model. Indeed, 2–3 months' resistance training in healthy young individuals resulted in increased muscle mass and decreased muscle mRNA and serum myostatin (Roth et al., 2003, Walker et al., 2004). To the best of these authors' knowledge, no reports on the effect of exercise (in any form) on GDF11 expression currently exists.

Recently, high intensity interval training (HIIT) has received much attention due to its physiological and sociological benefits. Indeed, HIIT is noted to be more enjoyable than traditional, continuous training (Thum et al., 2017), has higher compliance in patient populations than continuous training (Shiraev and Barclay, 2012), and is noted to have equal or improved clinical outcomes in a number of ageing-related cardiovascular or metabolic disorders (Cassidy et al., 2016, Ramos et al., 2015). Whilst not optimized for muscle hypertrophy, HIIT improves myofibrillar protein synthesis (Bell et al., 2015), muscle power (Sculthorpe et al., 2017), and fat free mass (FFM) (Herbert et al., 2017) in older males.

Therefore, in order to progress our understanding of the biological relationship between myostatin and myostatin-interacting peptides with ageing and exercise, the aim of this preliminary study was twofold: 1) To compare resting levels of plasma myostatin and myostatin-interacting peptides between lifelong sedentary (SED) and a positive control group of lifelong exercising (LEX) ageing men, and 2) to examine the influence of 6 weeks' HIIT on plasma myostatin and myostatin-interacting peptides in SED and LEX. We hypothesised that, on enrolment to the study, SED would exhibit higher myostatin, follistatin, free myostatin, and lower GDF11. We further hypothesized that 6 weeks' HIIT would decrease plasma myostatin, follistatin, and free myostatin in SED, and increase GDF11.

METHODS

Participants

Participants provided written informed consent prior to enrolment to a larger study (Hayes et al., 2017, Herbert et al., 2017, Knowles et al., 2015) which was approved by the University of the West of Scotland Ethics Committee (Reference: UEC16_042012/Herbert). Participants were familiarised with experimental procedures and approval to exercise was given by their general practitioner. Subsequently, a subgroup of 24 males were analysed for this pilot investigation. Thirteen males participated in the SED group, whilst 11 males participated in the LEX group (Table 1). Participants in the SED group did not participate in any formal exercise training and had not done so for >30 years. The LEX group were active exercisers and had been so for the previous >30 years. They consisted primarily of current masters competitors in sports including water-polo, triathlon, sprint cycling, road cycling and distance running. For six weeks prior to commencing HIIT training, LEX recorded their normal weekly exercise, which included type, frequency, intensity (recorded by heart rate telemetry), and duration of training. Time spent in low to medium intensity (<65% heart rate reserve [HRR]), and high-intensity (>65% HRR) training totalled 214 ± 131 $min \cdot wk^{-1}$ and 67 ± 52 $min \cdot wk^{-1}$ respectively. Group selection was affirmed by differences in aerobic conditioning (peak oxygen uptake; VO_{2peak}) between groups (table 1). Participants were tested pre- and post-HIIT at the same time of day, seven weeks apart. Order of measurements was blood sampling, body composition, peak power assessment, and determination of VO_{2peak}.

Table 1 about here

Blood draws and analysis

Participants arrived at the exercise physiology laboratory between 07.00–09.00 h, following an overnight fast and having abstained from strenuous exercise for a minimum of 48 h. Participants were reminded to maintain standardized conditions prior to each assessment point which included arriving in a hydrated state having abstained from caffeine and alcohol consumption for 36 h. Following 20 min supine rest blood was sampled from the nondominant arm using the standard venepuncture method into sterile serum separator vacutainer tubes (Becton Dickinson, Rutherford, NJ) that were kept at room temperature in the dark, for 30 min, to allow for clotting, after which samples were centrifuged at 1100 g at 4°C for 15 min. Serum was then extracted, aliquoted, and stored at −80°C until subsequent analysis. Blood samples were collected at the same time of day for each participant to control for biological variation and minimise inter-participant analytical variation.

Concentrations of serum myostatin protein (both total and free fractions) were quantified by ELISA (DGDF80, R&D Systems, UK). Briefly, aliquots of serum were brought to room temperature, before 100 µL of plasma was diluted with 1:4 diluent buffer (free myostatin) or activated with 50 µL HCl (6 mol, 10 minutes at room temperature) for removal of myostatin binding proteins, before neutralization (50 µL of NaOH 6 mol + 1.2 mol HEPES) and dilution with provided diluent buffer (200 µL) to produce a final 1:4 dilution. Recombinant myostatin was used as a standard (33.3-2,000 $pg \cdot mL^{-1}$). Concentrations of serum follistatin (DFN00, R&D Systems, UK) and serum GDF11 (DY1958, R&D Systems, UK) were quantified by ELISA, per manufacturer's instructions. Recombinant follistatin (250-16,000 $pg \cdot mL^{-1}$) and GDF11 (15.6 – 1000 $pg \cdot mL^{-1}$) was used as a standard. Plates were read spectrophotometrically at 450 nm and blanked to 570 nm (VersaMax, Molecular Devices,

USA). Coefficient of variability of standards and samples were 7% and 6%, 6% and 4%, and 4% and 8%, for myostatin follistatin, and GDF11, respectively.

Body composition and performance measures

Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK), and a multi frequency bioelectrical impedance analyzer (BIA; Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.) was used to determine body mass and body composition as described elsewhere (Hayes et al., 2013b). Participant peak power output was assessed using the Herbert 6 s cycle test (Herbert et al., 2015b) and participants' individual values were used to calculate the resistance (40% peak power output) during HIIT. VO_{2peak} was determined by indirect calorimetry as previously described (Knowles et al., 2015).

Exercise training

HIIT sessions were performed once every five days, for six weeks (nine sessions in total) as previously described (Hayes et al., 2017, Herbert et al., 2017, Knowles et al., 2015). Rationale for this programme is provided by our previous work which identified that five days of recovery was required for recovery of peak power output in ageing men (Herbert et al., 2015a). Each session consisted of 6 x 30 s sprints at 40% predefined peak power output interspersed with 3 min active recovery on a cycle ergometer (Wattbike Ltd., Nottingham, UK). Sessions were conducted in groups of between four and six participants and were the sole exercise performed by both groups during this time.

Statistical Analysis

Following confirmation of parametricity by a Shapiro-Wilk test of normality and Levene's test for homogeneity of variance, a mixed (between group [SED, LEX] x within individual

time [pre-HIIT, post-HIIT]) repeated measures analysis of variance (ANOVA) was used for differences in groups and time points with Bonferroni *post-hoc*. Non-parametric data were examined by Fishers exact test, with correction for multiple comparisons by Bonferroni's method. Alpha level was set *a priori* at $p < 0.05$, and effect size for paired comparisons is reported as Cohen's d throughout, interpreted as trivial (<0.2), small (≥0.2), moderate (≥0.5), and large (≥0.8). Parametric data sets are summarized in text as mean [SD], whilst non-parametric are given as median (upper - lower quartile). Figures are presented as grouped dot plots, as recommended by Drummond and Vowler (2012).

RESULTS

Pre-HIIT, SED individuals were heavier ($p = 0.131$, Cohen's $d = 0.66$) with a greater body fat percentage ($p = 0.120$, Cohen's $d = 0.66$) than LEX. SED had a lower VO_{2peak} ($p < 0.001$, Cohen's $d = 2.00$), absolute peak power output ($p = 0.036$, Cohen's $d = 0.90$) and relative peak power output (a surrogate for muscle quality; $p = 0.020$, Cohen's $d = 1.08$) than LEX (table 1).

There was no group x time interaction for total myostatin protein ($p = 0.750$), nor was there an effect of group ($p = 0.081$) or time ($p = 0.701$). However, large effect sizes were noted between SED and LEX total myostatin both pre-HITT (4217 [317] $pg \cdot mL^{-1}$ and 3394 [391] $pg \cdot mL^{-1}$ in SED and LEX respectively; Cohen's $d = 2.06$; Figure 1A) and post-HIIT (4163 [337] $pg \cdot mL^{-1}$ and 3678 [438] $pg \cdot mL^{-1}$ in SED and LEX respectively; Cohen's $d = 1.24$). Following HIIT, SED experienced only trivial increases in total myostatin (Cohen's $d = 0.17$) whilst LEX moderately increased total myostatin (Cohen's $d = 0.68$).

In a similar manner to total myostatin, there was no group x time interaction for free myostatin protein ($p = 0.790$), nor and effect of group (0.996) or time ($p = 0.601$). No notable effect size changes were observed for free myostatin pre-HIIT (1182.0 [372.2] pg·mL-1 and 1159.3 [418.1] pg·mL-1 in SED and LEX respectively; Cohen's d = 0.06; Figure 1B) or post-HITT (1203.3 [533.3] pg·mL-1 and 1224.5 [404.1] pg·mL-1 in SED and LEX respectively; Cohen's d = 0.05). Moreover, neither SED (Cohen's d = 0.05) nor LEX (Cohen's d = 0.16) had any more than a trivial effect on free myostatin from pre- to post-HIIT.

There was a significant main effect of group ($p = 0.002$), but not time ($p = 0.171$), or a group x time interaction ($p = 0.561$) for serum follistatin. SED follistatin was greater than LEX follistatin pre-HIIT (2508 [628] $pg \cdot mL^{-1}$ and 2102 [598] $pg \cdot mL^{-1}$ in SED and LEX respectively; $p = 0.132$, Cohen's d = 0.66). SED follistatin was also greater than LEX follistatin post-HIIT (3043 [676] $pg \cdot mL^{-1}$ and 2126 [809] $pg \cdot mL^{-1}$ in SED and LEX respectively; $p < 0.001$, Cohen's d = 1.22). The HIIT-induced increase in follistatin was large in SED ($p = 0.011$, Cohen's d = 0.82), whilst LEX experienced no change ($p = 0.443$, Cohen's d = 0.03).

GDF11 data were examined by Fishers exact test, and presented as median (upper - lower quartile). GDF11 was higher in LEX pre- ($p = 0.012$, Cohen's d = 0.49), and post- ($p = 0.009$, Cohen's d = 0.63) HIIT compared to SED. HIIT resulted in no change to GDF11 in SED (70.7 [52.6 – 193.1], 77.1 [73.1 – 104.3] $pg \cdot mL^{-1}$ pre- and post-HIIT respectively; $p = 0.74$, Cohen's d = 0.03) or LEX (272.7 [219.2 – 387.2], 305.0 [243.8 – 399.4] $pg \cdot mL^{-1}$ pre- and post-HIIT respectively; $p = 0.72$, Cohen's d = 0.00).

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243 *Figure 1 about here*

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246 As we have previously reported in a larger cohort (Hayes et al., 2013a), peak power output
247 was higher in LEX individuals relative to SED ($p = 0.036$, Figure 2A). There was no
248 correlation between peak power output and total myostatin ($p = 0.196$, $r = -0.273$), free
249 myostatin ($p = 0.812$, $r = 0.051$), or follistatin ($p = 0.569$, $r = -0.113$). However, strong
250 positive correlations were observed between GDF11 and both absolute peak power output (p
251 $= 0.002$, $r = 0.603$; Figure 2B) and relative peak power output ($p < 0.001$, $r = 0.636$; figure
252 2C).

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254

255 *Figure 2 about here*

DISCUSSION

The main finding of this preliminary study was that SED presented greater concentrations of serum total myostatin and follistatin, and lower concentrations of GDF11, compared with LEX pre-HIIT. Serum follistatin alone responded significantly to HIIT but was confined to the SED group. A notable and novel finding from this study is the observed association between peak power output and GDF11, which has not been previously demonstrated in the human. These data provide preliminary evidence that the role of GDF11 in healthy ageing observed in mice is maintained in humans.

With regards to healthy ageing, our finding that LEX displayed significantly higher GDF11 than SED at baseline is novel and noteworthy. It has been noted that older mice treated with plasma from younger mice show a younger phenotype (Horrington et al., 1960, Lunsford et al., 1963), which has since been partially attributed to GDF11 differences in older and younger mice. In mice, mid-life GDF11 is predictive of longevity (Zhou et al., 2016). Aged mice show a typical 'older muscle' phenotype which results in lower muscle volume, endurance, and grip strength relative to young mice. Moreover, treatment with recombinant GDF11 returned grip strength to near young levels, and improved running endurance performance (Sinha et al., 2014). However, it has also been noted that GDF11 may inhibit myoblast differentiation into mature myotubes in a myostatin-like manner (Egerman et al., 2015), perhaps unsurprising, as the myostatin and GDF11 peptide share ~90% homogeneity. It should be further noted that Egerman et al. (2015) used *in vitro* doses of 10-100 $ng \cdot mL^{-1}$, whilst both their data, and our data reported here, suggests circulating GDF11 in older males is 100-1000 $pg \cdot mL^{-1}$, an order of magnitude lower in concentration, possibly explaining the disparity of these findings.

This argument that GDF11 concentration play a role in successful ageing is supported by two separate findings we report here. Firstly, we note GDF11 is significantly higher in LEX than SED, with some overlap between these groups. Further, we note a significant moderate positive correlation between peak power output (both absolute power and relative to FFM) and GDF11, independent of grouping. Whilst our data does not allow us to suggest causality, it is exciting to note this correlative relationship. To the best of our knowledge, this is the first dataset linking successful ageing and improved muscle function in the human with GDF11, and directly links our findings with those of Sinha et al. (2014), that exogenous GDF11 protects older mice against ageing- and sedentarism-associated frailty. It is thus tempting to suggest GDF11 plays a similar role in ageing humans, and this hypothesis needs to be further explored with experimental approaches to increase GDF11 expression in humans.

Circulating myostatin is noted to correlate with lean muscle mass across both healthy and cachexic individuals (Gonzalez-Cadavid et al., 1998). As SED and LEX presented with different body composition at baseline, the moderately lower concentrations of total myostatin in LEX at baseline is understandable. Whilst others have reported decreases in plasma myostatin and gains in muscle mass following resistance exercise (Walker et al., 2004, Saremi et al., 2010), limited research regarding interaction between HIIT and myostatin exists. Pugh et al. (2015) reported reduced muscular myostatin mRNA in healthy individuals 2 and 6 hours following a single bout of HIIT (although a different protocol to that employed herein), yet we are the first group to report chronic changes to resting serum myostatin following HIIT. The aim of HIIT is not primarily to build muscle mass, so whilst our HIIT protocol did not significantly alter serum total or free myostatin, expectations of an alteration in this peptide may have been ambitious in the absence of muscle mass alteration.

Whilst our findings concerning GDF11 are noteworthy, we acknowledge certain limitations of the present investigation. Whilst we attribute differences in GDF11 to life-long activity differences, we acknowledge that we cannot separate how much exercise was required to produce these observed differences. The addition of a moderately active group (meeting physical activity guidelines), would allow for comparison of multiple exercise habits, rather than the two extremes presented here. Moreover, our lack of inactive control group (no HIIT) and relatively small sample size may limit interpretations. The present investigation formed part of a larger research study with other primary outcome variables (Grace et al., 2015, Herbert et al., 2017, Knowles et al., 2015), and therefore only a subset of participants were analysed. As such, our results remain preliminary until the influence of exercise habits on serum GDF11 is investigated with either a large-scale randomized control trial (RCT) or prospective observational trial.

To date, much attention has been placed on myostatin itself, with alterations in myostatin expression resulting in significant and striking alterations in muscle mass in animal models (Kambadur et al., 1997, Mosher et al., 2007). However, here we show that total myostatin only moderately differs in a model of successful ageing, suggesting the role of myostatin may not be as important in successful ageing as other factors reported here. Instead, greater focus may need to be placed on these myostatin-interacting factors, as we showed follistatin was lower, and GDF11 was higher in our LEX model of successful ageing. Further, the correlation between GDF11 and muscle quality is exciting, and may suggest a protective role of GDF11 against ageing-associated muscular frailty in the human.

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ADDITIONAL INFORMATION

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MANUSCRIPT TABLES

Table 1: Participant anthropometric and performance parameters on enrolment to the investigation in lifelong sedentary (SED), and lifelong exercising (LEX), older males. Data presented as mean [SD].

	SED (n=13)	**LEX (n=11)**
Age (years)	64 [6]	62 [6]
Stature (cm)	174 [6]	174 [6]
Body mass (kg)	91 [19]	80 [12]
Body fat (%)	24 [16]	16 [6]
FFM (kg)	66 [6]	66 [7]
Peak oxygen uptake ($ml \cdot kg \cdot min^{-1}$)	28 [6]	40 [7]*****
Peak power output (W)	663 [147]	831 [221]*****
Peak power output ($W \cdot kg\ FFM^{-1}$)	10 [2]	12 [2]*****

*****Denotes significantly different than SED ($p < 0.05$). FFM = fat free mass