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Age-related loss of skeletal muscle function and the inability to express the autocrine form of insulin-like growth factor-1 (MGF) in response to mechanical overload

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Abstract The response of insulin-like growth factor-1 (IGF-1) signalling and the capacity of skeletal muscle to adapt to mechanical overload was studied using synergistic muscle ablation. Overload of the plantaris and soleus resulted in marked hypertrophy and activation of satellite cells (as indicated by MyoD expression), particularly in young rats. Two muscle IGF-1 splice variants were measured and found to be differentially regulated at the RNA level. The significant changes associated with the inability of the older muscles to respond to mechanical overload included the considerably lower expression of the local splice variant mechano growth factor, and the failure to up-regulate IGF-1 receptor and MyoD mRNA.

Key words: Insulin-like growth factor-1 splice variant; Muscle; Overload; Ageing

1. Introduction

Loss of muscle mass (sarcopenia) is one of the major causes of disability in elderly people. The inability to generate enough power to adjust posture results in the elderly falling and breaking bones that are brittle due to osteoporosis [1]. The decline in function and plasticity of skeletal muscle is also characterised by impaired mechanisms of regeneration and repair [2,3]. One of the best definitions of ageing is the eventual breakdown of tissue maintenance [4], and because of the post-mitotic nature of skeletal muscle tissue, its ability to regenerate and effect repair depends on a population of quiescent mononucleated stem cells known as satellite cells [5]. Therefore, it is likely that the decline in the number of satellite cells and/or in their proliferative capacity in senescence contributes to the pathology of muscle ageing [7–9].

The post-natal growth spurt that occurs early in life is largely regulated by growth hormone (GH) produced in the pituitary, which in turn induces the production of insulin-like growth factor-1 (IGF-1) by the liver. In later life, the systemic IGF-1 is still important for general tissue maintenance and well being of the body as a whole. One noticeable change during ageing is the decline in the circulating GH and IGF-1 levels. However, GH therapy in elderly adults has had only moderate effects on the function of muscle [10]. On the other hand, virally mediated local expression of IGF-1 and the over-expression of muscle IGF-1 in transgenic rodents has been shown to induce hypertrophy [11] and reverse the age-related muscle atrophy [8,12].

Although IGF-1 yields a simple mature peptide, its gene structure and patterns of expression are complex. In addition to its gene transcription being regulated by two promoters, IGF-1 splice variants differ in their precursor mRNA sequences, resulting in mature transcripts encoding at least four IGF-1 precursor proteins [13]. Two forms produced locally in active muscle appear to be positive regulators of muscle hypertrophy [14]. One of these, IGF-1Ea, has a similar structure to the major endocrine form produced by the liver. It has been given various abbreviations including muscle liver-type IGF-1 [14] and muscle IGF-1 [15]. The other isoform, which is the IGF-1Eb in rodents and corresponds to IGF-1Ec in humans, is only markedly up-regulated in exercised and in damaged muscle and has been named mechano growth factor (MGF) [16,17].

Measurements of expression levels of IGF-1 at different ages, in resting muscle, have failed to show any differences in both mRNA and peptide levels [18]. However, this work has not involved exercise studies or distinguished between different IGF-1 isoforms. Muscle has an intrinsic ability to adapt to an increased load by undergoing hypertrophy. The role of IGF-1 in muscle hypertrophy is supported by the observation that over-expression of IGF-1 both in vitro and in vivo induces hypertrophy [15,19,20]. IGF-1 has also been shown to be involved in the synthesis of muscle-specific proteins, required for satellite cell proliferation and differentiation into mature myotubes [21]. It was therefore considered important to determine the expression of MGF, IGF-1Ea, and IGF-1 receptors in relation to the ability to adapt to mechanical overload at different ages. Activated satellite cells express MyoD, a member of muscle regulatory transcription factors [22]. Therefore, by measuring the expression of this myogenic factor, some light may be shed on the functional implications of the age-related ability of muscle to express MGF and IGF-1Ea.

As muscle mass is known to increase in response to overload and stretch, it was decided to use the method of synergistic muscle tenotomy, which stretches as well as increases the load on the intact muscle. The resulting hypertrophy, as shown by Goldberg [23] in hypophysectomised rats, is independent of the GH/IGF-1 axis. Therefore, this system ap-

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peared to be appropriate for studying the early response of autocrine IGF-1 splice variants to overload. Caloric restriction is the only intervention known to retard ageing in mammals. Studies in *Caenorhabditis elegans* suggest that the IGF-1/insulin system may integrate systems that define an organisms rate of ageing. Dietary intervention has been shown to delay the atrophic changes related to senescent muscle [24]. The effect of caloric restriction on the expression of local IGF-1s was therefore also investigated.

2. Materials and methods

2.1. Animal experiments

Male Sprague–Dawley rats were weaned at 21 days and at 3 months, randomly allocated into ad libitum-fed (AD) and diet-restricted (DR) groups, which were individually caged. AD animals had unlimited access to food, while DR rats were fed an identical diet to 70% of the AD food-group intake. All rats were kept in conditions of constant temperature and light-controlled environment (12:12 h light–dark cycles). Three different sub-groups of 18 rats each were classified in each of the AD and DR groups as 3 month (young), 12 month (mature) and 24 month (old). All procedures were performed while the animals were anaesthetised. Experiments were initiated by unilateral excision of the distal gastrocnemius tendon as described by DeVol [25], and sham operations were carried out on the contralateral leg, which would then act as the control. After the animals recovered from anaesthesia, they were observed to use both legs almost within 1 h. They all survived until time of death at 1, 2, 3 and 5 days, when the animals were again anaesthetised and then sacrificed. The plantaris and soleus were immediately removed, weighed, and one part snap-frozen in liquid nitrogen and stored at −70 °C.

2.2. RNA isolation and analysis

Total RNA was isolated by the guanidine thiocynate method of Chomczynski and Saachi [26]. The extracted RNA was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically with the Gene spec I (Naka Instruments). The integrity of the RNA was confirmed by visual inspection of ethidium bromide stained 18S and 28S ribosomal RNA under ultra violet light.

2.3. Reverse transcription

1 μg of each sample of RNA was reverse-transcribed using Superscript II reverse transcriptase (RT) (Gibco BRL) in a 20 μl volume. To facilitate the efficiency of RT of transcripts expressed at low levels such as MGF and IGF-1 receptor, short specific primers annealing 50–100 base pairs downstream of the polymerase chain reaction (PCR) reverse primers were used. Hence, a mixture of random hexamers and specific decamers were used in the same reaction. The RT primers for MGF and IGF-1 receptors are 5′-TTGCAGGTTGCT-3′ and 5′-GTACTACTACT-3′

2.4. Real-time quantitative PCR

Primers used for PCR are listed in Table 1. All the PCR primers were designed using Omega Version 2.0 (Oxford Molecular) and synthesised by Sigma Genosys. Real-time PCR was performed in the Roche Lightcycler using SYBR green detection.

3. Results

3.1. Effect of loading

Five days after tendon ablation, there was an increase in the wet weight of the synergistically overloaded plantaris muscle of 60%, 35% and 20% in the young, mature and old rats, respectively (Table 2).

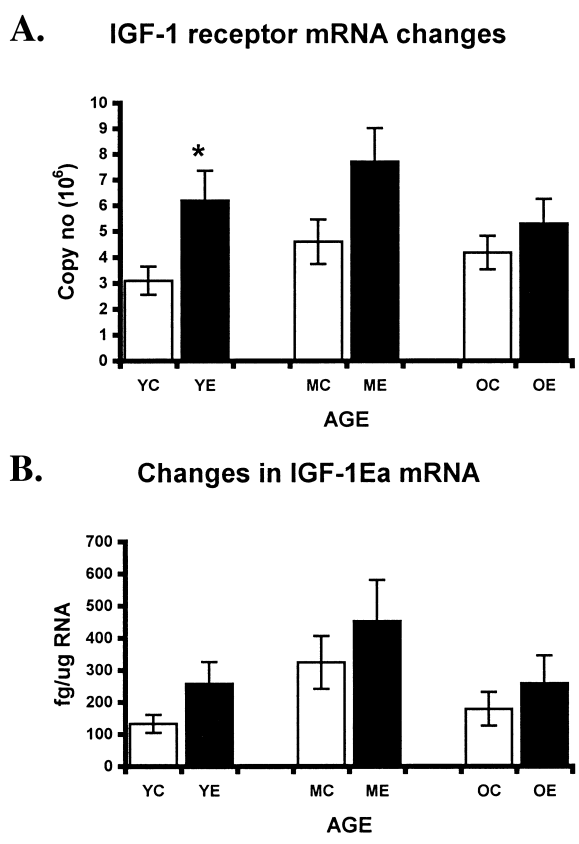

Fig. 1. IGF-1 receptor mRNA (A) and IGF-1Ea mRNA (B) quantification 5 days after tenotomy. Values are ±S.E.M; $n=4$/group. *$P<0.05$ significantly different from control. Experimental (E) is in reference to the overloaded muscle, while control (C) refers to the contralateral leg.

3.2. Effect of loading and age on IGF-1, MGF, IGF-1R, and MyoD gene expression.

A significant increase in IGF-1 receptor mRNA levels ($P<0.05$) was found in overloaded muscles of the young rats when compared to the control levels, but not in mature and old muscle (Fig. 1A). There was no significant change in IGF-1Ea mRNA levels during ageing or after tenotomy in the young, mature and old muscles. (Fig. 1B)

However, there was a significant increase in MGF mRNA at all ages in overloaded muscle relative to control muscle (Fig. 2A). This was very significant in young muscle ($P<0.001$), but less so for both mature and old muscles ($P<0.05$). The degree of increase in the old overloaded muscle was markedly less than that in the young muscles. (Fig. 2B)

With regard to MyoD expression, its baseline mRNA levels

Table 1
Primers used in the PCR in 5′ to 3′ direction

Gene	Forward primer	Reverse primer
IGF-1Ea	GCTTGCTCACTTTACCAGC	AATGTACTTCCTTCTGGGTCT
MGF	GCTTGCTCACTTTACCAGC	AAATGTACTTCCTTTCCTT
IGF-1 receptor	GCATTGACATCCGCAACG	ACTGTGAGGTTCGGAAGAGG
MyoD	GGAGACAATCCTCAAGCGATGC	AGCACCTGGTAAATCGGATTGG

Table 2
Contralateral leg as control (C) and overloaded leg as experimental (E) $n=6$

Samples	YC	YE	MC	ME	OC	OE
Weight (g)	0.128	0.188	0.325	0.414	0.351	0.411
Standard deviation	0.026	0.033	0.065	0.047	0.074	0.067

Key: Young (Y) 3 month old rats; mature (M) 13 month old; old (O) 24 month old.

were significantly higher in old muscle in comparison to those of young muscle ($P<0.05$; Fig. 3A). However, there was a significant increase in the expression of MyoD in both young ($P<0.001$) and old overloaded muscles ($P<0.05$). The degree of increase in the old muscle was attenuated and far less than that in the young muscle ($P<0.05$; Fig. 3B).

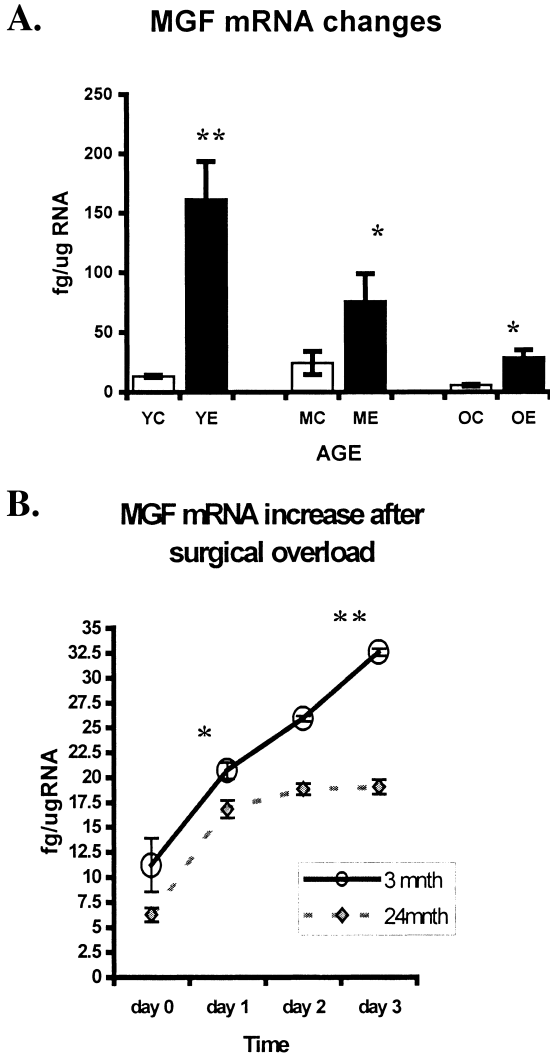

Fig. 2. Quantification of MGF mRNA levels. A: After 5 days in young (Y), mature (M) and old (O) samples. There is a significant increase in MGF levels in overloaded muscle (E) compared to controls (C) at all ages. B: Changes in MGF levels 1, 2 and 3 days after surgery in overloaded muscles of young and old rats. Day 0 represents the levels in the contralateral legs, which serve as the controls. The level of MGF increase in young is significantly higher than that in the old after 24 h. In comparison to the old muscles, MGF levels in the young continue to increase significantly after 1 day (**$P<0.001$ and *$P<0.05$).

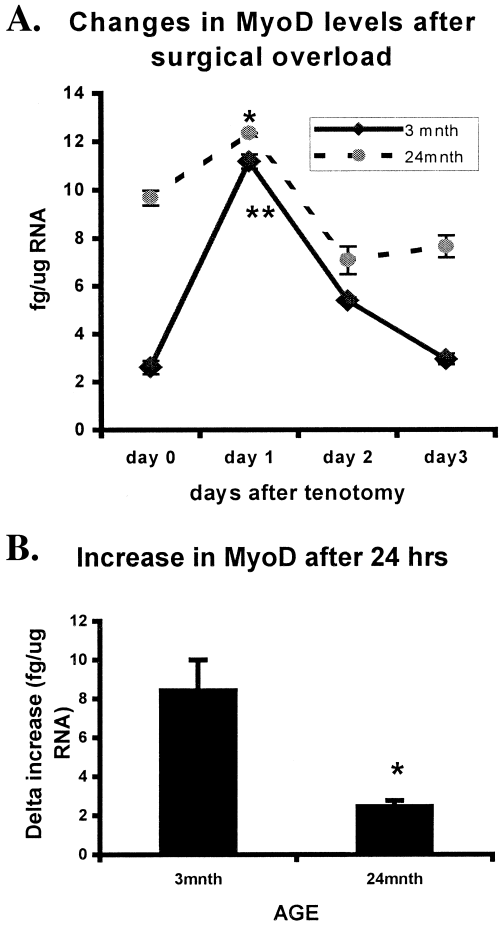

Fig. 3. Quantification of MyoD mRNA levels after tenotomy. A: Changes in MyoD mRNA after 1, 2 and 3 days in overloaded muscles of young and old rats. Day 0 corresponds to levels in control muscle. Baseline MyoD levels are significantly higher in the control muscle of old rats compared to young rats. There is a significant increase in MyoD levels after 1 day, which declines to control levels by day 2 (**$P<0.001$ and *$P<0.05$). B: Increase of MyoD in old muscle is significantly less in comparison to that in the young ($P<0.05$).

3.3. Effect of caloric restriction

Caloric restriction had no significant effect on the mRNA levels of IGF-1Ea, MGF or IGF-1 receptors, in both the control and overloaded muscles. Therefore, these results are not presented.

3.4. Statistical analysis

The Student's *t*-test was used to test the difference in the means of the overloaded and control muscles at the various age groups. One-way analysis of variance was used to determine significant differences among the means, with the level of significance set at $P<0.05$. For all groups $n=6$. Values are expressed as mean ± standard error of the mean (S.E.M.).

4. Discussion

This study showed for the first time that in muscle, IGF-1 mRNA isoforms are differentially regulated in response to mechanical overload. Increases in muscle IGF-1 have been observed during work-induced compensatory overload, in regenerating fibres after acute and chronic exercise and during

passive stretch in mammalian muscle [16,25,27–29]. However, in previous work, no distinction was made between the different transcripts. Indeed, the previously reported early increase in IGF-1 gene expression may have been a reflection of the increase in MGF rather than IGF-1Ea mRNA. Local IGF-1 expression is likely to be dissociated from hepatic/circulatory IGF-1 due to the localised effects of hypertrophy observed in only the overloaded muscle [29]. This suggests the presence of a transient and activity-sensitive local trophic factor. Accumulating evidence suggests that this is MGF, the mRNA of which increases markedly in response to stretch and stimulation [16,17].

MGF and IGF-1Ea splice variants, apparently yield the same mature peptide, which is derived from the highly conserved exons 3 and 4 of the IGF-1 gene. A mechanism of extracellular endoproteolysis of the IGF-1 prohormone, results in the same mature peptide, but different E peptides [30], which may function as independent growth factors [31]. It has been suggested that IGF-1 precursors could be pluripotenial, in a form analogous to that of prohormone proopiomelanocortin and proglucagon [31]. Of particular interest is the observation that a synthetic peptide derived from the rat Eb domain induces proliferation in epithelial cells [31]. The role of the growth promoting properties of the E peptide in MGF, acting as a separate growth factor, is supported by observation that muscle cell lines stably transfected with MGF show greater proliferative rates of mononucleated myocytes than in the control or in those cells transfected with IGF-1Ea (Yang et al., unpublished data). Furthermore, an antibody raised against the synthetic IGF-Eb-related precursor peptide can detect a protein product of immunological similarity in human and mammalian tissues extracts [31].

The early induction of MGF, following mechanical overload, indicates that this may play an important role in the increase in protein synthesis, proliferation and differentiation of satellite cells in response to stretch and damage. IGF-1 expressed by muscle is believed to increase protein synthesis and to activate satellite cells. These proliferate, differentiate and fuse with the pre-existing muscle fibres [19]. In γ-irradiated mouse skeletal muscle, where the proliferative capacity of satellite cells has been destroyed, vector-delivered IGF-1 is only able to induce partial hypertrophy [32]. Muscle response to overload occurs in two phases [7]. At early time points, the increase in muscle DNA and RNA content has been related to the proliferation and differentiation of satellite cells [19]. In agreement with previous observations, there was a marked transient increase in MyoD mRNA levels in both the young and old rats 24 h after tendon ablation. Concurrent with data from Musaro et al [33], old muscle expresses higher baseline levels of myogenic factors, and after exercise, does not achieve the same levels of increase as young muscle [33–36]. Although it is not known why resting levels of MyoD should be higher, it has been proposed that this may be a result of increased denervation and impaired reinnervation phenomena that is associated with ageing in muscle [34].

It is proposed that age-related loss in muscle mass is due to the failure to activate satellite cells, which are needed to repair the cumulative damage incurred by cycles of regeneration/degeneration [12]. Restoration of muscle strength using virally mediated over-expression of IGF-1 supports the hypothesis that a deficit in local trophic factor may be linked to age-related muscle atrophy. Exogenous locally infused IGF-1 restores the satellite cell proliferative potential of immobilised old skeletal muscle [8]. The significant decrease in baseline levels of MGF with increasing age may partially explain the decrease in proliferative capacity of satellite cells with ageing [3,6,36]. The attenuated increase of MGF mRNA levels in old muscle after tenotomy correlates well with the decreased expression of myogenic factors in senescent muscle subjected to exercise.

The reduced plasticity of senescent muscle is also reflected in the lack of increase in IGF-1 receptor mRNA levels after 5 days of overload in the old muscle, compared to the young animals. Both IGF-1 and IGF-II mediate their cellular effects by binding to the IGF-1 receptor. This activates a tyrosine kinase cascade that has been correlated with the different cellular effects of IGFs. In particular, IGF-1 has been shown to induce hypertrophy through the calcineurin/calmodulin pathway [20]. The observed increase in IGF-1 receptor transcription would serve to increase the effects of IGF-1 in muscle. The mechanism by which stretch may activate IGF-1 receptor transcription is as yet unknown.

Cellular ageing in muscle is likely to be due to multiple factors. However, the down-regulation of the IGF-1 system particularly, the decrease in MGF mRNA levels as well as IGF-1 receptor levels in response to overload, is likely to play an important role in the impaired regenerative potential of old skeletal muscle.

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