Regulation of IGF-I, IGFBP-4 and IGFBP-5 gene expression by loading in mouse skeletal muscle

Bonaventure Awede[a], Jean-Paul Thissen[b], Phillipe Gailly[a], Jean Lebacq[a,*]

[a] *Unité de Physiologie Générale des Muscles, Université Catholique de Louvain, 1200 Brussels, Belgium*
[b] *Unité de Diabétologie et de Nutrition, Université Catholique de Louvain, 1200 Brussels, Belgium*

Received 27 September 1999; received in revised form 22 October 1999

Abstract Gene expression of IGF-I, IGFBP-4 and IGFBP-5 was studied in hindhimb skeletal muscle of mice, which were either overloaded or unloaded for 8 days. Overloading induced a 15% hypertrophy in soleus muscle associated with a 60% increase of IGF-I transcript levels and a doubling of IGFBP-4 mRNA levels. IGFBP-5 mRNA levels were decreased to one third of the control value. Changes in IGFBPs mRNA always preceded changes in IGF-I gene expression. Unloading by hindlimb suspension resulted in atrophy of soleus muscle (20%) and phenotype change towards the fast type associated with a transient decrease of IGF-I mRNA (30%) and a sustained increase (×2) of IGFBP-5 transcript. These alterations in IGFBPs expression, in unloaded or overloaded soleus, suggest that they may play a role in skeletal muscle adaptation to changes in loading.

Key words: Loading; Hypertrophy; IGFBP; Phenotype change; Skeletal muscle

1. Introduction

Skeletal muscle is able to adapt to several external stimuli such as pattern of nerve or electrical stimulation, hormonal impregnation, stretch and changes in mechanical loading. Increased loading not only induces muscle hypertrophy but also phenotype changes towards the slow type whereas unloading is responsible for muscle atrophy and shift from slow to fast fiber.

Investigations of the signalling mechanisms involved in these processes suggest that insulin-like growth factor I (IGF-I) plays a major role in triggering hypertrophy induced by overload. First, stretch or overload of skeletal muscle induces an increase in local expression of IGF-I (mRNA and peptide) which is correlated with muscle hypertrophy, increased protein and DNA contents [1–4]. Second, local infusion or overexpression of IGF-I in skeletal muscle induces hypertrophy without any systemic effect [5,6].

While the chain of events triggering hypertrophy clearly involves IGF-I, the signalling mechanism inducing phenotype change is less clear. First, in individual fibers of stretched skeletal muscle, expression of IGF-I is correlated to that of slow or neonatal myosin [7]. Second, overexpression of IGF-I in vivo induces a shift in myofiber type towards a more oxidative fiber type [5]. Third, soleus muscles of growth hormone (GH)-deficient rats, which are lacking IGF-I, contain higher proportions of fast fibers than muscles from intact rats. Furthermore, treating these rats with GH increases the proportion of slow fibers in these muscles [8]. All together, these data suggest a possible role of IGF-I in fast to slow phenotype change.

The biological effects of IGF-I on muscle cells have mainly been studied in vitro. First, IGF-I not only induces myofiber hypertrophy [9] but also a phenotype change [10,11]. Second, both IGF-I and IGF-II stimulate proliferation and differentiation of myoblasts and the effects of IGFs are modulated by their binding proteins (IGFBPs) [12]. Amongst the seven IGFBPs described so far [12,13], four (IGFBP-2, -4, -5 and -6) are produced by different myoblast cell lines whereas only IGFBP-4, IGFBP-5 and IGFBP-6 are expressed by adult skeletal muscle [12,14]. IGFBP-4 is believed to inhibit proliferation and differentiation induced by IGF-I whereas IGFBP-5 has a dual role: inhibition of both processes, or stimulation of differentiation, depending on culture conditions [15,12,16].

The potential role of IGFBPs in the physiological response to loading changes has not yet been investigated. The goal of the present study was to analyze the regulation of gene expression of IGF-I and its main IGFBPs in skeletal muscle subjected to either functional overload or unloading.

2. Materials and methods

2.1. Animals manipulation

Male NMRI mice, 60 days of age at the beginning of the protocol, were used. The mice were housed in cages, maintained in light controlled environment (12:12-h light-dark cycle) and had unlimited access to food and water. Two separate sets of experiments were made: one for overloading and one for unloading. In each case, the mice were randomly assigned to two groups: control and treated. All procedures were performed while the animals were anaesthetized by subcutaneous injection of ketamine (100 mg/kg) and xylazine (10 mg/kg).

Overload was induced by inserting blocks of lead embedded in silicone underneath the skin of the hinder part of the back [17]. After a short medial incision in the skin and cutaneous detachment, four blocks, weighing together about 60% of body weight, were inserted, two on each side. Mice of the control group received the same treatment except that only silicone blocks weighing about 1.5 gram were inserted. A group of mice, which did not receive any treatment, served as a control at day 0 and their muscles were dissected at the beginning of the experiment.

A modified version of the protocol described by Criswell [18] was used to perform hindlimb unloading. A strip of adhesive bandage and a non-adhesive tape cut 15 cm×0.5 cm were used. The bandages were wrapped around the tail starting at the base of the tail. After recovery from anesthesia, a swivel hook was placed through the non-adhesive bandage, the tips of which were joined. The swivel hook was then raised so that the hindlimbs were elevated just of the cage floor (this

*Corresponding author. Fax: (32) (2) 7645580.
E-mail: lebacq@fymu.ucl.ac.be

Abbreviations: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; EDL, extensor digitorum longus; CTR, control; OLD, overloaded; ULD, unloaded

PII: S0014-5793(99)01469-6

produces a 30° head-down tilt). Forelimbs remained in contact with the cage floor allowing mice to move through a 360°-circle. Mice had at libitum access to food and water throughout the hindlimb suspension procedure.

After 2 and 8 days of treatment (overloading or unloading), mice were anaesthetized and their soleus muscles were dissected for analysis. Soleus was used because the procedure of overload applied affects especially this slow twitch muscle [17]. In a group of mice, which were not treated, EDL (extensor digitorum longus) muscles were also analyzed to perform comparison of gene expression with soleus muscles.

2.2. Electrophoretic analysis of myosin heavy chains (MHCs)

Frozen muscles were pulverized, extracted in Guba-Straub solution and MHCs were separated by SDS-PAGE as described previously [19]. Identification of the bands was based on their electrophoretic mobilities. Their proportions were quantified by densitometry. All measurements were made in duplicate for each mouse. The results are expressed as percentage of total MHCs.

2.3. RNA isolation and Northern blot hybridization

Total RNA was isolated by the guanidium thiocyanate/cesium chloride method [20]. Soleus or EDL muscles from five mice were pooled. Each sample of RNA (10 μg) was denatured in formaldehyde-MOPS and subjected to electrophoresis on 1.2% agarose gel. Homogeneity of RNA loading was assessed by UV transillumination of the gel after staining with ethidium bromide. The RNA was transferred to nylon membranes (Hybond, Amersham, Buckingham-shire, UK) by vacuum blotting (Vacugene, Pharmacia, Uppsala, Sweden). Levels of IGF-I, IGFBP-4 and IGFBP-5 mRNA were determined by hybridization with specific riboprobes. The 194-bp *Ava*II-*Hin*fI rat IGF-I exon 4 complementary DNA fragment was inserted into the plasmid vector Bluescript (pBSM13+) and linearized with *Eco*RI. A 221-bp rat IGFBP-4 gene fragment was ligated into the plasmid vector Bluescript (pBS SK+) and linearized with *Bam*HI. A 656-bp rat IGFBP-5 gene fragment was ligated into the plasmid vector Bluescript (pBS SK+) and linearized with *Eco*RI. The specific riboprobes were generated from linearized plasmids with uridine 5′-[^{32}P]triphosphate using T7 or T3 RNA polymerases.

The mRNA levels were quantified by densitometric scanning of the hybridization signal (LKB Ultroscan XL laser densitometry; LKB, Bromma, Sweden) using the software Gel scan (Pharmacia). All size-class of IGF-I mRNA transcripts were pooled together. The mRNA levels were normalized by assigning the mRNA level observed at the day of the beginning of the experiment (day 0) an arbitrary value of 100%.

2.4. Statistical analyses

Results were expressed as mean ± S.E.M. For RNA analysis, overloading experiments were performed four times ($n = 4$ for both control and overload groups) and 100 mice were used. Unloading experiments were performed five times and 140 mice were used. Differences between control and overloaded or unloaded groups were compared using a Student's *t*-test and statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of overloading

3.1.1. Effect of overloading on muscle mass. After 8 days of overload, a significant (15%, $P < 0.05$) increase in mass of soleus muscles was observed (10.9 ± 0.5 mg for overloaded soleus versus 9.5 ± 0.1 mg for control, $n = 6$). In a previous study, we showed that 1 month of overloading induced also in soleus muscle an increase in the ratio of type I to type II fibers [17].

3.1.2. Effect of overload on IGF-I, IGFBP-4 and IGFBP-5 gene expression.. As shown in Fig. 1, a significant increase in IGF-I mRNA levels (60%) was found in overloaded soleus muscles after 8 days, relative to control muscles.

Overload induced also an activation of IGFBP-4 gene expression in soleus muscles. As shown in Fig. 1, the levels of IGFBP-4 mRNA were significantly higher (100%) than control values both after 2 days and 8 days of overload. The effect of mechanical overload on IGFBP-4 gene expression was therefore earlier than the effect on IGF-I.

In contrast to changes observed in IGF-I and IGFBP-4 mRNAs, after 2 days of overload, IGFBP-5 mRNA levels were depressed by two thirds with respect to control values. After 8 days, the levels of IGFBP-5 mRNA in overloaded soleus muscles remained significantly lower than control values.

3.2. Effects of unloading

3.2.1. Effects of unloading on muscle mass and myosin heavy chains proportions. Hindlimb unloading induced a marked loss of soleus muscle mass. After 8 days, a 20% atrophy of soleus muscle was observed: 9.2 ± 0.5 mg for control muscle versus 7.3 ± 0.3 mg for unloaded soleus ($n = 5$, $P < 0.05$). The loss of muscle mass reached 31% after 15 days: 9.3 ± 0.3 mg for control versus 6.4 ± 0.1 mg for unloaded soleus ($n = 6$, $P < 0.01$).

Electrophoretic analysis of MHCs showed that hindlimb unloading for 15 days induced a shift from slow to fast phenotype in soleus muscles. Soleus muscles from control mice contained high proportions of MHC1 (52%) and MHC2a (39%) and a small proportion of MHC2x (9%). Unloading induced a significant decrease of MHC1 (43%) ($P < 0.05$) and MHC2a (24%) ($P < 0.001$) proportions associated with an increased proportion of MHC2x (27%) ($P < 0.001$) and an expression of MHC2b (6%). The most important change seemed to be the shift from MHC2a to MHC2x and 2b.

3.2.2. Effects of unloading on IGF-I, IGFBP-4 and IGFBP-5 gene expression. As shown in Fig. 2, 2 days of unloading induced a modest but significant decrease (30%) of IGF-I mRNA levels in soleus muscles. This change was transient since after 8 days of unloading the levels of IGF-I mRNA were not different from the control value. Hindlimb unloading had no significant effect on the levels of IGFBP-4 mRNA.

In contrast to the effects on IGF-I and IGFBP-4 mRNA levels, unloading increased the amount of IGFBP-5 mRNA by about 100%. This change in IGFBP-5 mRNA was already conspicuous after 2 days and persisted after 8 days of unloading.

3.3. Comparative gene expression of IGF-I, IGFBP-4 and IGFBP-5 in soleus and EDL muscles

As shown in Fig. 3, soleus muscles of control animals contained higher levels (35%) of IGF-I mRNA than EDL muscles. IGFBP-4 mRNA levels were similar in both muscles. In contrast, the level of IGFBP-5 mRNA was higher in EDL (40%) than in soleus muscles. Thus, soleus, a slow muscle, contained high level of IGF-I mRNA associated with low level of IGFBP-5 mRNA whereas EDL, a fast twitch muscle, expressed a low level of IGF-I with a high level of IGFBP-5.

4. Discussion

Our study showed for the first time that gene expression of IGF-I and its main binding proteins in muscle, IGFBP-4 and IGFBP-5, is acutely regulated during adaptative changes induced in skeletal muscle by overloading and unloading. Given their action on muscle cells, IGF-I and these IGFBPs probably play a role in hypertrophy and changes in phenotype induced in vivo by overloading and unloading.

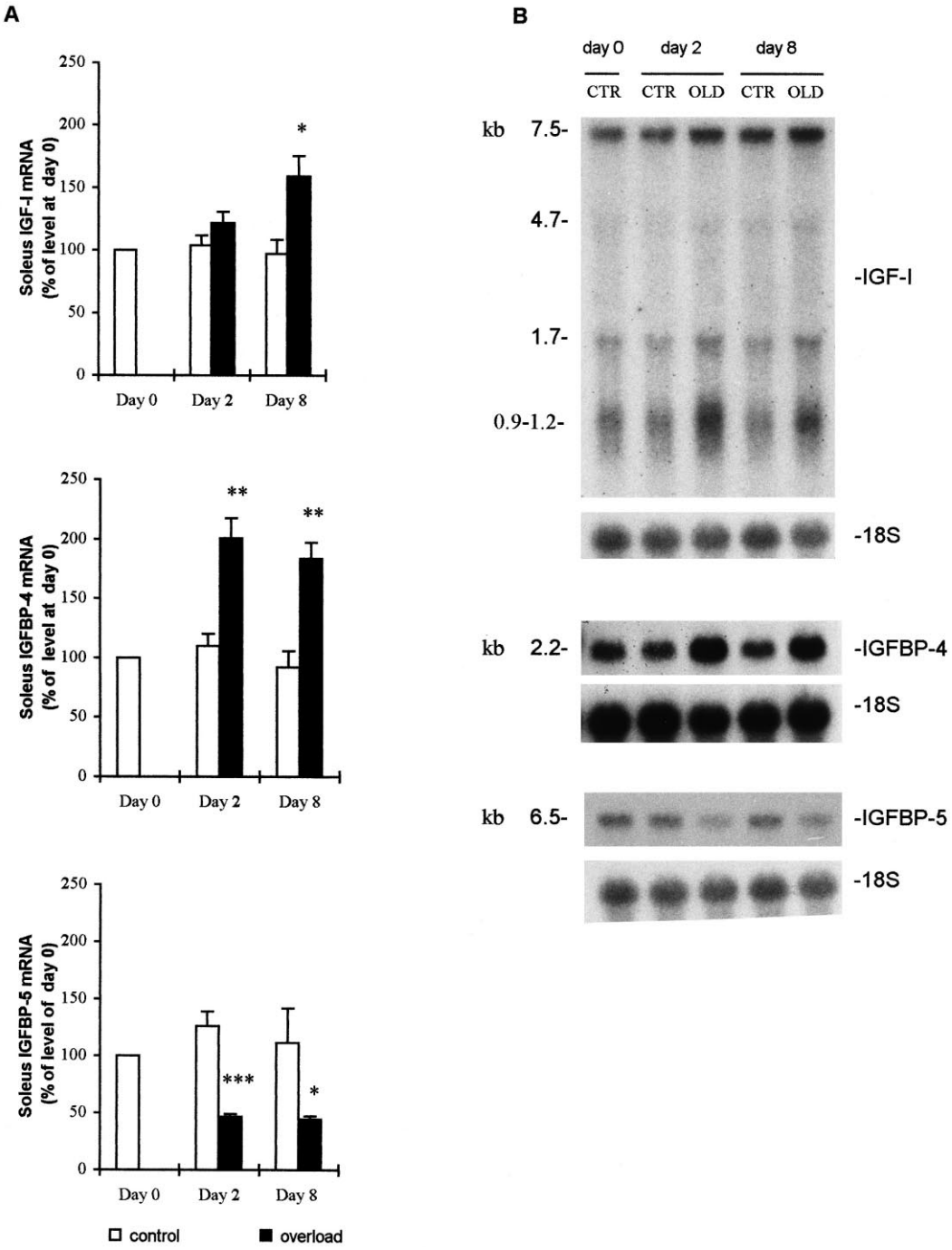

Fig. 1. IGF-I, IGFBP-4 and IGFBP-5 mRNAs detected by Northern blotting of 10 μg of total RNA from control and overloaded soleus muscles. A: Quantification of IGF-I, IGFBP-4 and IGFBP-5 mRNA levels by densitometry and expressed as percentage of the levels at day 0. Values are means ± S.E.M.; $n = 4$/group. * Significantly different ($P < 0.05$) from control, ** ($P < 0.01$), *** ($P < 0.001$). B: Autoradiographs showing expression of IGF-I, IGFBP-4 and IGFBP-5 mRNAs in control (CTR) at day 0, day 2 and day 8 and overloaded (OLD) soleus muscles (day 2 and day 8).

In the present study, overloading-induced hypertrophy of the soleus muscle is associated with increased IGF-I mRNA levels, as previously reported [1–3]. The role of IGF-I in this physiological response is supported by observations showing that overexpression of IGF-I induces muscle hypertrophy [5,6,10,11]. IGF-I is indeed believed to induce hypertrophy by activating satellite cells, which proliferate, differentiate and fuse with pre-existing myofibers, maintaining the size of the myonucleus domain [4]. Recently, it has been demonstrated that the IGF-I-induced myofiber hypertrophy is mediated by a calcium dependent calcineurin signalling pathway [21,11]. It would therefore be interesting to investigate the involvement of this pathway in hypertrophy of skeletal muscle induced by increased load. The mechanism responsible for increased expression of IGF-I in this model is not known. A mechano-transduction mechanism requiring an intact cytoskeleton could be involved in the increase of IGF-I expression in response to stretch and overload [22].

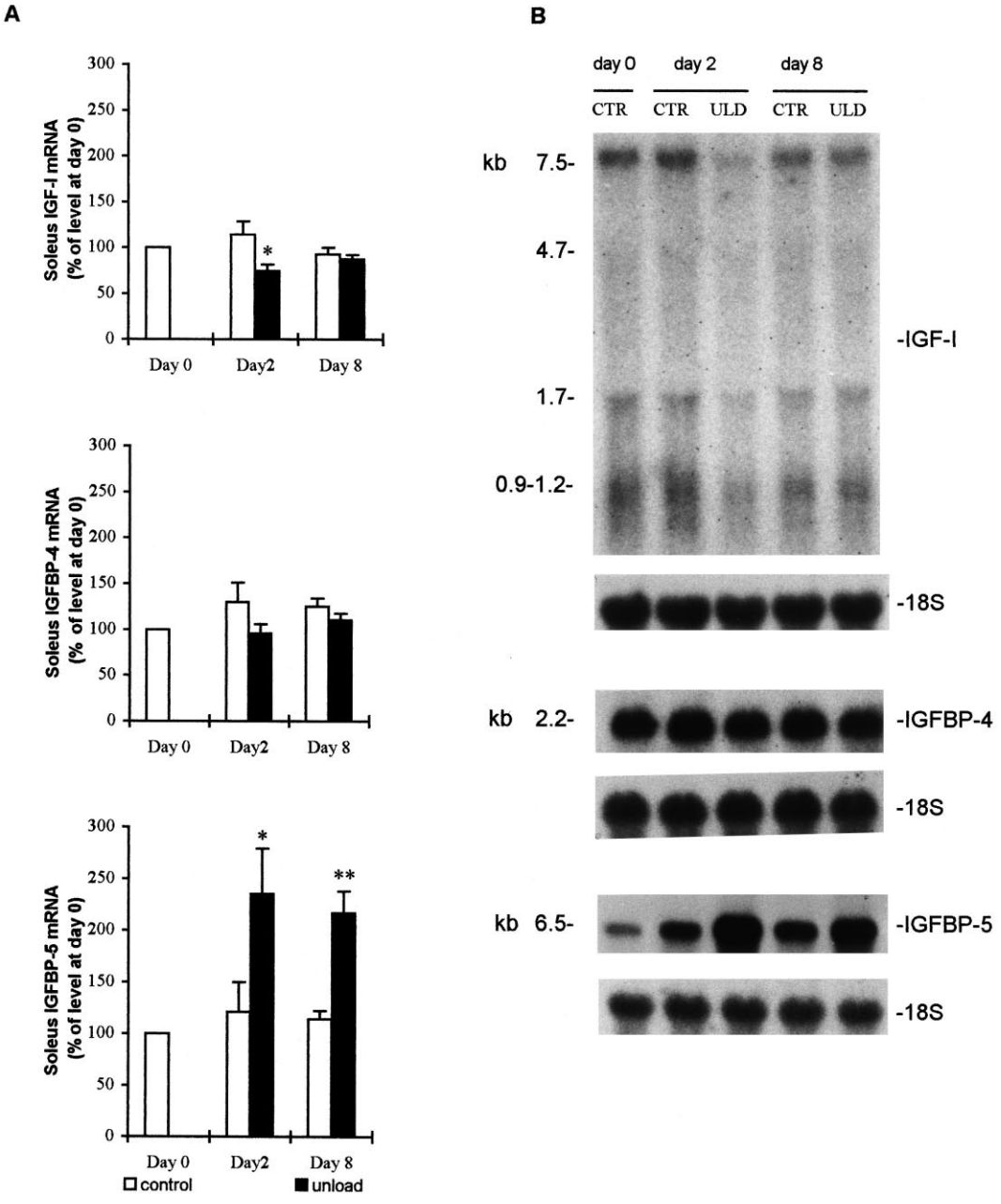

Fig. 2. IGF-I, IGFBP-4 and IGFBP-5 mRNAs detected by Northern blotting of 10 μg of total RNA from control and unloaded soleus muscles. A: Quantification of IGF-I, IGFBP-4 and IGFBP-5 mRNA levels by densitometry and expressed as percentage of the levels at day 0. Values are means ± S.E.M.; $n = 5$/group. * Significantly different ($P < 0.05$) from control, ** ($P < 0.01$). B: Autoradiographs showing expression of IGF-I, IGFBP-4 and IGFBP-5 mRNAs in control (CTR) at day 0, day 2 and day 8 and unloaded (ULD) soleus muscles (day 2 and day 8).

Reducing load on soleus muscle induces loss of muscle mass, but no role of IGF-I in atrophy induced by unloading has been established yet. Previous studies using different models of disuse did not report any changes in IGF-I expression [7,18], and overexpression of IGF-I in skeletal muscle did not prevent atrophy induced by unloading [18]. However the transient decrease of IGF-I mRNA levels induced by unloading could be involved in myonuclear apoptosis associated with unloading-induced atrophy, since IGF-I was shown to protect myonuclei against apoptosis in unloaded muscles [23].

Our work is the first that demonstrated the specific regulation of IGFBP-4 and IGFBP-5, the main IGFBPs expressed in skeletal muscle in mice, in response to loading or unloading. The increase in IGFBP-4 mRNA associated to overload-induced muscle hypertrophy seems paradoxical since IGFBP-4 is generally thought to inhibit IGF-I action [24,12]. This suggests that, in vivo, IGFBP-4 could act by a different mechanism on IGF-I action, or that, it prevents the proliferating effect of IGF-I on satellite cells, thus limiting the increase of muscle mass. The early increase of IGFBP-4 could explain the increase in IGF-I peptide independent of changes of its mRNA observed by others in muscle subjected to exercise [25]. In the early steps, IGFBP-4 could act by quenching circulating IGF-I within the muscle before any local IGF-I expression changes. The measurement of IGF-I and IGFBP-4 proteins in overloaded muscles would confirm this hypothesis.

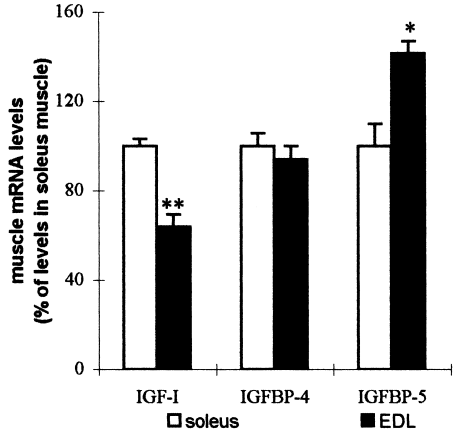

Fig. 3. IGF-I, IGFBP-4 and IGFBP-5 mRNAs detected by Northern blotting of 10 μg of total RNA from EDL and soleus muscles. IGF-I, IGFBP-4 and IGFBP-5 mRNA levels were quantified by densitometry and expressed as percentage of the levels in soleus muscle. Values are means ± S.E.M.; $n = 4$/group. * Significantly different ($P < 0.05$) from control, ** ($P < 0.01$).

In contrast to IGF-I and IGFBP-4, IGFBP-5 expression is down-regulated in overloaded muscles. This change in IGFBP-5 expression is not compatible with its potentiating effects described in vitro [24,12]. However, in view of the possible inhibitory effect of IGFBP-5 on myoblast proliferation induced by IGF-I [16], the decrease of its gene expression could facilitate the activating effect of IGF-I on satellite cells. In unloaded muscles, the increased expression of IGFBP-5 associated with the low level of IGF-I could promote muscle atrophy. On the other hand, the higher levels of IGFBP-5 expression in fast muscle compared to slow muscle together with its decrease in overloaded muscle, which undergoes fast to slow phenotype change, suggest that IGFBP-5 could promote the fast phenotype. Alternatively, IGFBP-5 could act independently of IGF-I by binding to its own receptor. Indeed, IGF-I-independent effects of IGFBP-5 have been described in osteoblasts [26,27]. Binding of IGFBP-5 to the extracellular matrix and its cleavage by specific proteases could be important factors of modulation of IGF action by IGFBP-5.

When the effects of overloading or unloading on IGF-I, IGFBP-4 and IGFBP-5 mRNAs are taken together, it appears that IGFBP-4 and IGFBP-5 are differentially regulated by loading and that significant effects on IGFBPs mRNA can precede that on IGF-I mRNA. Thus these effects are not caused by alterations in IGF-I expression and are probably directly induced by loading changes. This indicates that IGFBPs are probably important in the adaptation of skeletal muscle subjected to loading.

Acknowledgements: We thank Josiane Verniers and Anne-Laure Hubert for their technical assistance. B. Awede is supported by the 'Commissariat Général aux Relations Internationales' (Belgium). J.P. Thissen and Ph. Gailly are Research associates of the 'Fonds National de la Recherche Scientifique' (Belgium).

References

[1] DeVol, D.L., Rotwein, P., Sadow, J.L., Novakofski, J. and Bechtel, P.J. (1990) Am. J. Physiol. 259, E89–95.
[2] Czerwinski, S.M., Martin, J.M. and Bechtel, P.J. (1994) J. Appl. Physiol. 76, 2026–2030.
[3] Adams, G.R. and Haddad, F. (1996) J. Appl. Physiol. 81, 2509–2516.
[4] Adams, G.R. (1998) Exerc. Sport Sci. Rev. 26, 31–60.
[5] Coleman, M.E., DeMayo, F., Yin, K.C., Lee, H.M., Geske, R., Montgomery, C. and Schwartz, R.J. (1995) J. Biol. Chem. 270, 12109–12116.
[6] Adams, G.R. and McCue, S.A. (1998) J. Appl. Physiol. 84, 1716–1722.
[7] Yang, H., Alnaqeeb, M., Simpson, H. and Goldspink, G. (1997) J. Anat. 190, 613–622.
[8] Daugaard, J.R., Laustsen, J.L., Hansen, B.S. and Richter, E.A. (1998) Acta Physiol. Scand. 164, 119–126.
[9] Vandenburgh, H.H., Karlisch, P., Shansky, J. and Feldstein, R. (1991) Am. J. Physiol. 260, C475–484.
[10] Semsarian, C., Sutrave, P., Richmond, D.R. and Graham, R.M. (1999) Biochem. J. 339, 443–451.
[11] Semsarian, C., Wu, M.-J., Ju, Y.-K., Marciniec, T., Yeoh, T., Allen, D.G., Harvey, R.P. and Graham, R.M. (1999) Nature 400, 576–581.
[12] Florini, J.R., Ewton, D.Z. and Coolican, S.A. (1996) Endocr. Rev. 17, 481–517.
[13] Damon, S.E., Haugk, K.L., Swisshelm, K. and Quinn, L.S. (1997) Exp. Cell Res. 237, 192–195.
[14] Putzer, P., Breuer, P., Götz, W., Gross, M., Kübler, B., Scharf, J.G., Schuller, A.G., Hartmann, H. and Braulke, T. (1998) Mol. Cell. Endocrinol. 137, 69–78.
[15] Ewton, D.Z. and Florini, J.R. (1995) J. Endocrinol. 144, 539–553.
[16] Ewton, D.Z., Coolican, S.A., Mohan, S., Chernausek, S.D. and Florini, J.R. (1998) J. Cell. Physiol. 177, 47–57.
[17] Awede, B., Berquin, A., Wuytack, F. and Lebacq, J. (1999) Eur. J. Appl. Physiol., in press.
[18] Criswell, D.S., Booth, F.W., DeMayo, F., Schwartz, R.J., Gordon, S.E. and Fiorotto, M.L. (1998) Am. J. Physiol. 275, E373–379.
[19] Maréchal, G., Coulton, G.R. and Beckers-Bleukx, G. (1995) Am. J. Physiol. 268, C513–519.
[20] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[21] Musarò, A., McCullagh, K.J., Naya, F.J., Olson, E.N. and Rosenthal, N. (1999) Nature 400, 581–585.
[22] Goldspink, G. (1999) J. Anat. 194, 323–334.
[23] Allen, D.L., Linderman, J.K., Roy, R.R., Bigbee, A.J., Grindeland, R.E., Mukku, V. and Edgerton, V.R. (1997) Am. J. Physiol. 273, C579–587.
[24] Jones, J.I. and Clemmons, D.R. (1995) Endocr. Rev. 16, 3–34.
[25] Eliakim, A., Moromisato, M., Moromisato, D., Brasel, J.A., Roberts Jr., C. and Cooper, D.M. (1997) Am. J. Physiol. 273, R1557–1561.
[26] Andress, D.L., Loop, S.M., Zapf, J. and Kiefer, M.C. (1993) Biochem. Biophys. Res. Commun. 195, 25–30.
[27] Mohan, S., Nakao, Y., Honda, Y., Landale, E., Leser, U., Dony, C., Lang, K. and Baylink, D.J. (1995) J. Biol. Chem. 270, 20424–20431.