Vol. 198, No. 1, 1994
January 14, 1994

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 97-102

# Interactions between growth hormone and nutrition in hypophysectomised rats: skeletal muscle myosin heavy chain mRNA levels

**Paul T. Loughna and Peter C. Bates[1]**

Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, United Kingdom

[1]AFRC Institute of Animal Physiology & Genetics Research, Babraham, Cambridge CB2 4AT, United Kingdom

Received November 15, 1993

The aim of this study was to examine the role of growth hormone (GH) in regulating muscle phenotype and to determine how this is modulated by altered nutrition. Total RNA was extracted from gastrocnemius muscles of hypophysectomised rats treated with saline, GH or GH but fed a restricted intake. Type 1, 2A, 2B, embryonic and neonatal myosin heavy chain mRNA levels were estimated by slot blot hybridization. Hypophysectomy reduced the concentrations of types 1, 2A and embryonic mRNAs and dramatically elevated types 2B and neonatal compared to control levels, but this was time-dependant. All MHC mRNA levels were partially restored to control levels in the GH-treated rats except for type 1; the level of this transcript was only elevated by GH in the restricted intake group. Restricted food intake modulated the effects of GH administration for all other MHC mRNA concentrations. 

The primary role of GH in adult animals appears to be its involvement in the regulation of body composition. In hypophysectomised animals muscle protein synthesis rate is decreased and this may be partly restored by GH administration *in vivo*. The somatogenic effects of growth hormone are thought to be mediated, at least in part, by IGF-1 (1). Plasma concentrations of IGF-1 rise after treatment with GH, however the GH/IGF-1 axis is sensitive to changes in nutrition (2). Both short term and long term dietary changes alter the IGF-1 response to GH treatment; in malnourished humans and other species, plasma IGF-1 concentrations are low even though GH concentrations are increased (2; 3).

One of the most abundant and physiologically important contractile proteins, myosin heavy chain (MHC), exists as a number of different isoforms each of which is coded for by a

0006-291X/94 $5.00

different member of a multigene family. The expression of these genes in adult skeletal muscle can be altered in response to innervation, mechanical stimuli, workload and thyroid hormone status (4; 5; 6). Despite the considerable interest in GH and IGF-1 action their effects upon muscle phenotype have been little investigated. Large doses of GH have been shown to increase muscle fibre diameter and cellular proliferation in adult rats (7), and hypophysectomy has been observed to reduce the number of type 1 muscle fibres which could be reversed by GH administration (8). These changes in fibre type composition of skeletal muscle in response to GH suggest that the contractile protein components may be differentially affected by GH administration. The aim of the present study was to determine the effects of hypophysectomy and subsequent GH treatment upon MHC mRNA levels in skeletal muscle and how this is affected by restricted food intake.

## MATERIALS AND METHODS

Animals: Male rats (body wt. 191 ± 3g, mean ± S.E.M.) were hypophysectomised ($H_x$) and 14 days later were divided into 4 groups. The first group ($H_xO$; n=4) were killed and the remaining 3 groups (n=5 per group) were treated for 7 days with saline ($H_x7$), 60 mU/day of human pituitary growth hormone and fed *ad libitum* (GH) or with food intake restricted to match that of $H_x7$ (GH-PF). A fifth group of animals were non-hypophysectomised controls ($H_{norm}$). After the animals were killed serum was removed and stored at -20°C and gastrocnemius muscles were rapidly dissected and stored frozen in liquid nitrogen.

Preparation of synthetic probes: Oligonucleotides of 20 bases in length, specific for five MHC mRNA transcripts (types 1, 2A, 2B, embryonic and neonatal ) were obtained from Oswell DNA service. The sequences of these oligonucleotides, which are all specific for the 3' untranslated regions of the MHC transcripts have been reported previously (9) and have been demonstrated to be highly isoform specific (5; 9; 10). Oligothymidine (oligo-dt) of 18-24 bases in length was obtained from Sigma (Poole, Dorset, UK). The oligonucleotide probes were radioactively labelled at the 5' end, using T4 polynucleotide kinase, to a specific activity of approximately $10^7$ cpm/ g and separated from unincorporated [$^{32}$P]ATP (Amersham International, Amersham) using sephadex G25 columns.

RNA extraction and analysis: Total cellular RNA was extracted by a modification of the hot phenol procedure (11). Aliquots of 2, 4 and 8 µg of total RNA (in a solution of 50% formamide, 6% formaldehyde and 1X SSC), from each sample, were blotted onto Hybond N (Amersham International, Amersham, UK) using a standard slot blot procedure. It has been shown previously that the amount of bound synthetic $^{32}$P labelled probes increased linearly with increasing amounts of total cellular RNA of up to 10 µg per slot applied to the filter (5; 9). Filters were prehybridized and hybridized as described previously (5). In the case of hybridizations with oligo-dT probe, a 20X excess of unlabelled oligo-dT was added to the hybridization fluid. After washing the filters autoradiography was carried out at -70°C. Hybridization intensity was estimated by densitometric scanning using a Seescan densitometer.

Statistical analysis: The data is shown as mean ± S.E.M. and was analyzed by Student's *t*-test.

## RESULTS

Hypophysectomy caused catabolism of the gastrocnemius muscle (muscle weight change from $H_x0$ to $H_x7$, -4.0%); this was reversed by GH treatment regardless of food intake (GH, 7.6; GH-PF, 8.8% weight change from $H_x0$), even though the GH group ate 34% more food than the $H_x7$ or the pair-fed GH-PF group. As well as the lack of GH, hypophysectomy

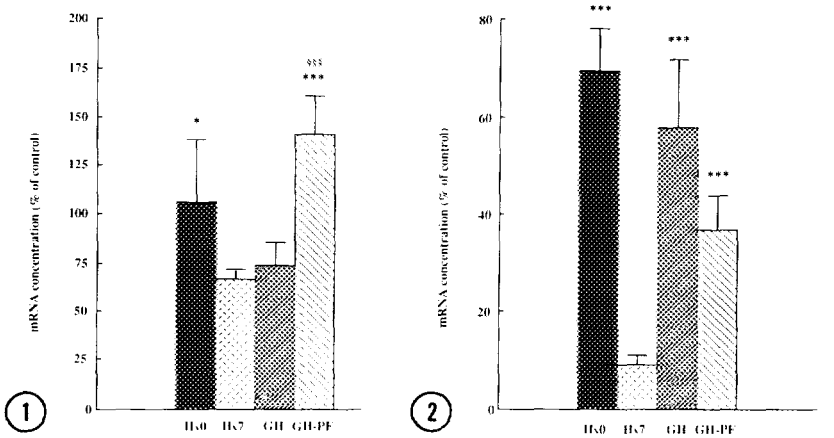


FIGURE 1. Concentration of mRNA for type 1 (slow oxidative) myosin heavy chain from gastrocnemius muscle of hypophysectomised rats killed at the start of the experiment (Hx0) or after 7 days treatment with saline (Hx7) or with growth hormone but fed ad libitum (GH) or given a food intake to match that of the Hx7 group (GH-PF). Values are shown as percent of the level in control non-hypophysectomised rats and are means ± S.E.M. from four animals in Hx0 and five animals in each of the other groups. *P<0.05, ***P<0.001 compared with Hx7; §§§P<0.001 compared to GH.

FIGURE 2. Concentration of mRNA for type 2A (fast oxidative glycolytic) myosin heavy chain from gastrocnemius muscle of hypophysectomised rats killed at the start of the experiment (Hx0) or after 7 days treatment with saline (Hx7) or with growth hormone but fed ad libitum (GH) or given a food intake to match that of the Hx7 group (GH-PF). Values are shown as percent of the level in control non-hypophysectomised rats and are means ± S.E.M. from four animals in Hx0 and five animals in each of the other groups. ***P<0.001 compared with Hx7.

results in the loss of thyroid hormones; this was only very marginally affected (P<0.05) by GH-treatment in the rats fed *ad libitum* (serum tri-iodothyronine concentrations: $H_x0$, 0.21 ± 0.01; $H_x7$, 0.21 ± 0.02; GH, 0.32 ± 0.03; GH-PF, 0.22 ± 0.02 μg/l).

Two weeks after hypophysectomy ($H_x0$) the levels of type 1 (slow oxidative) MHC mRNA were not significantly different from control $H_{norm}$ animals (Fig. 1). However three weeks following $H_x$ ($H_x7$) the level of this transcript had fallen to levels significantly below control levels. Administration of GH to animals fed an *ad libitum* diet (GH) did not produce significant changes in type 1 MHC mRNA concentrations compared to those in the $H_x0$ and $H_x7$ groups but restricting the increased food intake due to GH, in the GH-PF group, did result in an increase in the type 1 mRNA level.

Similarly to the type 1 transcript, all of the experimental groups had levels of type 2A (fast oxidative glycolytic) MHC mRNA significantly below control $H_{norm}$ levels (Fig. 2). The largest fall in type 2A MHC mRNA levels was observed in $H_x7$ where levels were less than 10% of $H_{norm}$ values and were also significantly lower than those found in $H_x0$. Growth hormone administration produced levels of this transcript significantly higher than $H_x7$ though this increase was more pronounced in the GH than the GH-PF group.

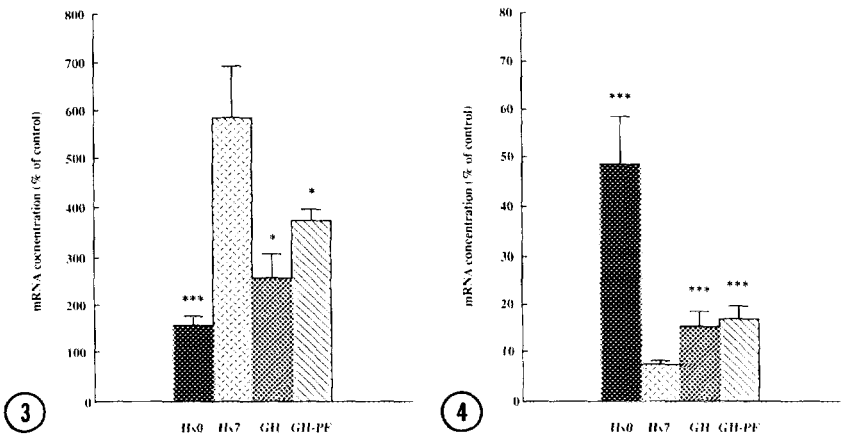


FIGURE 3. Concentration of mRNA for type 2B (fast glycolytic) myosin heavy chain from gastrocnemius muscle of hypophysectomised rats killed at the start of the experiment (Hx0) or after 7 days treatment with saline (Hx7) or with growth hormone but fed ad libitum (GH) or given a food intake to match that of the Hx7 group (GH-PF). Values are shown as percent of the level in control non-hypophysectomised rats and are means ± S.E.M. from four animals in Hx0 and five animals in each of the other groups. *P<0.05, ***P<0.001 compared with Hx7.

FIGURE 4. Concentration of mRNA for embryonic myosin heavy chain from gastrocnemius muscle of hypophysectomised rats killed at the start of the experiment (Hx0) or after 7 days treatment with saline (Hx7) or with growth hormone but fed ad libitum (GH) or given a food intake to match that of the Hx7 group (GH-PF). Values are shown as percent of the level in control non-hypophysectomised rats and are means ± S.E.M. from four animals in Hx0 and five animals in each of the other groups. ***P<0.001 compared with Hx7.

In contrast to type 1 and type 2A, the levels of type 2B (fast glycolytic) MHC mRNA were significantly elevated in $H_x0$ and dramatically increased in $H_x7$ when compared to $H_{norm}$ rats (Fig. 3). This increase was significantly reduced in GH rats and to a lesser extent in the GH-PF rats, although in both groups levels were still significantly above $H_{norm}$.

Embryonic MHC mRNA levels, which are low in gastrocnemius muscles of adult normal unhypophysectomised rats, were reduced further by hypophysectomy (Fig. 4). The reduction of the embryonic transcript level was significantly greater, however, in $H_x7$ than $H_x0$ rats. GH administration significantly increased the levels of this message to above those in the $H_x7$ rats but not to the levels found in the $H_x0$ group. There was no significant difference between the levels of embryonic MHC mRNA levels between the GH and the GH-PF groups.

Neonatal MHC mRNA was virtually undetectable in $H_{norm}$ but was dramatically increased in the $H_x7$ group. This large increase was reversed by GH treatment in both *ad libitum* fed (GH) and restricted intake (GH-PF) groups. The level was so much greater in the $H_x7$ group than any of the other groups, that the autoradiograph was overexposed if left long enough to quantify the other groups; as a result no statistical analysis could be performed on this transcript.

## DISCUSSION

This study has demonstrated that hypophysectomy produces dramatic changes in the mRNA levels for specific MHC isoforms in skeletal muscle. The levels of three MHC mRNAs, types 1, 2A and embryonic, were decreased in response to hypophysectomy whereas those of 2B and neonatal increased dramatically. Interestingly in the case of all MHC transcripts examined, the effects of hypophysectomy was far more pronounced in the $H_x7$ group (3 weeks after $H_x$) than in the $H_x0$ group (2 weeks after $H_x$). In particular the type 1 MHC mRNA levels are not significantly different in $H_x0$ when compared to control non-$H_x$ levels whereas in the $H_x7$ group levels of this mRNA fell significantly to two thirds of control levels.

Hypophysectomy causes a severe disruption to metabolism and many hormones other than GH, such as thyroid hormones and insulin, are decreased (12). Thyroid hormones in particular are known to directly affect MHC gene expression; however, although thyroid hormone may play a role in modulating MHC gene expression following hypophysectomy it seems unlikely to be the major influence with regard to the data presented in this study. All four experimental groups of animals were considerably hypothyroid and yet exhibited dramatically different MHC mRNA levels, in some cases one group having levels of a particular transcript lower than euthyroid control animals whereas in another group levels may be higher. In fact although only one group had significantly higher tri-iodothyronine levels than the other groups (GH) the GH-PF had markedly different MHC mRNA levels to the $H_x$ groups and often similar levels to the GH group.

The dramatic reversal of the effects of hypophysectomy by GH administration suggests that this hormone is highly influential in the regulation of skeletal muscle MHC gene expression, but whether its effects are direct or indirect cannot be resolved by the data presented here. Anabolic effects of GH in muscle are known to be mediated, at least in part, by IGF-1, and GH-treatment increased IGF-1 mRNA levels in muscle even with restricted food intake (13). The observation that nutrition could modulate the effects of GH administration on muscle MHC mRNA levels could be explained if these effects were mediated by the action of IGF-1. Evidence that IGF-1 can directly affect MHC gene expression has been obtained by Florini and coworkers (14). *In vivo* studies showed that long-term treatment with GH elevated the relative amount of ß-MHC mRNA in the myocardium of rats (the cardiac ß- and skeletal muscle type 1-MHC mRNAs are coded for by the same gene in the rat). This elevation of cardiac ß-MHC was shown to be mediated by the action of IGF-1 in subsequent *in vitro* studies where administration of this peptide to cardiac myocytes in culture greatly increased ß-MHC gene expression while that of α-MHC was unaffected. In contrast thyroid hormone administration produced opposite effects upon the expression of these two genes.

We have, therefore demonstrated that hypophysectomy and GH-treatment produce significant changes in the relative levels of different transcripts of myosin heavy chain, hypophysectomy producing a shift towards 2B and neonatal and away from type 1, 2A and embryonic isoforms. GH-treatment prevented this shift but the effects of GH were partially

modulated by restricting the increased food intake induced by GH. These changes could be a direct effect of GH but may be mediated through local production of IGF-1.

## REFERENCES


1. Daughaday, W.H. (1981) in: Endocrine Control of Growth (Daughaday, W.H., ed) pp. 1-24, Elsevier, New York.
2. Pell, J.M. & Bates, P.C. (1990) Nutrition Research Reviews 3, 163-192.
3. Elsasser, T.H., Rumsey, T.S. & Hammond, A.C. (1989) Journal of Animal Science 67, 128-141.
4. Izumo, S., Nadal-Ginard, B. & Mahadavi, V. (1986) Science 231,597-600.
5. Morgan, M.J. & Loughna, P.T. (1989) FEBS Letters 255, 427-430.
6. Loughna, P.T., Izumo, S., Goldspink, G. & Nadal-Ginard, B. (1990) Development 109, 217-233.
7. Ullman, M. & Oldfors, A. (1989) Acta Physiologica Scandinavica 135, 531-536.
8. Ayling, C.M., Moreland, B.H., Zanelli, J.M. & Schulster,D. (1989) Journal of Endocrinology 123,429-435.
9. Gustafson, T.A., Markham, B.E. & Morkin, E. (1985) Biochemical and Biophysical Research Communications 130, 1161-1167.
10. Gustafson, T.A., Markham, B.E. & Morkin, E. (1986) Circulation Research 59, 194-201.
11. Soeiro, R.H., Birnboim, C. & Darnell, J.E. (1966) Journal of Molecular Biology 19, 362-372.
12. Brown, J.G., Bates, P.C, Holliday, M.A. and Millward, D.J. (1981) Biochemical Journal 194, 771-782.
13. Bates, P.C., Loughna, P.T., Pell, J.M., Schulster, D. & Millward, D.J. (1992) Journal of Endocrinology 139, 117-126.
14. Florini, J.R. & Ewton, D.Z. (1992) Growth Regulation 2, 23-29.