Bed rest increases the amount of mismatched fibers in human skeletal muscle

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Andersen, J. L., T. Gruschy-Knudsen, C. Sandri, L. Larsson, and S. Schiaffino. Bed rest increases the amount of mismatched fibers in human skeletal muscle. J. Appl. Physiol. 86(2): 455–460, 1999.—The effects of a 37-day period of bed rest on myosin heavy chain (MHC) expression on both mRNA and protein level in human skeletal muscle fibers were studied. Muscle biopsies from vastus lateralis muscle were obtained from seven healthy young male subjects before and after the bed-rest period. Combined in situ hybridization, immunocytochemistry, and ATPase histochemistry analysis of serial sections of the muscle biopsies demonstrated that fibers showing a mismatch between MHC isoforms at the mRNA and protein level increased significantly after the bed-rest period, suggesting an increase in the amount of muscle fibers in a transitional state. Accordingly, fibers showing a match in expression of MHC-1 and of MHC-2A at the mRNA and protein level decreased, whereas fibers showing a mismatch between MHC-2X mRNA and protein increased after bed rest. Overall, there was an increase in fibers in a transitional state from phenotypic type 1 → 2A and 2A → 2X. Furthermore, a number of fibers with unusual MHC mRNA and isofiber combinations were observed after bed rest (e.g., type 1 fibers with only mRNA for 2X and type 1 fibers negative for mRNA for MHC-β/slow, 2A, and 2X). In contrast, no changes were revealed after an examination at the protein level alone. These data suggest that the reduced load-bearing activity imposed on the skeletal muscles through bed rest will alter MHC gene expression, resulting in combinations of mRNA and MHC isoforms normally not (or only rarely) observed in muscles subjected to load-bearing activity. On the other hand, the present data also show that 37 days of bed rest are not a sufficient stimulus to induce a similar change at the protein level, as was observed at the gene level.

HUMAN SKELETAL MUSCLE contains different fiber types, best characterized by the specific myosin heavy chain (MHC) isoforms. Adult human skeletal muscle fibers mainly express three distinct MHC isoforms (β/slow, 2A, and 2X) (32). In addition to fibers containing one of these three MHC isoforms, a number of “hybrid” fibers coexpressing various amounts of two MHC isoforms can be recognized. In a recent paper we have suggested that a combined detailed analysis of the distribution of MHC isoforms and transcripts in serial sections of normal human skeletal muscle will enable the identification of transitional fiber phenotypes and also enable the determination of the direction of change in MHC gene expression by correlating in situ hybridization, immunocytochemistry, and ATPase histochemistry (3).

Periods without weight bearing have significant effects on skeletal muscle (4). Most prominent is a decrease in protein synthesis (17), loss of muscle mass, and loss of muscle strength (6, 15, 16, 27). Experimental animal models inducing unloading of the muscle are limited. Besides bed rest (5, 15, 24, 28), exposure to microgravity in space (16, 37), voluntary immobilization (6, 7, 9, 36), or immobilization after surgery (20, 21) have been applied. Although unloading of muscle in animal models has been shown to induce fiber-type transformation within a 5- to 6-wk period (11, 35), such transformations have not been obtained in human bed-rest (5, 8, 18, 24) or unloading (1, 6, 23) experiments as of now. This does not necessarily signify that fiber-type transformation is not in progress during a 5-wk bed-rest period but only that significant changes cannot be obtained at the protein level. Therefore, the purpose of the present study was to study how 5 wk of bed rest would affect the expression of MHC isoforms in human skeletal muscle on both the protein level and the mRNA level.

METHODS

Subjects. Seven subjects [age 28 ± 1 yr, height 176 ± 1 cm, body weight 74 ± 3 kg (before) and 73 ± 3 kg (after), not significant] completed a 37-day period of −6°head-down-tilt bed rest. Weight-bearing physical exercise or other countermeasures were not allowed during the bed-rest period. The subjects volunteered for the study and gave their written consent (for further details, see Ref. 18).

Muscle biopsies. Muscle biopsies were obtained from vastus lateralis muscle by the percutaneous conchothome method under local anesthesia before and on day 37 of bed rest (18, 27). The muscle biopsies were trimmed, mounted, and frozen in Freon, cooled by liquid nitrogen, and kept at −80°C until further analysis.

In situ hybridization, immunocytochemistry, and ATPase histochemistry. In situ hybridization, immunocytochemistry, and ATPase histochemistry were performed in serial cryosections (10 μm). In situ hybridization was performed as previously described by using probes specific for human MHC-β/slow, MHC-2A, and MHC-2X (33). The final concentration of adenosinetriphosphatase histochemistry; fiber-type transitions; hybrid fibers; immunocytochemistry; in situ hybridization; myosin; simulated microgravity.

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RESULTS

Histochimistry and immunocytochemistry. No significant change in fiber-type distribution, as determined by ATPase histochimistry and immunocytochemistry, was observed after the bed-rest period (Table 1). Overall fiber atrophy (~11.2%) was observed after bed rest (4,625 ± 407 vs. 4,108 ± 219 µm², P < 0.05). The decrease in size was similar for the various fiber populations (4,397 ± 336 vs. 3,903 ± 472, 5,251 ± 318 vs. 4,516 ± 603, 4,502 ± 427 vs. 4,138 ± 600, and 4,286 ± 516 vs. 3,729 ± 461 µm² for type 1, type 2A, type 2A/2X, and 2X fibers, respectively); however, the differences were not statistically significant when single fiber types were compared.

In situ hybridization. The proportion of fibers positive for mRNA for β/slow alone decreased after bed rest (53.2 ± 5.2 vs. 40.2 ± 4.8%, P < 0.05). Similarly, the proportion of fibers positive for mRNA for 2A alone decreased after bed rest (31.7 ± 5.3 vs. 19.1 ± 4.3%, P < 0.05). In contrast, the proportion of fibers positive for mRNA for both 2A and 2X (10.0 ± 2.1 vs. 15.8 ± 2.4%, P < 0.05) and fibers positive solely for mRNA for 2X (4.5 ± 1.5 vs. 19.1 ± 4.1%, P < 0.05) increased after the bed-rest period (Table 2). A small proportion of fibers positive for mRNA for all three MHCs (β/slow, 2A, and 2X), negative for mRNA for both β/slow and 2X but negative for mRNA for 2A was observed (Tables 2 and 3). All these phenotypically atypical fibers were found in the post-bed-rest biopsies and were all typical type 1 fibers at the protein level.

Transitional fiber phenotypes. According to our earlier findings (3), human skeletal muscle fibers can be separated into fibers with corresponding expression of MHC at the mRNA and protein levels and fibers that display a mismatch between MHC expression at the mRNA and protein levels, the interpretation being that the former represents fibers in a stable state, whereas the latter may represent fibers in a transitional state (for detailed characterization, see Ref. 3). The proportion of type 1 fibers showing corresponding expression of mRNA and protein for MHC decreased after bed rest (53.2 ± 5.1 vs. 40.2 ± 4.8%, P < 0.05). Similarly, the proportion of type 2A fibers showing corresponding expression of mRNA and protein for MHC increased after bed rest (26.5 ± 4.3 vs. 16.3 ± 3.8%, P < 0.05). The proportion of type 2X fibers showing corresponding expression of mRNA and protein for MHC increased after bed rest (3.8 ± 1.5 vs. 9.1 ± 3.8%, P < 0.05) (Table 3). The proportion of fibers that, according to the above-mentioned scheme, can be determined as transitional in the direction 1 → 2A (1.1 ± 0.5 vs. 4.5 ± 1.7%, P < 0.05) and in the direction 2A → 2X (1.7 ± 0.6 vs. 18.3 ± 3.2%, P < 0.05) increased after bed rest. The proportion of fibers determined as transitional in the direction 2X → 2A decreased (10.2 ± 2.1 vs. 1.6 ± 1.0%, P < 0.05). The proportion of fibers characterized as atypical type 1 “jump” fibers (i.e., fibers positive only for MHC-1 protein but positive for the MHC-2X transcript) increased after bed rest (0.0 ± 0.0 vs. 4.0 ± 1.9%, P < 0.05) (Table 3, Fig. 1). Furthermore, after bed rest a number of fibers negative for β/slow, 2A, and 2X transcripts were observed in three of the seven subjects (Fig. 2). All of these “negative” fibers were positive only for MHC-1 at the protein level.

DISCUSSION

The major result of the present study is that bed rest, covering a period of 37 days, can induce significant changes in the MHC mRNA levels, with an increase in the number of fibers expressing mRNA for MHC-2X and a decrease in the number of fibers expressing mRNA for MHC-1, without significant changes at the protein level, thus producing a significant increase in the proportion of “mismatched” fibers. This finding may reflect the existence of an incomplete transitional process because of the much longer turnover of the MHC
isoproteins compared with that of the corresponding transcripts, as well as the slow incorporation of the newly formed isoproteins into the myofibrils (3). If this interpretation is correct, one could predict that MHC isoform changes at the protein level would be detectable after longer periods of bed rest.

Bed rest compared with spaceflight. Bed rest has been proposed as the best ground-based model to simulate the microgravity experienced by astronauts during spaceflight (14). The few studies conducted examining fiber-type composition by ATPase histochemistry (18, 24) or both ATPase histochemistry and SDS-PAGE for MHC isoforms (8) all indicate that no significant changes occur after 5–6 wk of bed rest. These results are confirmed by the present data, showing no change in fiber type, when these are evaluated by ATPase histochemistry and immunocytochemistry. The most detailed results dealing with the effects of spaceflight on MHC expression in human skeletal muscle give some indications that exposure to microgravity in as short a time period as 11 days induced a significant decrease in type 1 fibers although the global MHC profile was not changed significantly (16, 37), thus indicating that microgravity in spaceflight can be a somewhat more potent unloading stimulus than the ground-based bed-rest model. This is to some extent confirmed by animal studies in which changes in MHC expression have been recorded after only 6 days of spaceflight (13). Similarly, additional studies of rat muscle exposed to either 14 days of spaceflight or 14 days of hindlimb suspension indicate that spaceflight initiates a fiber-type transition faster than do the ground-based unloading models (26, 30). In humans, substantial evidence of this assumption still needs to be confirmed by data from more extended periods (5–6 wk) of exposure to spaceflight. It should be stressed that there are important differences between the situation of spaceflight and the bed-rest model, both with respect to general conditions, e.g., stress, that may affect muscle properties, and with respect to muscle activity per se, e.g., the astronauts’ performance of long daily exercises but with contractions that are almost completely unloaded.

Transcriptional or posttranscriptional regulation of MHC expression. It might be somewhat surprising that protein changes are not detected after 5 wk of bed rest. An alternative interpretation explaining this rigidity in MHC expression at the protein level could be that MHC isoforms are mainly or partly regulated at the posttranscriptional level. For a posttranscriptional transition-controlled process to occur, this would mean that, e.g., a commencing transition from a phenotypic type 1 to phenotypic type 2 fiber; i.e., a fiber with only MHC-1 protein present, would have to be positive for both mRNA for β/Slow and 2A but for some reason would only translate the β/Slow mRNA, and not the 2A mRNA, into protein, whereas a fiber containing only MHC-1 protein but negative for β/Slow mRNA (and positive for mRNA for 2A or 2X or both) would be transcriptionally controlled. Because we frequently find fibers of this latter phenotype in the post-bed-rest biopsies, our results seem to support the notion that MHC isoform expression in adult human skeletal muscle is transcriptionally controlled, although some form of posttranscriptional modulation cannot be excluded.

Atypical type 1 fibers in post-bed-rest biopsies. The present data suggest that the shift in MHC mRNA and protein isoform expression induced by the deactivation of the muscles is more easily evoked between some isoforms than between others. This could be called the “responsiveness” of the muscle fibers to changes in stimuli going to the muscle fibers. The concept of responsiveness may be taken beyond the simplistic assumption that the conversion of phenotypes is progressive, following an MHC-1 → MHC-2A → MHC-2X pathway. In a recent study, Talmadge et al. (34) observed that 30 days after a spinal cord transection a smaller number of fibers or myonuclei in the rat soleus muscle apparently have the ability to jump the phase of MHC-2A expression and convert directly from expres-

Table 2. Fiber-type distribution before and after bed rest, on the basis of in situ hybridization for MHC transcripts

<table>
<thead>
<tr>
<th></th>
<th>Β/Slow</th>
<th>Β/Slow + 2A</th>
<th>2A</th>
<th>2A + 2X</th>
<th>2X</th>
<th>Β/Slow + 2A + 2X</th>
<th>Β/Slow + 2X</th>
<th>Negativet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before bed rest</td>
<td>53.2±5.1</td>
<td>0.6±0.4</td>
<td>31.7±5.3</td>
<td>10.0±2.1</td>
<td>4.5±1.5</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>After bed rest</td>
<td>40.2±4.8</td>
<td>2.7±1.3</td>
<td>19.1±4.3</td>
<td>15.8±2.4</td>
<td>19.1±4.1</td>
<td>0.2±0.2</td>
<td>0.5±0.5</td>
<td>2.4±1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. *Significant difference (P < 0.05) between before and after bed-rest values. †Fibers negative for all 3 MHC transcripts (β/Slow, 2A, and 2X).

Table 3. Fiber types and transitional fibers before and after bed rest on the basis of combined analysis for MHC isoprotein and transcripts

<table>
<thead>
<tr>
<th></th>
<th>Type 1</th>
<th>Type 2A</th>
<th>Type 2X</th>
<th>Type 1/2A</th>
<th>Type 2A/2X</th>
<th>1→2A</th>
<th>2A→1</th>
<th>2A→2X</th>
<th>2X→2A</th>
<th>Jumps Fibert</th>
<th>Negativet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before bed rest</td>
<td>53.2±5.1</td>
<td>26.5±4.3</td>
<td>3.8±1.5</td>
<td>0.2±0.2</td>
<td>3.3±2.4</td>
<td>1.1±0.5</td>
<td>0±0</td>
<td>1.7±0.6</td>
<td>10.2±2.1</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>After bed rest</td>
<td>40.2±4.8</td>
<td>16.3±3.8</td>
<td>9.1±2.6</td>
<td>0±0</td>
<td>3.6±0.9</td>
<td>4.5±1.7</td>
<td>0±0</td>
<td>18.3±3.2</td>
<td>16.1±1.0</td>
<td>4.0±1.9</td>
<td>2.4±1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. *Significant difference (P < 0.05) between before and after bed-rest values. †Fibers only positive for MHC-1 at protein level but positive for 2X transcript. We interpret these fibers as transitional fibers, changing rapidly in direction 1→2A→2X. For further description of the combined analysis for MHC isoprotein and transcripts, see Ref. 3. For detailed explanation of Table 3, see RESULTS and Fig. 1.
ing MHC-1 to expressing MHC-2X. The finding of the present study that a small number of fibers post-bed rest only contain MHC-1 at the protein level, but express only mRNA for 2X or mRNA for β/slow and 2X (Fig. 1), might indicate a conversion from type 1 to type 2X fibers with only a transient type 2A phase or even without any type 2A phase. From both the observations of Talmagde et al. and the present study, it is evident that a considerable number of muscle fibers will continue to express the MHC isoform that they expressed before intervention, thus suggesting that there might be a limit to the plasticity of the MHC expression in certain fibers (10). It has been suggested that the reason why apparently analogous slow fibers within the same muscle respond very differently to the same unloading stimulus has to do with differences in developmental pathways (10). This seems reasonable, at least when normal physiological increases or decreases in muscle activity are considered. Although, for example, paralysis of human vastus lateralis muscle after a spinal cord injury eventually will lead to a situation in which the β/slow gene is nearly or completely turned off, such muscles as soleus and tibialis anterior in the same subjects will maintain at least some expression of β/slow, suggesting that the responsiveness of type 1 fibers is related to not only developmental history but also muscle origin (Ref. 2; Andersen, unpublished data). A minor number of fibers were found to be nonreactive with all of the three mRNA probes (β/slow, 2A, and 2X). All of these fibers contained solely MHC-1 protein. In theory, these negative fibers could express 1) neonatal or embryonic mRNA for MHC, 2) no mRNA for myosin, or 3) a presently unidentified MHC mRNA. The negative fibers were
found to be nonreactive with antibodies for embryonic MHC and neonatal MHC (data not shown). Furthermore, we have on a previous occasion observed corresponding fibers in a vastus lateralis muscle biopsy from a normal young subject recovering from heavy resistance training, and, on several occasions, in biopsies from very elderly subjects (J. L. Andersen, V. Smerdu, and S. Schiaffino, unpublished observations). On all of these earlier occasions these negative type 1 fibers were also nonreactive with mRNA probes for embryonic MHC, neonatal MHC, and MHC α-cardiac. If these negative fibers do not express any mRNA for MHC, one would believe that they have been transcriptionally “silent” for at least 2 days before the biopsy because the turnover time for MHC mRNA can be estimated to be in the range of 2–3 days (29). Another explanation for this phenomenon could be that some myonuclei within these fibers, because of the unloading of the muscle, temporally pass through a phase of lethargy. What seems to contradict this theory is the fact that these fibers could be confirmed negative in further serial sections in the same biopsy sample. Similarly, we have observed that these negative fibers are not more frequent in muscle biopsies from patients with paralysis caused by spinal cord injury, leading to a more permanent non-weight-bearing state (Andersen, unpublished observations).

Animal models of unloading skeletal muscle indicate that the magnitude of atrophy is more severe in type 1 than in type 2 fibers (25, 31, 35). Similarly, in unloading of animal muscle it appears that antigravity muscles, e.g., soleus, are more affected by atrophy than are muscles primarily used for locomotor and burst activity (10). This more pronounced atrophy of type 1 than of type 2 fibers after bed rest is at least partly confirmed in some human bed-rest studies (8, 18). If this apparently increased atrophy in type 1 fibers compared with type 2 fibers holds true, it could explain why some phenotypic type 1 fibers, as observed in the present study, have no expression of any mRNA for MHC after bed rest. This will leave them with no opportunity to substitute wasted MHC, leading to a slow, imperceptible atrophy of the type 1 fibers, at least in this particular period. Our data could not confirm a more pronounced atrophy of the type 1 fibers compared with the type 2 fibers. Finally, these phenotypic type 1 fibers could express some yet unidentified (assumed) MHC-1 isoform. In support of this view, an additional MHC-1 has been postulated to occur in both developmental and adult mammalian skeletal muscle in general (32). Similarly, two very recent papers give a more detailed description of the existence of two different MHC 1 isoforms in rabbit skeletal muscle (19, 22).

In conclusion, the present study is, to our knowledge, the first to demonstrate that non-weight bearing of human skeletal muscle will cause a smaller percentage of the type 1 fibers to rapidly switch to expression of mRNA for MHC-2X or pass through an apparent period of lethargy, during which no mRNA for the three major MHC isoforms is transcribed. Furthermore, the apparent upregulation of the gene expression of the 2X isoform, leading to an increased amount of mismatched fibers, which indicates a probable transition toward increased expression of MHC-2X, was not completed at the end of the experimental period. It remains to be seen whether a complete change in phenotype will occur in these apparently transitional fibers if the bed-rest period is extended further. It can, nevertheless, be concluded that, although 5 wk of bed rest are not enough to evoke a significant change in MHC isoform expression at the protein level, 5 wk will induce a shift in gene expression, leading to a relative increase in the transcription of mRNA for MHC-2X.

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