Qualitative and quantitative adaptations of muscle fibers and muscle protein pattern to 35-days bed rest

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Abstract

Ten healthy sedentary subjects underwent 35-days bed rest (BR) and needle biopsy samples of the vastus lateralis muscle were collected pre-BR and post-BR. One portion of the biopsy was glycerinated and used to dissect individual muscle fibers, which were studied for measuring fiber size, myosin content and myosin actin ratio by quantitative electrophoresis. Another portion of the biopsy was immediately frozen and used to determine myosin heavy chain (MHC) isoform distribution and to perform proteomic analysis by two-dimensional gel electrophoresis. As expected on the basis of previous findings, muscle fibers were found to go through a significant degree of atrophy. Myosin concentration was found to be lower post-BR than pre-BR in individual muscle fibers, whereas in the same fibers myosin actin ratio was unchanged. The latter findings indicate a disproportionate loss of myosin with respect to fiber CSA and a proportional loss of myosin and actin, suggesting a decrease in myofibrillar density within the fibers. MHC isoform distribution showed a shift in the direction MHC-1 → MHC-2A → MHC-2X as expected mainly on the basis of previous findings in rat models of disuse. The proteomic analysis identified several differentially expressed proteins post-BR, which mainly belonged to antioxidant defense systems and energy metabolism. The antioxidant defense systems were down-regulated suggesting that oxidative stress could occur in disused human muscle as previously showed in rat models. Both an oxidative and four glycolytic enzymes were down-regulated post-BR suggesting a general downsizing of energy metabolism.

Key Words: bed rest, muscle fibers, muscle atrophy, myosin content, proteomic analysis

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Head down bed rest, which is the most widely used model of microgravity, causes several systemic adaptations which resemble those occurring during spaceflight: upward fluid shifts, reduced energy requirements, decreased sensory stimulation, cardiovascular changes and adaptations of the locomotor system. Interestingly, bed rest is a very frequent condition being still used during medical therapy. In fact, although it is now fully understood that long periods in bed can have adverse effects, they are unavoidable in some patients [54]. The relevance of bed rest studies, therefore, goes far beyond the developing of approaches to enable astronauts to withstand long duration spaceflights.

The adaptations of the locomotor system to bed rest (BR), and in general disuse are the most important and have been extensively studied. Muscle mass is known to decrease following BR [1,25,34,36], and a disproportionate loss of force with respect to size has been observed determining a loss of specific force [11,28,34,36]. Besides quantitative adaptations, i.e. adaptations in muscle mass, disuse atrophy is generally believed to determine qualitative adaptations such as a shift in the contractile and metabolic phenotype towards that of a fast-twitch muscle. A slow to fast shift in fiber type or myosin heavy chain isoform expression and an oxidative → glycolytic shift in energy metabolism have been, in fact, observed [5-7,26,34].

However, notwithstanding the large amount of work devoted to the analysis of disuse muscle atrophy, mostly through bed rest, but also using other models such as lower limb suspension, immobilization and spaceflight itself, several open questions still remain. The slow to fast shift in fiber type has been rather well documented in small mammals [8,34,67], but information are less and not fully consistent in humans [28,30,42,72]. Even more scanty information is available on the impact of disuse on the metabolic profile of human muscles. It is, in fact, unclear whether an oxidative → glycolytic shift in metabolic enzymes actually occurs [30,34]. It is also unclear whether in

disuse atrophy the decrease in myofibrillar protein content is proportional to the decrease in muscle mass. The latter issue is relevant as a disproportionate loss in contractile proteins could account for the lower specific force of muscles [11] and individual muscle fibers [46,70,72] observed in several studies following disuse. Very limited information on protein content was available in humans till a recent work, which suggested no change in myosin concentration in whole vastus lateralis muscle following bed rest and unilateral hind limb suspension [41]. Finally, no information is available on whether oxidative stress, i.e. an unbalance between the cellular antioxidant systems and free radical (ROS) production, actually occurs in disused human muscles [10]. The issue is relevant. Several findings [47,48,56,61] have, in fact, suggested that oxidative stress have a pivotal role in determining the unbalance between protein synthesis and degradation ultimately bringing to disuse atrophy [14,16,37,69].

In this study the above open questions have been addressed taking advantage of the organization of a bed rest campaign by the OSMA (osteoporosis and muscle atrophy) project supported by the Italian Space Agency (ASI).

Ten subjects were subjected to 5 weeks bed rest and needle biopsy samples were taken from their vastus lateralis muscle pre-BR and post-BR. The biopsy sample was divided in several portions, which were used by several OSMA research groups. Two portions, one frozen and stored and -80 °C and the other glycerinated and stored at -20 °C were used for this study. The glycerinated samples were used for dissection of individual muscle fibers whose cross sectional area, and myosin and actin concentration were precisely determined. The frozen samples were used for the analysis of myosin heavy chain isoform distribution, which is a very precise index of muscle contractile phenotype, and for proteomic analysis. The proteomic analysis appears particularly suited to address the complexity of phenotype adaptations as by using two dimensional electrophoresis can separates hundreds of proteins providing a very comprehensive picture of the protein pattern of a muscle sample. In this study the proteomic approach, by comparing 2D maps pre-BR and post-BR, enabled to identify the differentially expressed proteins, which can be considered the targets of the disuse process, in a very large pool of proteins (~800)

Materials and Methods

Subjects

The bed rest campaign was organized by the research group OSMA (Osteoporosis and Muscle Atrophy), supported by the Italian Space Agency (ASI), at the Orthopedic Hospital of Valdoltra (Slovenia) in July 2007. Ten healthy men (age=24±1 years; body weight=78±3), which did not have a previous history of neuromuscular or cardiac diseases, gave written, informed consent to participate in the study. The study was approved by the ethical committee of the University of Ljubljana and conformed to the principles of the Declaration of Helsinki on human experimentation.

Muscle biopsies

Needle biopsies were taken from the vastus lateralis muscle the day before starting BR according to Bergstrom [12]. Muscle samples were divided in several portions.

Two such portions were used for this study. One portion was immediately frozen in liquid nitrogen and used for analysis of myosin heavy chain (MHC) isoform distribution and for proteomic analysis. One portion was divided in smaller bundles, stored at -20°C in skinning solution plus 50% glycerol and used for dissection of individual muscle fibers. Single muscle fibers were used for determination of CSA and myosin content. The amount of material available did not enable all analyses to be performed in all subjects. The sample of one subject was only viable pre-BR and therefore the subject was discarded. MHC isoform distribution was determined in the remainder 9 subjects. Single muscle fiber CSA and myosin content was determined in 7 out of the 9 subjects. Proteomic analysis was performed in 5 of the 9 subjects.

Determination of cross sectional area and myosin concentration of individual muscle fibers

MHC isoforms were separated using an approach based on densitometry of SDS-PAGE myosin bands, stained by Coomassie blue, which has been routinely used in our laboratory for years [18,22,40].

Proteomic analysis

The proteomic analysis was performed using a previously described approach [19].

Sample preparation

Muscle samples, dissected and frozen in liquid nitrogen, were pulverized in a small steel mortar and immediately suspended in a lysis buffer [19]. After the incubation with DNAase and RNAase and the determination of sample protein concentration (with 2D Quant kit protein assay; GE Healthcare), equal protein quantities from each biopsy sample were pooled to obtain 3 repetitions of 2D-electrophoresis gels. Isoelectrofocusing was carried out by the IPGphor system (Ettan IPGphor isoelectric focusing system; GE Healthcare) using immobilized pH gradient (IPG) gel strips, 13 cm, pH 3–11 nonlinear rehydrated for 14 hours, at 30 V and 20°C, in 250 μL of reswelling

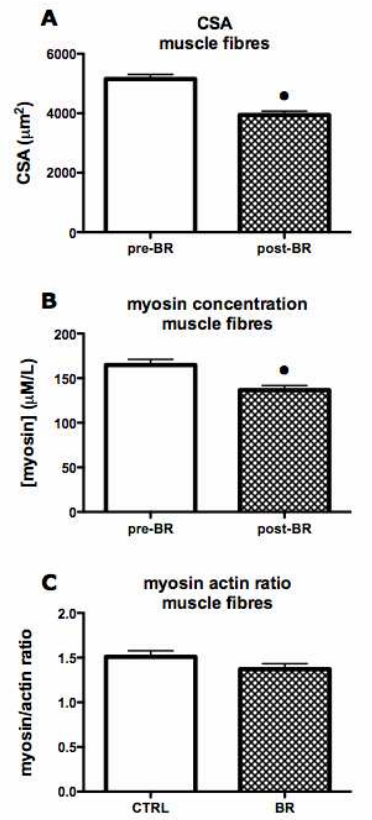

Fig. 1 *Quantitative adaptations of individual muscle fibres to five weeks bed rest. (A) mean values (±S.E.M.) of cross sectional area (CSA) of individual muscle fibres (n=140 pre-BR and n=138 post-BR) dissected from biopsy samples of 7 subjects; (B) mean values (±S.E.M.) of myosin concentration of the same fibres as in panel A determined by quantitative electrophoresis; (C) myosin actin ratio of the same fibres of panel A and B determined by densitometry of myosin and actin bands in the same gels used for myosin quantification.*

buffer [19]. Strips were loaded with 150 µg of protein sample and focused at 20,000 Volt/hour (Vhr), at a constant temperature of 20°C. After isoelectrofocusing, the strips were equilibrated for 10–12 minutes in 5 mL of equilibration buffer [19] and finally applied to 15% acrylamide gels without a stacking gel. The separation was performed at 70 V for 18 hours at room temperature. The 2D gels were fixed and stained with a fluorescent stain (flamingo by Biorad).

Quantitative analysis of protein pattern.

Triplicate gels of each sample were visualized using a Typhoon laser scanner (GE Healthcare) and analyzed with Platinum Software (GE Healthcare). The software provides normalized volume for each spot (representing protein amount); the volumes of each spots in the triplicate gels are averaged and spots

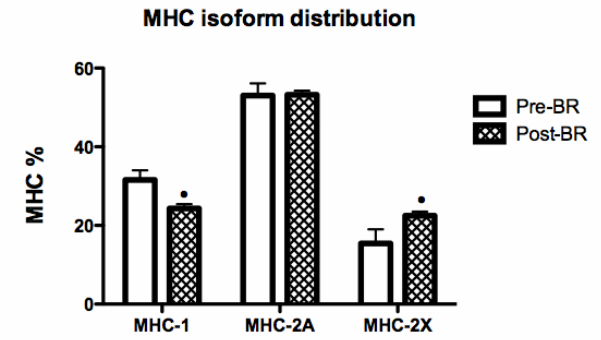

Fig. 2 *Myosin heavy chain (MHC) isoform distribution of biopsy samples from 9 subjects pre-BR and post-BR. MHC distribution was determined by densitometry of MHC bands in SDS-PAGE gels.*

statistically changed were obtained ($P < 0.05$). The average volumes of each differentially expressed spot are used to determine the volume ratios reported in the figures and tables.

Protein identification.

Electrophoresis fractionation and in situ *digestion*

For protein identification, 2D gels were loaded with 300 µg of proteins *per strip*; the electrophoretic conditions were the same described above. After staining with Colloidal Comassie spots were excised from the gel and washed in 50mM ammonium bicarbonate pH 8.0 in 50% acetonitrile to a complete destaining. The gel pieces were treated to obtain the peptide mixtures [19].

MALDI MS analysis

The peptide samples were analyzed with an Applied Biosystem Voyager DE-PRO mass spectrometer equipped with a reflectron analyser and the mass signals provided were then used for database searching using the MASCOT peptide fingerprinting search program (Matrix Science, Boston, USA) available on the net.

Statistical analysis

Data were expressed as mean ± S.E.M. Statistical significance of the differences between means was assessed by Student's t-test. A probability of less than 5% was considered significant ($p<0.05$).

Results and Discussion

1. Bed rest and muscle fiber atrophy

Fig 1A shows the mean values of cross sectional area (CSA) of individual muscle fibers dissected from biopsy samples of 7 subjects pre- and post-bed rest (BR). A total of 140 individual muscle fibers pre-BR and 138 fibers post-BR were dissected and their CSAs were very precisely determined using the approach described in the methods. Mean CSA was significantly lower post-BR than pre-BR. Both BR and spaceflight

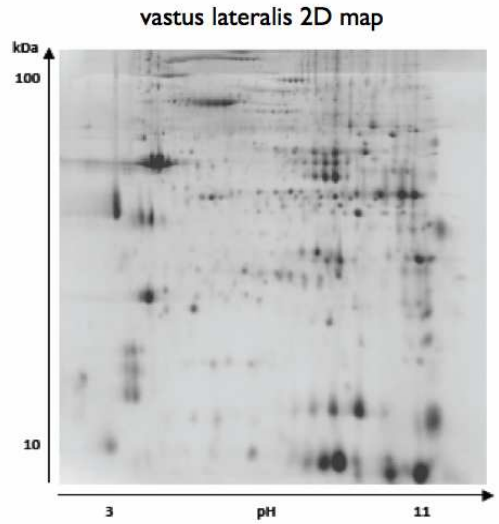

Fig. 3 *A representative 2D proteomic map of vastus lateralis muscle pre-BR. 761 spots were found to be common to 2D maps pre-BR and post-BR and were compared.*

are well known to determine muscle atrophy [25,34]. However, the extent of muscle fiber atrophy was found to vary from 9% to 16% according to the duration of disuse, and to the muscle (soleus, vastus lateralis, gastrocnemius) and muscle fiber type considered. Type 1 fibers went through a loss of CSA of 9% in soleus following 17 days spaceflight [72], 6% in soleus following 17 days BR [73] and 16% in vastus lateralis following 84 days BR [70]. Type 2A fibers went through a 9% decrease in CSA following 84 days BR [70]. The degree of muscle fiber atrophy (~23%) observed in this study is, therefore, among the largest observed so far and can be partially explained by the duration of BR (5 weeks). In this study muscle fiber type has not been identified and the values reported refer to a pool of different fiber types, which was very likely composed of mostly type 1 and 2A fibers given the MHC isoform distribution of the samples (Fig 2).

2. Bed Rest and myofibrillar protein concentration

The same fibers, whose mean CSA values are reported in Fig 1A, were individually subjected to quantitative electrophoresis to determine myosin concentration. Fig 1B shows that the mean value of myosin concentration in individual muscle fibers was significantly lower (~17%) post-BR than pre-BR. The latter is the first evidence of a disproportionate loss of myosin with respect to CSA in individual muscle fibers following disuse in young subjects. Interestingly, specific force of muscle fibers has been mostly found to be lower not only in small mammals following hindlimb suspension [63], but also in humans following BR [46,70] and spaceflight [72]. The observation of a lower myosin concentration in individual muscle fibers following BR could account

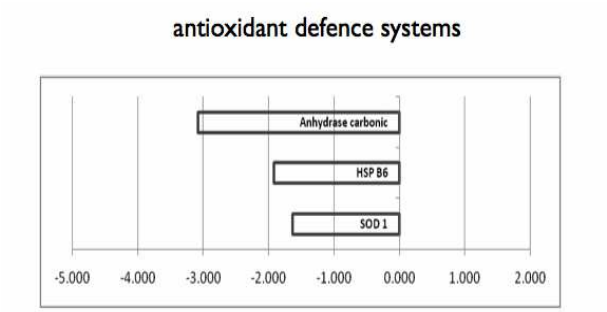

Fig. 4 *Histogram of volume ratios of differentially expressed proteins belonging to antioxidant defence systems in vastus lateralis (VL) muscle post-BR in comparison to pre-BR. The numbers on the x axis indicate the ratio between the average volume of a given protein expressed in VL pre-BR and the average volume of the same protein post-BR. Positive numbers (on the right) indicate up-regulation of a protein following BR, whereas negative numbers (on the left) indicate down-regulation. Analysis could be performed on samples from 5 of the 9 subjects*

for such, so far unexplained, findings. In fact, information on protein content was contradictory in small mammals, showing either no change [39] or a significant decrease [9] and almost absent in humans till a very recent study [41]. The latter study has shown no myosin loss following either 90 days BR or 35 days unilateral lower limb suspension in whole muscle samples from vastus lateralis and soleus muscles [41]. The discrepancy could depend on the different experimental approach used or on variability in the response to disuse of different populations of subjects. In this study myosin determination was performed on individual muscle fibers using an approach, which requires few, although very precise, determinations. In the study by Haus et al. [41] myosin determination was performed on whole muscle samples using a more complex procedure requiring several subsequent steps to obtain myofibrillar protein fraction to load in SDS-PAGE gels. Densitometry was applied to myosin bands, which were silver stained, and not Coomassie stained as in this study. It should be noted that a disproportionate loss of myosin has been also observed in atrophic muscle fibers of elderly subjects: myosin concentration was found to be lower and a linear relation was observed between myosin concentration and specific force [23].

Several evidences have suggested that in disuse atrophy a disproportionate loss of actin filaments occurs and that such phenomenon can determine atrophy induced increase in unloaded shortening velocity of individual muscle fibers. In keeping with the latter observations we hypothesized that the myosin

actin ratio would be increased following BR. The availability of gels to determine myosin concentration enabled to assess myosin actin ratio (M/A ratio) by densitometry in each single muscle fibers analyzed as regard CSA and myosin content. Fig 1C shows that mean M/A ratio was not different pre-BR and post-BR indicating that no preferential loss of thin filaments could occur. The latter finding suggests a decrease in the number of sarcomere in parallel in the CSA of muscle fibers and does not support the idea of an altered ratio between thin and thick filaments within sarcomeres. The discrepancy could depend on the technique used, on the duration of disuse and on the muscle and species considered. A disproportionate loss of thin filaments was observed by electron microscopy [58-60]. In humans, the duration of unloading was much shorter, namely 17 days, both in BR [59] and spaceflight [60] studies and the muscle analyzed was different (soleus vs vastus lateralis in this study). In rat, duration was also shorter (14 days) and loss of thin filaments was found in soleus and not in adductor longus muscle notwithstanding that both muscles underwent significant atrophy [58]. A possible way to reconcile the different findings is to hypothesize that myosin and actin are lost at different rates and that after 5 weeks of disuse the extent of myosin and actin loss is similar, whereas after 2 weeks actin loss overcomes myosin loss.

3. Bed rest and muscle phenotype

3.1. Adaptations in myosin heavy chain isoform distribution

Fig 2 shows the average myosin heavy chain (MHC) isoform distribution pre- and post-BR of the biopsy samples of vastus lateralis muscle from 9 subjects. The MHC-1 content was significantly lower post-BR (24%) than pre-BR (31%), whereas the MHC-2X content was significantly higher post-BR (22%) than pre-BR (15%). No change was observed in the MHC-2A content (53% pre-BR and post-BR). The latter findings indicate a MHC-1 → MHC-2A → MHC-2X shift in MHC expression consistently with the generally accepted idea that disuse causes a slow to fast shift in muscle phenotype [6,7,26]. The latter belief is mainly based on experiments on rodents (mice and rats) [34], whereas evidences in humans were less numerous and more scanty [32]. A decrease in the relative content of type 1 fibers and an increase in the relative content of hybrid (type 2AX and type 1-2AX) fibers have been observed following long duration (84 days) BR [70]. However, a much less clear and subject dependent slow to fast shift in fiber type distribution has been observed following 17 days spaceflight [72] and no increase in type 2X fibers was observed following 11 days spaceflight [30]. The clear slow to fast shift observed in this study could be due to several causes. The duration of bed rest (5 weeks) was longer than in most other studies in which fiber type distribution was determined. The number of subjects was also rather large for this kind of studies (n=9) and enabled a more precise and statistically relevant analysis. Care was taken to standardize the subjects as regards age (24±1) and nutrition during BR. Finally, the approach used to assess MHC isoform distribution, i.e. densitometric analysis of MHC bands in SDS-PAGE gels, provides a very precise assessment of MHC isoform expression of the whole muscle sample being not affected by a possible bias due to the dissection of individual muscle fibers from the samples [70,72] or to the histological analysis of a small portion of the sample [30]. Interestingly, so far the clearest example of a slow to fast shift in muscle phenotype comes from a study in which the knee of a large number of subjects (n=48) have been strictly immobilized for 3 weeks and muscle phenotype was assessed by both fiber type distribution and analysis of mRNA for MHC isoforms [42].

3.2. Adaptations in protein pattern

Proteomic maps of vastus lateralis (VL) muscles of 5 subjects pre-BR and of the same subjects post-BR were obtained using 2D gel electrophoresis as described in the Methods. Subjects were not analyzed individually, but equal amounts of protein from the biopsy sample of each subject were pooled together to obtain pools, which equally represented all subjects pre-BR and post-BR. Figure 3 shows an example of a VL 2D map pre-BR.

A large number of spots (n=761) were found to be present in both VL samples pre-BR and post-BR and were compared. The percent of differentially expressed spots was small, 3.95% (n=30). Of the 30 spots found to be differentially expressed, 5 were up-regulated and 25 down-regulated. So far, eleven of such spots could be identified by MALDI-Tof (Fig. 4-6). Identified proteins were grouped on the basis of their functional role in the following categories: antioxidant defense systems; energy production systems (glycolytic metabolism, oxidative metabolism); other proteins.

Antioxidant defense systems

Three proteins involved in cellular defense systems against reactive oxygen species or free radicals (ROS), superoxide dismutase (Zn/Cu SOD), carbonic anhydrase 3 (CAH III) and Heat Shock Protein B6 (HspB6), were down-regulated following BR (Fig. 4). Zn/Cu SOD catalyzes the dismutation of superoxide (a free radical) in hydrogen peroxide which is converted into water by catalase, peroxiredoxin and glutathione peroxidase. A lower content in Zn/Cu SOD following BR suggests a lower capacity to buffer free radicals. CAH III protects against oxidative damage by binding free radicals [20]. Hsps work downstream of the other antioxidant defense systems by removing products caused by free radicals formation [50] and protecting cell against oxidative stress [48]. Therefore, collectively, the adaptations observed are consistent with an impairment of the antioxidant defense systems

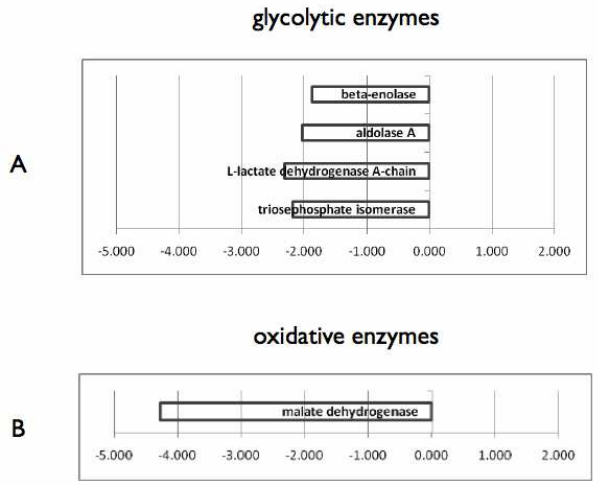

Fig. 5 *Histogram of volume ratios of differentially expressed metabolic enzymes in vastus lateralis (VL) muscle post-BR in comparison to pre-BR. (A) glycolytic enzymes; (B) oxidative enzymes. The same subjects and the same approach to represent differentially expressed proteins was used as in Fig 4.*

following BR in VL of humans and suggests that oxidative stress could occur in disused human skeletal muscle.

Disuse atrophy is widely believed to depend on an unbalance between protein synthesis and degradation [14,16,37,69]. Oxidative stress, due to an unbalance between the cellular antioxidant systems and free radical (ROS) production, has been indicated as a major trigger of the latter phenomenon [47,48,56,61]. Accordingly, amelioration of disuse muscle atrophy following antioxidant administration has been observed [3,4,43]. Interestingly, a pivotal role is now widely recognized to oxidative stress in the pathogenesis of muscle wasting not only in disuse, but also in a variety of pathologic conditions [52]. However, most studies so far had been performed in small mammals and it was still unknown whether similar phenomena occurred in disused human muscle [10].

Energy production systems

Fig 5 shows the differentially expressed glycolytic and oxidative enzymes. Malate dehydrogenase, a major oxidative enzyme and four glycolytic enzymes (triosephosphate isomerase, L-lactate dehydrogenase A-chain, aldolase A and beta-enolase) were 2-4 fold down-regulated following BR. The lower content in the oxidative enzyme is consistent with the slow to fast shift in MHC isoform expression as type 1 fibers are known to rely mostly on oxidative metabolism, whereas type 2X fibers are mostly glycolytic [53,66,71]. The lower content in oxidative enzymes might also be partially independent from a fiber type shift as a change in the metabolic profile of slow fibers

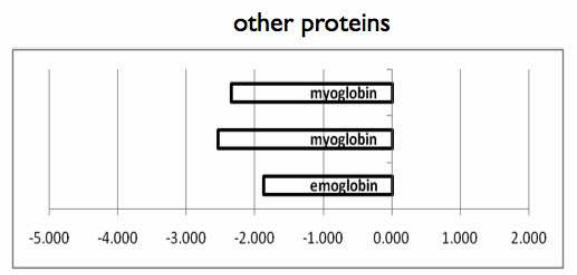

Fig. 6 *Histogram of volume ratios of differentially expressed "other proteins" in vastus lateralis (VL) muscle post-BR in comparison to pre-BR. The same subjects and the same approach to represent differentially expressed proteins was used as in Fig 4 and 5.*

towards that of fast fibers has been observed in rat [38]. A down-regulation of oxidative metabolism following disuse is consistent with previous observations in rats and is a widely recognized phenomenon in disuse atrophy [15,35,64]. On the contrary, the lower content in glycolytic enzymes cannot find an explanation in the slow to fast shift in MHC isoform expression and is not in agreement with their higher activity observed in rat following HU or space flight [33,34,64]. However, it should be underlined that much less information is available on the adaptations of metabolic enzymes to disuse in humans than in rat and that, in agreement with the present data, a very recent study has shown a down-regulation of glycolytic enzymes following 4-11 days immobilization in humans by microarrays analysis [21]. Moreover, Edgerton et al. [30] found no change in enzymes involved in oxidative metabolisms (succinic dehydrogenase and glycerol 3-phosphate dehydrogenase) in human VL muscle following 11 days spaceflight. Altogether the adaptations of metabolic enzymes observed in this study do not confirm in humans the disuse induced oxidative → glycolytic shift observed in rat. On the contrary the present findings suggest a general downsizing of energy metabolism. It can be hypothesized that the latter might not be just a mere consequence of muscle atrophy, but could concur to its pathogenesis.

Several evidences, in fact, support a potential role of impaired energy metabolism in the pathogenesis of disuse atrophy. Two recent gene expression studies one in mice [24] and one in humans [21] also indicated a general downsizing of energy metabolism. Moreover, a general impairment in energy production was also observed in a number of pathologic conditions in which muscle wasting occurs such as muscular dystrophy [55], cancer, fasting, diabetes, uremia [49] and in burn sepsis [29]. A general impairment of energy production could decrease the rate of protein synthesis, as the latter is an ATP-requiring process.

Interestingly, the decrease of protein synthesis rate, which is later on followed by an increase in protein degradation, is an early major phenomenon underlying the unbalance between protein anabolism and catabolism in disuse both in rat [68] and humans [13,31,62]. Moreover, AMPK, a cellular sensor of energy balance, has been suggested to regulate myofiber size as its inhibition counteracted myofiber atrophy following energy unbalance [2]. Consistently with their potential pathogenetic role, metabolic adaptations were found to precede muscle atrophy and fiber type shift in rat SOL [45], a phenomenon observed also in disused heart [65].

Other proteins

Two spots identified as myoglobin and one identified as hemoglobin were found to be significantly down-regulated following BR (Fig 6). The lower content in myoglobin following BR could be at least partially accounted for by the slow to fast shift in MHC isoforms as type 1, slow fibers have higher myoglobin content than fast fibers [74]. Myoglobin is not just a short-term oxygen reservoir, but also plays critical roles in energy metabolism by directly stimulating oxidative phosphorylation and by linking blood oxygen supply to mitochondria, facilitating oxygen diffusion within the cell [51]. The adaptation in myoglobin expression could, therefore, contribute to the impaired oxidative metabolism (Fig 4B) through down-regulation of oxidative phosphorylation and mitochondrial number.

Hemoglobin found in our proteomic maps come from capillaries and very small blood vessels comprised in the biopsy. Its lower content following BR (Fig 6) finds a possible explanation in the lower muscle vascularization that has been suggested to occur following disuse at least in rat [27].

Summary and conclusions

Five weeks bed rest determined both quantitative and qualitative adaptations in vastus lateralis muscle of the young, healthy subjects enrolled in the study.

The quantitative changes comprised muscle fiber atrophy (~23%) and a disproportionate loss of myosin and actin, the major contractile proteins of the sarcomere, with respect to muscle fiber size.

Muscle fiber atrophy, which was largely expected on the basis of previous findings [6,28,34,57], indicates that BR successfully induced disuse in the vastus lateralis muscle of the subjects enrolled in the study.

The observation that the content in myosin and actin decreased more than muscle fiber size determining a decrease in myosin and actin concentration within individual muscle fibers is a novel observation in disuse muscle of young healthy subjects. It could account for the loss of specific force generally observed in single muscle fibers following disuse, which had remained so far unexplained.

The qualitative changes comprised a slow to fast shift in MHC isoform content and complex changes in proteins of the antioxidant defense systems and in metabolic enzymes. A shift in the contractile and metabolic phenotype towards that of a fast-twitch muscle is generally believed to occur in disuse [5-7,26]. However, in humans a clear slow to fast shift of fiber type or of MHC isoforms had not been invariably observed. The present study confirms and strengthens the idea that a clear slow to fast shift actually occurs not only in small mammals, but also in humans, provided that the duration of disuse and the approach used to determine phenotype adaptations are appropriate. Moreover, the present study does not suggest an oxidative → glycolytic shift, but a general downsizing of energy metabolism prompting the hypothesis that it could play a role in the pathogenesis of disuse atrophy.

Oxidative stress is widely considered a major trigger of disuse atrophy mostly based on experiments on small mammals [47,48,56,61]. On the contrary, evidences that such phenomenon could be involved in disuse atrophy in humans were scanty [10]. This study does support the idea of a potential role of oxidative stress in disuse atrophy in humans. However, the nature of this study does not enable to establish whether oxidative stress is a consequence or a cause of muscle atrophy.

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