Bed rest reduces metabolic protein content and abolishes exercise-induced mRNA responses in human skeletal muscle

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The oxidative capacity of skeletal muscle contributes to determining the ability of skeletal muscle to oxidize both carbohydrate and fat. Changes in skeletal muscle oxidative capacity can thus influence whole body metabolism (25). The plasticity of human skeletal muscle oxidative capacity is evident by the increased content and activity of oxidative enzymes/proteins and increased capillarization with exercise training and a corresponding decrease during detraining (16, 17, 19). Training-induced skeletal muscle protein adaptations are thought at least in part to originate from cumulative effects of transient increases in gene expression in response to each single exercise bout (46), as shown in human skeletal muscle for both metabolically related proteins (29, 40, 41, 44) and the angiogenic protein vascular endothelial growth factor (VEGF) (18, 21). Increased physical activity has been shown to reduce the exercise-induced responses of mRNA’s encoding metabolic (41) and angiogenic (21) proteins, but an increased peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA response to an acute exercise bout was evident after 4 wk of one-legged knee extensor exercise training (41), potentially reflecting a sensitizing effect of training.

Exercise training-induced changes in the protein content may also occur without any changes in transcription, for example, through regulation mediated by microRNAs (miRs). miR-1, miR-29b, and miR-133a have been shown to be implicated in myogenesis (45). Acute exercise has been reported to decrease and increase the miR-1 and miR-23 content, respectively, in mouse skeletal muscle (42) and to enhance miR-1 and miR-23 content in human skeletal muscle (39). In addition, miR-133a content has been demonstrated to be lowered in human skeletal muscle both with exercise training (39) and in type 2 diabetes (T2D) patients (14). Therefore, both mRNA and miR regulation are likely contributing mechanisms in skeletal muscle adaptations with repeated exercise.

The previous findings that overexpression of the transcriptional coactivator PGC-1α in mouse skeletal muscle converted otherwise white glycolytic muscles to red oxidative muscles (31) and that knockout of PGC-1α reduced expression of oxidative proteins (30) convincingly show the impact of PGC-1α on mitochondrial biogenesis in skeletal muscle. Similarly, the increased capillarization with PGC-1α overexpression (6) and decreased capillarization in PGC-1α knockout mice (28), as well as the effect of PGC-1α on VEGF expression (6, 28), demonstrate a role of PGC-1α in angiogenesis. PGC-1α transcription and mRNA (41) as well as PGC-1α protein content (32) have been shown to be upregulated in human skeletal muscle in response to a single exercise bout. In addition, the PGC-1α protein has been reported to be regulated by various posttranslational mechanisms, including activation by AMP-activated protein kinase (AMPK)-mediated phosphorylation (20) and by sirtuin 1 (SIRT1)-mediated deacetylation (15). AMPK is known as an intracellular energy sensor, which is phosphorylated and thereby activated by a single exercise bout (23). Furthermore, the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) has

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been shown to increase PGC-1α mRNA in mouse skeletal muscle (24) and repeated AICAR treatments to increase mitochondrial proteins in mouse skeletal muscle in a PGC-1α-dependent manner (28). Exercise training has also been shown to increase SIRT1 protein in humans (33), and PGC-1α deacetylation has been reported in mouse skeletal muscle in response to a single exercise bout (12). Together, this suggests that PGC-1α is a likely coordinator of exercise training-induced adaptations in skeletal muscle oxidative capacity, potentially involving both AMPK- and SIRT1-mediated regulation (12). Recent findings suggest that miR-29b may target PGC-1α mRNA (45), and such a mechanism could thus also play a role in PGC-1α-mediated gene regulation.

Although a physically inactive lifestyle is known to be an important risk factor in many diseases, and physical inactivity is an increasing problem in most parts of the world, less is known about the impact of physical inactivity on skeletal muscle oxidative capacity and on the ability of skeletal muscle to induce adaptive responses to an exercise bout. Therefore, the aim of the present study was to test the hypothesis that 7 days of bed rest will reduce expression and activity of oxidative proteins in skeletal muscle but not reduce the exercise-induced intracellular signaling, mRNA, and miR responses. This was addressed by placing young healthy subjects in bed for 7 days with a single exercise bout performed before and after the bed rest period.

METHODS

Ethical approval. Subjects were given both written and oral information about the experimental protocol and procedures and were informed about any discomfort that might be associated with the experiment before they gave their written consent. The study was performed according to the Declaration of Helsinki and approved by the Copenhagen and Frederiksberg Ethics Committee, Denmark (H-A-2008-0024).

Subjects. Twelve healthy, physically active male subjects with an average (means ± SD) age of 26.2 ± 5.3 yr, weight 75.5 ± 11.3 kg, height 181.7 ± 6.1 cm, and body mass index 22.8 ± 2.7 kg/m² participated in this study. Six of these subjects participated in an exercise trial. The average (means ± SD) age, weight, height, and body mass index of these subjects was 28.7 ± 5.3 yr, 82.2 ± 12.3 kg, 183.1 ± 7.6 cm, and 24.4 ± 2.2 kg/m², respectively.

Bed rest. The subjects were placed in hospital beds with manual head and leg elevation adjustments. During the bed rest period, the subjects were allowed to sit up for 5 h/day, and they were at all times transported in a wheelchair. During the bed rest period, subjects were served regular healthy food (10–20% energy from protein, 50–60% energy from carbohydrates, 25–35% energy from fat) ad libitum from the kitchen at Rigshospitalet, Copenhagen, Denmark.

Body composition. Six to 10 days before and immediately after the bed rest period, fat and fat-free tissue mass of the whole body, trunk, and extremities were measured on all 12 subjects, using a dual-energy X-ray absorptiometry (DEXA) scanner (Lunar Prodigy Advance; GE Healthcare, Madison, WI).

Oral glucose tolerance test. An oral glucose tolerance test (OGTT) was performed on all 12 subjects between 6 and 10 days before the onset of bed rest and 6 days into the bed rest. Each subject consumed 1 g/kg body wt glucose, with each gram of glucose dissolved in 6.67 ml of water. After consumption, blood was sampled from an arm vein after 30, 60, and 120 min, and the samples were analyzed for plasma insulin and glucose (Department of Clinical Biochemistry, Rigshospitalet).

Performance tests. Maximal oxygen uptake (Vo2max) and muscle endurance were determined for all 12 subjects 6–10 days before the onset of bed rest and at the end of the bed rest period. Vo2max was determined by an incremental bicycle test and leg muscle endurance by a one-legged knee extensor exercise test, using a modified ergometer bicycle (Monark Ergomedic 839E; Monark Exercise, Vansbro, Sweden). In the endurance test, the same absolute intensity was used before and after bed rest and starting with 15 min at 75% of maximal load (wattmax; before bed rest level) followed by 90% wattmax (before bed rest level) until exhaustion.

Each of the six subjects taking part in the acute exercise protocol performed an additional one-legged knee extensor exercise performance test 1 wk before the first experimental day to determine the workload to be used during the acute exercise experiments. The workload was gradually increased every 2 min, and the highest load, which could be sustained for 2 min, was set as the wattmax, as described previously (40).

Acute exercise experiment. The six subjects in the acute exercise protocol completed an identical experimental trial 4–10 days before initiation of the bed rest as well as on the 7th day of bed rest.

The day prior to the experimental day before bed rest, the subjects refrained from intense and prolonged exercise. The subjects were physically inactive the day before the experimental day after bed rest, because this was within the bed rest period. The day before both experimental trials, the subjects consumed a prepackaged dinner and evening snack, which was standardized based on the body weight of the subject (60 and 12 kJ/kg body wt, respectively).

On the experimental day before bed rest, the subjects arrived at the laboratory by minimum of physical activity, and on the experimental day after bed rest they were transported in wheelchairs. On the morning of both experimental trials, the subjects consumed a standardized breakfast regulated for body weight (30 kJ/kg body wt). A muscle biopsy was obtained from the middle portion of the vastus lateralis muscle, using the percutaneous needle biopsy technique (8) with suction 3.5 h after breakfast (Pre). This was followed by 45 min of one-legged knee extensor exercise at ~60% of wattmax (before bed rest level and thus the same absolute intensity before and after bed rest), using a modified ergometer bicycle (Monark Ergomedic 839E; Monark Exercise). Three of the subjects worked with their dominant leg and three with their nondominant leg. Additional muscle biopsies were obtained from the exercised leg immediately after exercise (Post) and at 3 h of recovery (3 h rec). All muscle biopsies were taken through separate incisions, quickly frozen in liquid nitrogen (<15 s), and stored at ~80°C until they were analyzed. A small part of the Pre biopsies was mounted in embedding medium frozen in isopentane precooled in liquid nitrogen and stored at ~80°C. Furthermore, a catheter was placed in the femoral artery of one leg, and blood samples were obtained before (Pre), during (20 min), and immediately after (Post) exercise. An article based on this experiment has recently been published, where body composition, OGTT, and performance data were reported for six of the subjects (26).

Plasma adrenaline. Plasma adrenaline was measured using an adrenaline RIA kit (Millipore, Bedford, MA).

Muscle glycogen. Muscle glycogen content was determined as glycosyl units after acid hydrolysis (34) using 400 μg of muscle homogenate protein and an automatic spectrophotometer.

Fiber type and capillarization. The mounted biopsies were cut in serial transverse sections, which were stained for myofibrillar adenosine triphosphatase to identify fibers as myosin heavy chain (MHC) type I, type IIa, or type IIx (11) or with the amylase-p-aminosalicylic acid method to visualize capillaries (5). Fiber type, fiber area, and capillary density were determined using a COMFAS image scanner (SBsysCOMFAS; Scan Beam, Hadsund, Denmark).

DNA isolation. Total DNA was isolated from ~10 mg of muscle tissue from Pre biopsies, as described previously (40). The DNA pellet was resuspended in 25 μl of distilled water. The isolated DNA was later used to determine the ratio between mtDNA and nuclear DNA content by real-time PCR.
RNA isolation, reverse transcription, and cDNA content. Total RNA for mRNA and miR was isolated from ~20 mg of muscle tissue by a modified guanidinium thiocyanate-phenol-chloroform extraction method adapted from Chomczynski and Sacchi (12a), as described previously (40), except that the tissue was homogenized for 2 min at 30 s⁻¹ in a TissueLyserII (Qiagen, Valencia, CA).

Superscript II RNase H⁻ system and Oligo dT (Invitrogen, Carlsbad, CA) were used to reverse transcribe the mRNA to cDNA, as described previously (40). The amount of single-stranded DNA (ss-DNA) was determined in each cDNA sample by use of OliGreen reagent (Molecular Probes, Leiden, The Netherlands), as described previously (35). Before reverse transcription of miR, RNA samples were diluted to 2 ng RNA/µl. Each specific miR was then reverse transcribed to cDNA by using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and miR-specific primers (Applied Biosystems). The reaction was run in a thermal cycler (PTC-200; MJ Research, Waltham, MA).

Real-time PCR. The mRNA content of cytochrome c (cyt c), hexokinase II (HKII), PGC-1α, and SIRT1 as well as miR DNA (represented by cyt c oxidase II) and nDNA (represented by cyt c) content were determined by real-time PCR using the fluorogenic 5′ nucleic assay with TaqMan probes and universal mastermix with UNG (ABI PRISM 7900 Sequence Detection System; Applied Biosystems), as described previously (35). All TaqMan probes were 5′-6-carboxyfluorescein and 3′-6-carboxy-N,N,N′,N′-tetramethylrhodamine labeled (Table 1). The obtained cycle threshold (Ct) values reflecting the initial content of the specific transcript in the samples were converted to an arbitrary amount by using standard curves obtained from a serial dilution of a pooled sample made from all samples. The amount of a given mRNA was normalized to the ssDNA content of the cDNA samples, whereas mRNA results were presented as miDNA normalized to nDNA content (miDNA/nDNA).

The content of miR-1, miR-23a, miR-29b, and miR-133a was determined by real-time PCR (as described above) using predesigned miR assays containing specific primers and TaqMan probe labeled with 5′-6-carboxyfluorescein and minor groove binder quencher (nonfluorescent) (Applied Biosystems). The obtained cycle threshold (Ct) values reflecting the initial content of the specific miR in the samples were converted to an arbitrary amount by using standard curves obtained from a serial dilution of a pooled sample made from all samples. The amount of a given miR was normalized to either the RNU6B content or the RNU48 content of the sample. RNU6B was unaffected by the acute exercise but affected by bed rest in resting samples, whereas RNU48 was unaffected by bed rest in resting samples but changed by acute exercise. Therefore, RNU6B was used as endogenous control for the acute exercise samples and RNU48 for resting miR levels.

Muscle lysate preparation. Freeze-dried muscle specimens were dissected free of blood, fat, and connective tissue under the microscope and homogenized in an ice-cold buffer as described previously (10), except the tissue was homogenized for 2 min at 30 s⁻¹ in TissueLyserII (Qiagen). Protein content in lysates was measured by the bicinchoninic acid method (Pierce Biotechnology, Rockford, IL). Lysates were prepared with sample buffer containing sodium dodecyl sulfate (SDS) and boiled for 3 min at 96°C and analyzed by SDS-PAGE and Western blotting.

SDS-PAGE and Western blotting. AMPK and acetyl-CoA carboxylase (ACC) phosphorylation as well as AMPKα2, ACC, cyt c, HKII, SIRT1, VEGF, and β-actin protein content were measured in muscle samples by SDS-PAGE (Tris-HCl, 5 and 10% gel; Bio-Rad) and Western blotting using PVDF membrane and semidry transfer, as described previously (10). Protein content and phosphorylation are expressed in units relative to control samples loaded on each gel. Primary antibodies used were phospho-ACC (no. 07-303; Upstate Biotechnology, Lake Placid, NY), phospho-AMPK (no. 2535S; Cell Signaling Technology, Beverly, MA), AMPKα2 and AMPKα1 pro-
Statistical calculations were performed using SigmaStat version 3.1. 

### Results

#### Anthropometric parameters

Total body weight, total fat mass, and percent fat did not change in response to 7 days of bed rest, but leg muscle mass was on average reduced ($P \leq 0.05$) after bed rest (Table 2).

#### Performance

$V_{O2max}$ was 4% lower ($P \leq 0.05$) after bed rest, whereas time to exhaustion decreased only nonsignificantly (11%; decreased in 7 of the 12 subjects) in response to bed rest (Table 2).

#### Oral glucose tolerance test

Fasting plasma glucose concentration decreased ($P \leq 0.05$) from 4.9 ± 0.1 to 4.5 ± 0.1 mM, and fasting insulin concentration increased ($P \leq 0.05$) from 29.8 ± 4.3 to 45.7 ± 3.6 pmol/l as a result of the bed rest period. Two hours after the oral glucose intake, the plasma glucose concentration tended to be higher ($0.05 < P \leq 0.1$) after bed rest (5.4 ± 0.3 mM) than before bed rest (4.9 ± 0.4 mM), and plasma insulin concentration was higher ($P \leq 0.05$) after bed rest (259.5 ± 23.0 pmol/l) than before bed rest (106.2 ± 27.5 pmol/l). The area under curve (AUC) was calculated for plasma glucose and insulin in response to the OGTT before and after bed rest. The AUC for the plasma glucose response was unchanged, whereas AUC for plasma insulin was 1.4-fold larger ($P \leq 0.05$) after bed rest than before bed rest.

#### MHC fiber type composition, fiber size, and capillarization

Despite the reduced muscle mass with bed rest, muscle fiber size did not change. In addition, percentage of type I, IIa, and IIx fibers and capillarization per fiber were similar before and after bed rest (Table 2).

#### DNA content, enzyme activity, and protein content

mtDNA/nDNA tended to be 15% lower ($0.05 < P \leq 0.10$) after bed rest than before bed rest (Fig. 1). A similar tendency for decreased ($0.05 < P \leq 0.10$) mtDNA/nDNA with bed rest was evident when only the six subjects in the exercise protocol were included (data not shown).

The protein content of cyt c and VEGF was similar before and after bed rest, but HKII protein content was 50% lower after bed rest than before bed rest (Fig. 2A). No significant change was apparent in CS or HAD activity with bed rest when only the six subjects in the exercise protocol were included (data not shown).

The protein content of cyt c and VEGF was similar before and after bed rest, but HKII protein content was 50% lower ($P \leq 0.05$), and SIRT1 protein content was 40% lower ($P \leq 0.05$) after bed rest than before bed rest (Fig. 2B). Analysis of protein content for the six subjects in the exercise protocol showed that SIRT1 protein content decreased ($P \leq 0.05$) and HKII and cyt c protein content tended to decrease ($0.05 < P \leq 0.10$), whereas VEGF protein content did not change significantly (data not shown).

#### Plasma adrenaline

The plasma adrenaline level was unaffected by bed rest, and plasma adrenaline during exercise did not change significantly either before or after bed rest (Fig. 3A).

#### Muscle glycogen

The muscle glycogen level tended to be higher ($0.05 < P \leq 0.10$) after bed rest than before bed rest, but muscle glycogen breakdown during exercise was similar before (139 mmol/kg dry wt) and after bed rest (145 mmol/kg dry wt) (Fig. 3B). Muscle glycogen was lower ($P \leq 0.05$) after bed rest than before bed rest.
levels in skeletal muscle. AMPK phosphorylation/AMPKα2 protein was 2.7-fold higher \((P \leq 0.05)\) immediately after exercise than Pre before bed rest but did not change with exercise after bed rest (Fig. 4A). A similar pattern was evident both for unnormalized AMPK phosphorylation and when normalized to AMPKα1 protein content (data not shown). ACC phosphorylation/ACC protein increased \((P \leq 0.05)\) similarly, approximately fivefold, in response to exercise before and after bed rest (Fig. 4B).

**mRNA content.** Bed rest did not affect the resting levels of HKII, cyt c, SIRT1, PGC-1α, or VEGF mRNA in skeletal muscle. Before bed rest, PGC-1α and VEGF mRNA content increased \((P \leq 0.05)\) 3.3- and 2.5-fold, respectively, at 3 h of recovery from exercise relative to Pre but did not change with exercise after bed rest (Fig. 5, A and B). HKII, cyt c, and SIRT1 mRNA content was not affected by the acute exercise.

**miR content.** The resting content of miR-133a was 8% lower \((P \leq 0.05)\), and miR-1 tended to be 9% lower \((0.05 < P \leq 0.10)\) after bed rest than before bed rest, whereas the resting levels of miR-23a and miR-29b were unaffected by bed rest (Fig. 6A).

Whereas the miR-23a content was unchanged in response to exercise before bed rest, miR-23a was 27% lower \((P \leq 0.05)\) at 3 h of recovery than Pre after bed rest (Fig. 6B). miR-1, miR-29b, and miR-133a content did not change in response to the exercise bout either before or after bed rest (data not shown).

**DISCUSSION**

The main findings of the present study are that only 7 days of bed rest abolished the exercise-induced PGC-1α and VEGF mRNA responses and reduced the mtDNA/nDNA content, HAD and CS activity, and HKII and SIRT1 protein content as well as the miR-1 and miR-133a content in human skeletal muscle, but without significant changes in cyt c and VEGF protein content or capillarization.

The present observations that bed rest resulted in loss of leg muscle mass and induced whole body glucose intolerance as well as reduced \(V_{\text{O2max}}\) are in accord with previous bed rest (2, 37, 43) and physical inactivity studies (27). However, this is the first study to examine the impact of 7 days of bed rest on oxidative capacity and acute exercise-induced adaptive responses in skeletal muscle. Based on previous reports showing decreased activity of metabolic proteins in human skeletal muscle with detraining (17), it was expected that bed rest would induce similar changes. The present findings that HKII and SIRT1 protein content as well as HAD and CS activity were reduced by bed rest are thus in line with this anticipation and suggest that only 7 days of bed rest results in opposite changes of those previously shown to occur with training (16, 17, 48). The observed reductions in protein content/activity of the cytosolic HKII, the nuclear SIRT1, and mitochondrial HAD and CS indicate that proteins in several different cellular compartments were affected by bed rest. But the lack of significant change in cyt c protein as such does not support a general decrease in all metabolic proteins with 7 days of bed rest. The decreased mt/nDNA ratio with bed rest in the present study provides evidence for a lower mitochondrial content in skeletal muscles after only 7 days of total physical inactivity and is in accord with training-induced mitochondrial biogene-
sis (16), although human studies in general report a lack of changes in mtDNA/nDNA with training (22, 47). In addition, this indication for decreased mitochondrial content and the observed lower HAD and CS activity suggests that the non-significant change in cytc protein may be due to longer half-life of cytc than the major part of mitochondrial proteins. But larger individual variation in the cytc response to the bed rest period is also a possibility. The similar resting HKII, cytc, PGC-1α, SIRT1, and VEGF mRNA levels before and after bed rest in the present study are in contrast to the reduction recently demonstrated for HKII, PGC-1α, and VEGF mRNA with 9 days of bed rest (1). This difference between the studies may be due to the duration of the bed rest and/or the number of subjects studied (12 subjects in the present study and 20 subjects in the previous study).

The lack of change in skeletal muscle capillarization after 7 days of bed rest in the current study is in accord with the results from a previous bed rest study (38). Moreover, the unaffected VEGF protein content is in accord with the unchanged capillarization and may support that regulation of capillarization is less sensitive to physical inactivity than at least some metabolic proteins. This suggestion is in line with previous findings.

Fig. 3. Arterial plasma adrenaline and muscle glycogen content. A: arterial plasma adrenaline before (Pre), after 20 min, and immediately after (Post) 45 min of one-legged knee extensor exercise BB and AB. B: muscle glycogen in vastus lateralis Pre, Post, and 3 h into recovery (3 h rec) from 45 min of one-legged knee extensor exercise BB and AB. Values are means ± SE; n = 6. *Significantly different from Pre in given trial, P ≤ 0.05; †significantly different from BB at given time point, P ≤ 0.05; (*)tendency to be significantly different from Pre in given trial, 0.05 < P ≤ 0.10; (†)tendency to be significantly different from BB at given time point, 0.05 < P ≤ 0.10.

Fig. 4. AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation (phos). AMPK phos (A) and ACC phos (B) in vastus lateralis Pre, Post, and 3 h rec from 45 min of one-legged knee extensor exercise BB and AB. The phosphorylation level is normalized to AMPKα2 protein and ACC protein content. C: representative Western blots from Pre, Post, and 3 h rec BB and AB. Values are means ± SE; n = 6. *Significantly different from Pre in given trial, P ≤ 0.05; (†)tendency to be significantly different from BB at given time point, 0.05 < P ≤ 0.10.
showing that detraining had a more dramatic effect on metabolic enzyme activities than on capillarization (17). However, it may be noted that VEGF protein content was reduced nonsignificantly, −50%, by bed rest, maybe indicating individual variation in the sensitivity to physical inactivity of this angiogenic regulator.

The unchanged capillarization despite decreased mitochondrial content may explain that the exercise endurance was not significantly reduced by bed rest in the present study and suggests that muscle endurance during such moderate-intensity exercise may not be that sensitive to short-term physical inactivity. An additional possibility is that some of the subjects are more sensitive to physical inactivity than others. However, the subjects showing no decrease in endurance with bed rest did exhibit reduced activity of CS and HAD as well as reduced mitochondrial DNA content in skeletal muscle, making this possibility less likely. Factors not related to muscle endurance, such as psychological factors, may also have influenced time to exhaustion for some subjects more than others and thereby contributed to the lack of significant effect of bed rest on endurance.

The observed exercise-induced increases in PGC-1α and VEGF mRNA in skeletal muscle before bed rest in the present study are in line with previous studies (18, 21, 41). Because the exercise-induced VEGF mRNA response has been shown to be reduced and the PGC-1α mRNA content to be increased in response to knee extensor exercise after a period of exercise training (21, 41), it was anticipated that the exercise-induced VEGF and PGC-1α mRNA responses would be elevated and reduced, respectively, after bed rest. Therefore, the findings that the exercise-induced PGC-1α and VEGF mRNA responses were totally blunted after bed rest were unexpected and, very interestingly, indicate that 7 days of total physical inactivity of the muscles abolishes the ability of the muscles to induce mRNA changes in response to an acute exercise bout.

Fig. 5. mRNA content. Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α (A) and VEGF mRNA content in vastus lateralis (B) Pre, Post, 3 h rec from 45 min of one-legged knee extensor exercise BB and AB. The mRNA content is normalized to the single-stranded DNA content in the sample. Values are means ± SE; n = 6. *Significantly different from Pre in given trial, P ≤ 0.05; †significantly different from BB at given time point, P ≤ 0.05; (†)tendency to be significantly different from Pre in given trial, 0.05 < P ≤ 0.10.

Fig. 6. miR content. Resting microRNA (miR) content normalized to RNU48 in vastus lateralis BB and AB (A) and miR-23a content normalized to RNU6B in vastus lateralis (B) Pre, Post, and 3 h rec from 45 min of one-legged knee extensor exercise BB and AB. Values are means ± SE; n = 12 in A and n = 6 in B. *Significantly different from Pre in given trial, P ≤ 0.05; †significantly different from BB at given time point, P ≤ 0.05. (†)tendency to be significantly different from BB, 0.05 < P ≤ 0.10.
Because previous studies in mice indicate that AMPK regulates the expression of PGC-1α and VEGF in skeletal muscle (24, 28), the observation that exercise-induced AMPK phosphorylation was also only present before bed rest may suggest that the lack of changes in AMPK phosphorylation after bed rest led to the absent PGC-1α and VEGF mRNA responses. In addition, the similar pattern of PGC-1α and VEGF mRNA content both before and after bed rest in the present study supports a possible AMPK/PGC-1α-mediated regulation of both PGC-1α and VEGF mRNA expression. On the other hand, other upstream factors may also be involved because it has been demonstrated that AMPKα2 is not required for exercise-induced PGC-1α mRNA increases in mouse skeletal muscle (24). Of notice is the similar exercise-induced phosphorylation of ACC at an assumed AMPK site (4) before and after bed rest in the present study. This may reflect that the in vivo AMPK activity was higher than indicated by the AMPK Thr172 phosphorylation due to allosteric regulation of AMPK (23) or that ACC was regulated by factors other than AMPK. Importantly, the maintained exercise-induced ACC phosphorylation in the present study and the recently shown exercise-induced pyruvate dehydrogenase regulation both before and after bed rest (26) together underline the fact that the muscles were indeed able to elicit some typical phosphorylation events in response to exercise after bed rest, indicating a specificity of the blunted AMPK phosphorylation after bed rest.

Because exercise-induced AMPK phosphorylation has been shown to be enhanced when muscle glycogen is reduced (36), it is possible that the elevated muscle glycogen level after bed rest in the present study contributed to the observed blunted AMPK phosphorylation and concomitantly the absent mRNA responses. However, it should be noted that muscle glycogen was increased only 20% by bed rest, indicating that this explanation may not be that likely, and the lack of mRNA responses may also be caused by an AMPK-independent mechanism. Hence, 9 days of bed rest has been shown to increase PGC-1α promoter methylation (1) and increased methylation of the PGC-1α promoter to be associated with reduced PGC-1α mRNA expression (1, 7). In addition, the PGC-1α promoter has been reported to be hypermethylated in skeletal muscle of T2D patients (7), and a smaller exercise-induced PGC-1α mRNA response has also been demonstrated in T2D patients (13). Therefore, it is possible that the lack of exercise-induced PGC-1α mRNA increase after bed rest in the present study was due to physical inactivity-induced hypermethylation of the PGC-1α promoter.

The present findings that resting miR-133a and miR-1 levels were reduced after bed rest are as such opposite of the expected based on the previously reported decrease after exercise training (39) but in accord with the decrease in miR-133a in mouse skeletal muscle upon spaceflight (3) as well as the reduced miR-133a content in skeletal muscle of T2D patients (14). The miR-133a content has previously been suggested to correlate with fasting glucose levels in T2D patients, but no correlations were present between miR-133a and fasting plasma glucose in the present study. This difference between the previous finding in T2D patients and the present finding in healthy subjects even after bed rest likely reflects the ability of the subjects in the present study to compensate for a reduced ability to remove glucose in the periphery (9, 38) by increased insulin secretion (37). The decreased miR-23a content observed 3 h into recovery after bed rest is in accordance with the previous observation that an acute exercise bout downregulated miR-23a content in mouse skeletal muscle (42). Because the subjects in the present study exercised at the same absolute workload before and after bed rest, the relative exercise intensity is expected to have been higher after bed rest than before bed rest. Although there are to our knowledge no studies yet investigating the importance of exercise intensity on exercise-induced miR responses, a lower relative exercise intensity is a likely explanation for the lack of changes in miR-23a before bed rest.

In conclusion, the lack of exercise-induced PGC-1α and VEGF mRNA responses after 7 days of bed rest suggests that total physical inactivity abolishes exercise-induced gene responses and thus in part the ability of human skeletal muscle to adapt to acute exercise. In addition, the observed reduced miR-1 and miR-133a content in skeletal muscle after bed rest further implies that posttranscriptional regulation may also be influenced by physical inactivity. Finally, the findings that bed rest reduced mitochondrial DNA content and HAD and CS activity as well as HKII and SIRT1 protein content indicate that just 7 days of total physical inactivity lowers metabolic capacity and affects metabolic regulation in human skeletal muscle. Together, the present results provide evidence that a physically inactive lifestyle as well as short-term bed rest due to trauma or surgery induce unfavorable changes in skeletal muscle, potentially affecting not only functional capabilities but also the adaptability to exercise.

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DISCLOSURES

The authors have nothing to declare.

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