Greater loss in muscle mass and function but smaller metabolic alterations in older compared to younger men following two weeks of bed rest and recovery

Running head title

Ageing, inactivity and rehabilitation

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ABSTRACT

This investigation aimed to compare the response of older adult and young men to bed-rest and subsequent rehabilitation. Sixteen older (OM: 55-65 years) and seven young men (YM: 18-30 years) were exposed to 14-day bed rest (BR) followed by 14-day rehabilitation (R). Quadriceps muscle volume (QVOL), force (QF) and explosive power (QP) of leg extensors, single fiber isometric force (Fo), peak aerobic power ($\dot{V}O_2$peak), gait stride length, and three metabolic parameters, Matsuda index of insulin sensitivity, postprandial lipid curve and homocysteine plasma level, were measured before and after BR and after R. Following BR, QVOL declined more in OM (-8.3%) than in YM (-5.7%, P=0.031), QF (-13.2%; P=0.001), QP (-12.3%; P=0.001) and gait stride length (-9.9%; P=0.002) declined only in OM. Fo significantly declined in both YM -32.0% and OM -16.4% without significant differences between groups. $\dot{V}O_2$peak declined more in OM (-15.3%) than in YM (-7.6%, P<0.001). Instead, Matsuda index showed greater decline in YM than in OM (-46.0% vs. -19.8%, respectively; P=0.003), while increase in postprandial lipid curve (+47.2%; P=0.013) and homocysteine concentration (+26.3%; P=0.027) were observed only in YM. Importantly, after R, the recovery of several parameters, among them QVOL, QP and $\dot{V}O_2$peak, was not complete in OM, while Fo did not recover in both age groups. The results show that the impact of inactivity on muscle mass and function is greater in OM, while the metabolic alterations are greater in YM. Furthermore, these findings show that the recovery of pre-inactivity conditions is slower in OM.

Key Words: Ageing–Prolonged Physical Inactivity–Functional Decline-Reconditioning

New and noteworthy

We compared the responses of elderly and young males to 14-d bed-rest and subsequent rehabilitation. The impact of inactivity on muscle mass and function (muscle force and power, fiber strength, $\dot{V}O_2$peak) was greater in the elderly, while metabolic alterations (insulin sensitivity, postprandial lipid curve, homocysteine levels) were greater in the young. The recovery of pre-inactivity conditions was slower in elderly. The results emphasize the importance of avoiding or minimizing periods of inactivity in old age.

INTRODUCTION

Physical inactivity of elderly people is a relevant health problem since its deleterious effects involve not only muscle mass and function (22) but also energy metabolic balance (21,24) and cardiovascular function (4). A sudden decrease in physical activity can also significantly worsen health already impaired by the aging process. In this perspective, the negative impact of hospitalization on the health of elderly people (25) could be partly explained by the disease-related problems and changes in their life habits (8), but could be also due to a sudden reduction of physical activity.

Due to their clinical implications, the effects of physical inactivity in older subjects have been widely studied in the last years. Bed rest (BR) protocol, which was originally designed to study the effect of microgravity during space flight (30), offers a realistic and useful model to study the impact of the decreased physical activity and muscle disuse experienced by bedridden patients. A number of BR experiments on older subjects have been carried out in the last ten years (6,12,14,15,22,23,36). Taking into account the risk of thromboembolic complications (4), alternative protocols, less systemically hazardous, such as unilateral leg immobilization (ULLI) (17,34) or unilateral leg suspension (ULLS) (11,18,19) have been also applied in older subjects. These latter experimental protocols proved to be very useful to study changes in muscle mass, strength or single muscle fiber properties, but are less convenient for exploring metabolic and other systemic changes induced by prolonged inactivity (39). The results obtained were ~~are~~ to some extent controversial. In elderly, prolonged disuse was found to be associated with an accelerated age-related deterioration of neuromuscular function (34,38), of the decline of contractile properties of vastus lateralis single muscle fibers (17-19) and of isometric contractile performance of quadriceps muscle (34). Other studies, however, showed that only high-velocity muscle actions are differentially affected (11) or that changes in maximal isometric and dynamic muscle strength are similar in young and elderly (17). The earlier ~~first~~ BR studies (12,14,15,22,23) carried out in older adults showed significant decline in muscle mass, strength

and power and maximal aerobic power. The decline in muscle mass was explained by impaired amino acid transport and by altered mTOR-related signaling (14) and was accompanied by altered IL-6 and TLR4 expression (15). Those studies, however, lacked of a control group of younger individuals and did not consider the rehabilitation or recovery phase. Both these issues were considered in a recent study (36), where a group of 9 elderly and a group of 14 young subjects were exposed to the same protocol, consisting of 5 days of BR followed by 8 weeks of rehabilitation based on resistance training. The results showed that 5 days were sufficient for inducing significant atrophy in the elderly but not in the young and that 8 weeks of rehabilitation were sufficient for complete recovery also in the elderly. In our view, this important and careful study leaves open several relevant questions. In the first place how the different response between the young and the elderly develops on a longer time frame, sufficient to induce significant atrophy in the young too. In other words, it is still unclear whether the difference between young and old reported by Tanner et al. (36) is a difference in time course or in severity of the atrophic response to disuse. In the second place, given that a very prolonged rehabilitation phase allows a complete recovery both in young and old (36), it is interesting to assess whether a difference ~~diversity~~ can be detected in the initial phase of the recovery. Finally, the BR involves also a metabolic and systemic adaptations, which deserve to be carefully analyzed and were not until now considered in their comparative aspects in the published papers.

All these considerations prompted us to design a BR study in which young and old participants were exposed to precisely the same protocol of inactivity in bed on a time frame of 14 days, sufficient to induce significant atrophy also in young healthy participants, and to a subsequent rehabilitation with moderate training for a corresponding period of 14 days, likely suitable to detect a diversity in the recovery response. We hypothesized a greater deterioration of muscle and metabolic functions in the elderly vs. the young subjects during bed rest, and a ~~n~~ slower ~~incomplete~~ recovery in the elderly vs. the young subjects during the subsequent rehabilitation period.

METHODS

PARTICIPANTS: Twenty-three healthy men, of which 7 young (YM; aged 18-30 years) and 16 older adults (OM; aged 55-65 years) were recruited for the study. All participants underwent medical examination and routine blood and urine analysis. Basic anthropometric parameters of the two groups are reported in Table 1. Exclusion criteria were: smoking; regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer > 500 $\mu g \cdot L^{-1}$; acute or chronic skeletal, neuromuscular, metabolic and cardiovascular disease conditions; pulmonary embolism. Participants were informed of the purpose, procedures and potential risk of the study before signing the informed consent. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and was approved by the National Ethical Committee of the Slovenian Ministry of Health on April 17, 2012 under the acronym: IR-aging 1200.

Insert table 1 approximately here

STUDY DESIGN: The study was conducted in controlled medical environment of the Orthopedic Hospital of Valdoltra, Slovenia. The participants were housed in standard air-conditioned hospital rooms and were under constant surveillance with 24-hour medical care. For 14 days, the participants performed all daily activities in bed and received eucalorically controlled ~~standard hospital~~ meals three times a day. ~~All participants followed an individually controlled eucaloric diet during BR period.~~ Dietary energy requirements were designed for each subject multiplying resting energy expenditure by factors 1.2 and 1.4 in BR and ambulatory period, respectively (2). The resting energy expenditure was calculated as done in previous studies (2). The macronutrient food content set at 60% of carbohydrates, 25% fat, and 15% of proteins, according to the scheme of the so called "mediterranean diet" and adopted also in previous bed rest protocols (2). Energy balance was checked weekly by fat mass assessment. The

daily protein intake was 1.1-1.25 g kg^{-1} in both groups, in BR and recovery period respectively, except the subgroups of OM who received a supplementation (see below).

After the BR, participants underwent a rehabilitation protocol (R) that consisted of 2-week supervised multimodal exercise program with 3 sessions per week. In each session, participants performed 12-minute warm-up, 15-20 minutes of balance and strength training, and 20-30 minutes of endurance training. The protocol included two interventions, as eight randomly selected OM followed daily 45 minutes of computerized cognitive training by navigating through virtual mazes with the use of a joystick during the BR period and then received a nutritional support based on 0.4 g whey protein/kg body weight/day at breakfast during the rehabilitation period. Since no significant differences were detected between the 8 OM receiving the interventions and the other 8 OM on the parameters reported in this study, the two OM groups were pooled for statistical analysis.

PROCEDURES: All assessments were performed before baseline data collection (BDC) starting BR, after 14 days of BR (BR14) and on 14th day of R (R+14).

Anthropometric determinations comprised body mass index and fat mass calculation using bio impedance with a tetra-polar impedance-meter (BIA101, Akern, Florence, Italy).

Quadriceps muscle volume (QVOL) of the right leg was measured from turbo spin-echo, T1-weighted, magnetic resonance images (MRI) obtained with 1.5 T (Magnetom Avanto; Siemens Medical Solution, Erlangen, Germany). On each MRI slice, contours corresponding to the quadriceps muscles were delineated by an expert of MRI imaging, using the image processing tools OsiriX (Pixmeo Sarl, v.4.1.2). QVOL was then derived by summation of a series of evenly spaced truncated cones comprised between each two axial images, a process that included an average of 25 images (range 23-28) and covered the entire length of the quadriceps.

Peak knee extensors muscle force (QF) of the right leg was estimated from maximal voluntary isometric contraction (MVC) at a 110° knee angle, with hip fixed at 90°. Force was measured by an electrical transducer (TSD121C, BIOPAC Systems), with 1kHz sampling

frequency, implemented on a custom built chair for isometric contractions of knee extensor muscle groups. After a familiarization, participants performed two MVC, with two minutes rest. The MVC with highest QF was taken into account for further analysis.

Lower limb peak explosive power (QP) of the bilateral efforts was determined by means of the Explosive Ergometer (EXER), described in detail by Lazzer et al. (26). Participants were secured on a seat by a safety belt tightened around shoulders and abdomen, placed the soles of their feet against the force platforms (LAUMAS PA 300, Parma, Italy), and were asked to accelerate the seat backward by pushing from force platforms. Prior explosive efforts knee angle was set at 110°. After familiarization, participants performed four maximal explosive jumps, with two minutes rest in between. The attempt with the highest QP, normalized by body weight, was taken for further analysis.

Peak aerobic power ($\dot{V}O_2peak$) was determined by a graded exercise test on a mechanically braked cyclo-ergometer (Monark Ergomedic 839E). During the test, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy). After determining rest values, participants warmed-up at 80W and thereafter, 20W increments were imposed every minute until volitional exhaustion. Values obtained at exhaustion were considered peak values and expressed normalized to body weight.

Gait analysis was performed while participants walked at their preferred speed with the 10 meters OptoGait system (Microgate, Bolzano, Italy). Each participant walked for 60 seconds and an average stride length was taken for further analysis.

A simple orthostatic test was performed at BDC and at the very end of BR, measuring heart rate with a strap heart rate monitor (Polar) which allowed a continuous record for 10 minutes.

Single muscle fiber analysis was performed from samples obtained from the mid-region of the left vastus lateralis muscle. Biopsy was done after anesthesia of the skin, subcutaneous fat tissue, and muscle fascia with 2 ml of lidocaine (2%). A small incision was then made to penetrate skin and fascia, and the tissue sample was harvested with a purpose-built rongeur (Zepf

Instruments, Tuttlingen, Germany). A part of the sample, used for single fiber analysis, was quickly placed into storage (high potassium, EGTA and glycerol, see 13) solution and stored at -20 °C, while another part was frozen in isopentane cooled with fluid nitrogen. Single muscle fiber segments were dissected from the samples stored in high potassium, EGTA and glycerol and mounted in the set-up as previously described (13). Cross Sectional Area (CSA) was measured from three diameters assuming a circular shape and isometric force (Fo) was measured in four subsequent maximal activations (pCa 4.6, sarcomere length 2.6 µm, temperature 12°C) see Doria et al. (13).

Insulin sensitivity was assessed in postprandial condition, after a standard meal test (500 mL, 500 kcal, vanilla flavour, Nutricomp ®, B.Braun) composed by 15% of protein, 30% of fat and 55% of carbohydrate. Blood samples were collected hourly for 6 hours after meal load, to assess plasma concentrations of insulin, glucose and triglycerides. Insulin sensitivity in postprandial condition was assessed using the Matsuda index or ~~of~~ insulin sensitivity index (ISI), calculated as follows:

$$\frac{10000}{\sqrt{glyT0 \cdot insT0 \cdot insmean \cdot glymean}} \qquad (1)$$

where gly_{T0} is plasma glucose concentration at baseline; ins_{T0} is plasma insulin concentration at baseline; gly_{mean} is the mean glucose concentration measured during the 6 hours of meal test; ins_{mean} is the mean insulin concentration measured during the 6 hours of meal test; and 10000 is a constant which provides numbers that are easy to deal with.

Changes in triglycerides concentrations during the first 3-h meal test were assessed calculating the area-under-the curve (AUC) of triglycerides plasma concentration versus time, using the linear trapezoidal method.

Fasting plasma homocysteine concentration was determined as previously reported (27).

STATISTICAL ANALYSIS: After assuring normal distribution by visual inspection (histogram, Q-Q plot) and Shapiro-Wilk's test, the data were analyzed with SPSS 19.0 software (IBM, Chicago, USA). Non-significant interactions in 3 (BDC, BR14, R+14) x 3 (YM, OM with interventions, OM without interventions) repeated measurements General Linear Model (GLM) ~~ANOVA~~ lead us to proceed with 3 (BDC, BR14, R+14) x 2 (YM, OM) repeated measures GLM, ~~ANOVA~~ with time as "within factor" and group as "between factor". After establishing significant main effects we performed post hoc analysis with the Bonferroni corrections. BDC values were used as covariates in case of different BDC values. ~~In case of different BDC values between both groups ANCOVA was used to check group differences at BR14.~~ Statistical significance for all analysis was set at 0.05.

RESULTS

All participants were able to comply with the study protocol. There was no dropout and no medical complications occurred beside transient hypotension during the 10 minutes orthostatic tolerance testing on BR14 in seven participants (3 in OM (18.8%) and 4 in YM (57.1%)).

As expected significant differences were present at BDC between OM and YM. In particular QVOL was 16.2% lower in OM compared to YM (OM: 1666 ± 234 ml vs. YM: 1987 ± 270 ml; P = 0.010), QF was 19.9% lower in OM compared to YM (OM: 546 ± 111 N vs. YM: 681 ± 115 N; P = 0.014) and QP was 34.9% lower in OM compared to YM (OM: 32.7 ± 5.76 W kg^{-1} vs. YM: 50.2 ± 4.54 W kg^{-1}; P < 0.001). $\dot{V}O_2$peak was lower in OM than in YM (-31.4%: OM: 26.8 ± 4.92 mlO_2 kg^{-1} min^{-1} vs. YM: 39.2 ± 5.06 mlO_2 kg^{-1} min^{-1}; P < 0.001). A significant difference was detectable also in single muscle fibres as Fo was 33.7% lower in OM compared to YM (OM: 0.667 ± 0.192 mN vs. YM: 0.814 ± 0.099 mN; P = 0.024), whereas the difference in CSA was below statistical significance (OM: 5760 ± 1383 μm^2 vs. YM: 6452 ± 2036 μm^2; P = 0.43).

Some difference between elderly and young was present at BDC also in plasma metabolic parameters. GlyT0 (OM: 96 ± 9.9 mg dl^{-1} vs. YM: 87 ± 11 mg dl^{-1}) and insT0 (OM: 5.87 ± 4.89 U ml^{-1} vs YM: 5.14 ± 2.91 U ml^{-1}) were not significant different in OM compared to YM, whereas significant differences were detectable in plasma lipid concentrations. Plasma cholesterol was 51.1% higher in OM compared to YM (OM: 198 ± 38 mg dl^{-1} vs. YM: 131 ± 10 mg dl^{-1}; $P < 0.01$), and the same was true for triglycerides that were 110% higher in OM compared to YM (OM: 118 ± 41 mg dl^{-1} vs. YM: 56 ± 16 mg dl^{-1}; $P < 0.01$) and also LDL was 69.6% higher in OM compared to YM (OM: 134 ± 37 mg dl^{-1} vs. YM: 79 ± 13 mg dl^{-1}; $P < 0.01$), whereas no significant difference was present in HDL levels (OM: 40 ± 7 mg dl^{-1} vs. YM: 40 ± 6 mg dl^{-1}). Finally, a significant difference was detectable in postprandial triglycerides AUC, as it was 123% higher in OM compared to YM (OM: 393 ± 140 h·mg dl^{-1} vs. YM: 176 ± 56.9 h·mg dl^{-1}; $P = 0.001$). No difference was present in the insulin sensitivity as assessed with Matsuda index ~~test~~ (see below).

Figure 1: Lower limb muscle volume and function here

All functional and metabolic parameters measured were affected by BR and R. The changes of the structural and functional parameters related with lower limb muscles are reported in Figure 1. QVOL (Figure 1A) showed a decrease at BR14 in YM and OM by -5.7 ± 3.9% ($P = 0.017$) and -8.4 ± 3.7% ($P < 0.001$), respectively. Taking into account the existing difference at BDC, QVOL decreased significantly more in OM than in YM at BR14 ($P = 0.031$) and, moreover, in OM remained lower than the BDC values also at R+14 ($P = 0.013$). The two parameters expressing the contractile function, QF (Figure 1B) and QP (Figure 1C), declined significantly at BR14 only in OM by -13.2 ± 12.4% ($P < 0.001$) and -12.3 ± 10.4% ($P < 0.001$), respectively; it is important to underline that at R+14 QF recovered completely, while QP remained lower compared to BDC by -7.8 ± 8.9% ($P = 0.009$).

Figure 2: $\dot{V}O_2$*peak and Gait stride length here*

$\dot{V}O_2$peak (Figure 2A) decreased significantly at BR14 in YM and OM by -7.6 ± 4.8% ($P = 0.007$) and -15.3 ± 11.0% ($P < 0.001$), respectively. At R+14 $\dot{V}O_2$peak fully recovered in YM,

whereas in OM remained lower compared to BDC (-9.3 ± 13.4%; P = 0.020) and different than in YM. Gait stride length (Figure 2B) decreased only in OM at BR14 (-9.9 ± 11.3%, P = 0.002), but fully recovered at R+14.

Figure 3: Single fibre parameters here

Single fibre CSA showed a BR-related decrease which did not reach statistical significance due to the large inter-individual variability (Figure 3A). In contrast isometric force Fo (Figure 3B) showed a marked and significant decrease at BR14, greater in YM (-32.0 ± 27.6%, P = 0.030) than in OM (-23.4 ± 40.9%, P = 0.045), with an interaction effect close to significant (P = 0.132). Interestingly, at R+14, Fo was still lower compared to BDC both in YM (-34.3%, P = 0.030) and OM (-27.0%, P = 0.043).

Figure 4: Metabolic parameters here

Relevant parameters of the glucose and lipid metabolism were explored in relation to a standardized mixed meal, as described in Methods. At BDC in the post-prandial state, OM exhibited comparable insulin sensitivity (measured by Matsuda index), but greater lipaemia (i.e., AUC triglycerides) than YM. Interestingly, this was associated with higher triglycerides and cholesterol concentrations in the plasma in fasting conditions in OM compared to YM (see above), but it was not related to a different body composition as, at baseline, the two groups did not show significant differences in percent body fat (Figure 4A). Body composition was altered by inactivity only in OM as indicated by the determination of fat mass percentage which increased at BR14 only in OM by 2.3 ± 3.2% (P = 0.022), see Figure 4A. Body mass, however, was not significantly altered by BR and rehabilitation: BMI, expressed in kg m^{-2}, was: in YM 24.0 ± 2.3, 23.1 ±2.1, 24.1±2.2, and in OM 26.6 ± 4.0, 25.5 ± 4.6, 26.4 ± 4.7, at BDC, end of BR and end of the rehabilitation, respectively.

The Matsuda index (Figure 4B) which measures postprandial insulin sensitivity showed a decrease with inactivity in both YM and OM. Surprisingly, the decrease of Matsuda index at BR14 was significantly greater in YM (-46.0 ± 11.9%; P = 0.003) than in OM (-19.8 ± 22.5%; P = 0.006) with an interaction effect P = 0.003 (see Figure 4B). In OM, Matsuda index fully

recovered at R+14. The post-prandial AUC of triglycerides (Figure 4C) showed at BR14 a minor and not significant variation in OM (P = 0.121), while it significantly increased in YM (47.2 ± 40.0%; P = 0.013) with an interaction effect P = 0.015.

Finally, at BR14 plasma homocysteine concentration in the fasting state (Figure 4D) increased from BDC only in YM (26.3 ± 21.4%; P = 0.027), whereas it did no change in OM.

DISCUSSION

~~The deleterious effects of muscle inactivity and reduced mechanical loading involve not only changes of skeletal muscle structure and contractile function, but also of bone, cardiovascular system and energy metabolism. Clinical evidence suggests that acute and long-term effects of reduced motor activity are more pronounced in the elderly than in the young (4,8). Such greater impact of a period of inactivity might be attributed to the aging process itself, but might be also due to concomitant chronic diseases or to the long lasting effects of previous diseases or traumatic events. To answer the question, several studies have been designed in the last decade with the aim of quantifying the impact of disuse and inactivity in healthy elderly. Three main experimental protocols have been adopted to reach this goal: bed rest (BR) (7,12,14,15,22,23,36), unilateral lower limb immobilization (ULLI) (17,34) and unilateral lower limb suspension (ULLS) (11,18,19). The latter two protocols have the advantage of a direct comparison of the muscles of the limb with reduced motor activity with the muscles of the contralateral limb, thus allowing a careful characterization of the muscle response. However, ULLI and ULLS protocols, have a very limited impact at whole body level on cardiovascular function and metabolism (39). In contrast, BR protocol not only reproduces directly the conditions of bedridden patients, but also allows the study of both muscle and whole body response. Among the few BR studies on elderly presently available, some lack a direct comparison with young subjects (12,14,15,23), whereas in the most recent study (36) which introduced a group of young subjects, the period of inactivity was so short that young subjects did~

~~not show any significant muscular adaptation in terms of loss of mass and contractile performance. Taking this into account, in the present study a BR experiment was designed ensuring that that i) young and elderly participants were exposed to exactly the same inactivity and rehabilitation protocols, ii) the inactivity or BR period (14 days) was sufficiently long to induce a response in the young as well as in the elderly, iii) the recovery was analyzed after a period (14 days) of rehabilitation of duration suitable to detect possible differences among the two age groups.~~

~~At the beginning, before BR, significant differences were present within the two groups of subjects. Such differences were expected as they can be considered expression of physiological aging. Quadriceps volume and contractile function of leg extensor muscles both in isometric conditions and during an explosive effort were lower in the OM (31). A lower force was detectable also in single fibre dissected from biopsy samples of vastus lateralis (10). Peak aerobic power, which during cycle ergometer exercise is mainly limited by cardiovascular function (16), was also lower in the OM, in accordance to expectations. The body composition was not significantly different, insulin resistance was not significantly greater in the OM, but AUC of triglycerides was significantly greater suggesting a moderate age-dependent metabolic impairment (5).~~

We designed a BR experiment to compare the response to disuse and rehabilitation in young and elderly subjects, according to the following principles: i) young and elderly participants were exposed to exactly the same inactivity and rehabilitation protocols, ii) the inactivity or BR period (14 days) was sufficiently long to induce a response in the young as well as in the elderly, iii) the recovery was analyzed after a period (14 days) of rehabilitation of duration suitable to detect possible differences among the two age groups.

In order to get an integrated or holistic view we aimed to detect changes at multiple levels from skeletal muscles which are obvious direct target of inactivity (QVOL, force, power, single fibres), to cardio-respiratory performance ($\dot{V}O_2peak$), to motor control (gait), to metabolism (Matsuda Index, Triglycerides AUC, plasma homocysteine) and to anthropometric parameters.

As expected, age-related differences were present among YM and OM at BDC, i.e. before the BR experiment started, and this made the comparison more complex. Therefore, for many parameters we determined the fractional or percent variations during BR and rehabilitation. Quadriceps volume and contractile function of leg extensor muscles both in isometric conditions and during an explosive effort were lower in OM (see also Ref. 31). A lower force was detectable also in single fibre dissected from biopsy samples of vastus lateralis (see Ref. 10). Peak aerobic power, which during cardio-respiratory maximal test at cycle ergometer is usually mainly limited by cardiovascular function (see Ref. 16), was also lower in the elderly, in accordance to expectations. In contrast, body composition was not significantly different, insulin resistance was not significantly higher in OMy, although AUC of triglycerides was significantly greater in the OM, suggesting a moderate age-dependent metabolic impairment (see 5).

Both YM and OM experienced a significant decrease of muscle volume (QVOL) during the two weeks of BR, but this was accompanied by an impaired contractile function in terms of maximal voluntary isometric force and explosive power (QF and QP) only in the elderly. The loss of QVOL per week in OM was approximately 4%, thus fully comparable to the values reported by Drummond et al. (14,15), and 30% greater of the weekly loss of QVOL observed in YM. The greater atrophic response in OM likely finds an explanation in the altered amino acid transport and mTOR signaling recently described by Tanner et al. (36). Moreover, while two weeks of rehabilitation were sufficient for a complete recovery of the QVOL in the YM, this was not the case in the OM. In fact, after two weeks of rehabilitation QVOL was still significantly lower compared to BDC in OM. This can be partly attributed to the peculiar increase of anabolic resistance observed in the elderly in relation to reduced locomotor activity (3). The contractile impairment of the knee extensor muscles (QF and QP) was consistent with the decrease in the force generating capacity of single muscle fibre (Fo), but might also imply a reduced neural drive. This is suggested also by the alteration of the gait parameters, observed only in the OM and not in the YM. Impairment of locomotor activity after BR in the elderly has been recently reported (7) and evidence of reduced neural drive in lower limb contraction during BR is also

available (29). The decrease in force developed by single muscle fibres was present both in YM and in OM and was comparable to findings in young subjects exposed to bed rest (28,37). Interestingly, the decrease in force was more pronounced in the YM, although statistical significance was not reached. Moreover, both in YM and in OM, the recovery period of two weeks was not sufficient to regain the BDC values. This is in accordance with recent data of Hvid et al. (18), who showed in a different disuse model (ULLS), that recovery of single muscle fibre after disuse occurs with a much slower time course than the decrease ~~that~~ during the inactivity period.

$\dot{V}O_2$peak was lower at BDC in OM compared to YM, as expected, and underwent a significant decline during BR in both groups. Similar results were reported by Kortebein et al. (23) where 10-day BR resulted in significant decline of -12.2% in maximal aerobic capacity in 11 older individuals. Importantly, our results showed that decline in the YM was 50% smaller than the decline in the OM and only in this latter group a recovery period of two weeks was not sufficient to regain the BDC values. Recent studies (32,33) have suggested a significant role of an impairment of skeletal muscle oxidative metabolism in limiting whole body oxidative function during exercise in young subjects exposed to bed rest. The specific role of skeletal muscles in limiting oxidative function following bed rest (and the subsequent rehabilitation) in the elderly remains to be elucidated.

At baseline, although OM exhibited a lower QVOL than YM, the overall body composition was similar in the two groups, as indicated by the similar percent fat mass. As stated above, a difference was present in the concentrations of cholesterol and triglycerides in fasting conditions and in the post-prandial handling of triglycerides, as shown by the higher post-prandial triglycerides AUC in the OM. This suggests a moderate age-dependent alteration of lipid metabolism in the post-prandial state (5). As expected according to previous BR studies (1,9), at BR14 in the young subjects insulin sensitivity significantly decreased, while postprandial lipaemia increased. Surprisingly, in OM the BR-induced insulin resistance was less pronounced than in YM. In fact, the Matsuda index of insulin sensitivity decreased at BR14 in OM only by

19.8% (versus 46% in YM) and post-prandial lipaemia did not change significantly from BDC values. ~~Two explanations could be considered for the lower impact of the BR on glucose and lipid metabolic in the elderly: a lower metabolic effect of inactivity or a higher ability to compensate such effect. In both cases, the changes in muscle volume do not seem to play a role. Actually, in the OM QVOL was lower before BR and underwent a further greater decrease at BR14. It is likewise unlikely to be due to the changes in muscle fiber type distribution, which was characterized by a greater abundance of slow fibers in the OM compared to the YM at BDC (P = 0.044; data not shown) and a trend, albeit statistically not significant, to a slow-to-fast shift in both group (data not shown). Also, such relative conservation of postprandial metabolic functions after BR cannot be explained by changes in adipose tissue. In fact, percent fat mass content increased slightly but significantly in the OM and remained unchanged in the YM. It seems, thus, to be the result of intrinsic metabolic changes in muscle and/or liver tissues, which are the major players in glucose and fat postprandial metabolism. Interestingly, homocysteine plasma level, which is considered a risk factor for coronary, cerebral and peripheral artery diseases (20), showed an increase only in YM and not in OM. Further work is required to understand the mechanism of such relative metabolic protection of the elderly during physical inactivity.~~

Interestingly, homocysteine plasma level, which is considered a risk factor for coronary, cerebral and peripheral artery diseases (20), showed an increase only in YM and not in OM. The lower impact of the BR on glucose and lipid metabolic parameters in the elderly is a very interesting finding and points to a lower sensitivity of the metabolism to inactivity in the elderly.

Although skeletal muscles play an important role in lowering glycemia, the observed changes in muscle volume in OM do not play in the direction of contrasting an increase in insulin resistance. Actually, in the OM, QVOL was lower before BR and underwent a further greater decrease at BR14. The same is true for the changes in muscle fiber type distribution, which was characterized by a greater abundance of slow fibers in the OM compared to the YM at BDC (P = 0.044; data not shown) and a trend, albeit statistically not significant, to a slow-to-fast shift in both groups (data not shown). A most likely explanation for the lower metabolic impact of BR in

the OM compared to YM is that the elderly were more close to a metabolic syndrome and already less active than the young and, therefore, the effects of a change in life style on metabolism were less marked. In our opinion, however, the issue deserves further investigations

It is worth to further underline that the analysis of recovery phase also provided important information, potentially relevant for translational medicine. While in YM all parameters describing muscle volume and function showed a complete recovery at the end of 14 days of rehabilitation ~~AR~~, in OM an incomplete recovery was detectable in QVOL and contractile performance as measured by QP. In addition, also VO_2peak showed at the end of the two weeks of rehabilitation a value lower than that determined at BDC before beginning BR. Finally, Fo measured on single muscle fibers in vitro did likewise not recover in a time interval of rehabilitation equal to the time interval of inactivity, in accordance with recent observations of Hvid et al (18). In our view, the lack of recovery, or at least, the slower recovery in OM is one of the most important messages given by the present study. Actually, the present data suggest that it is strictly necessary not only to pay special attention to the rehabilitation phase in the elderly, but also to design protocols to keep the elderly active even when hospitalization is strictly required.

In conclusion, two main and partially contradictory findings are the outcome of this comparative study on the adaptations to BR in healthy young and healthy old subjects, both exposed to precisely the same conditions of inactivity. The impact on muscle mass and function is greater in the older, while the metabolic alterations are greater in the younger adult men. The comparative analysis of the recovery period unambiguously highlights ~~ed~~ how in the older participants it was difficult to regain the pre-inactivity conditions. The greater detrimental effect of physical inactivity and the delayed recovery in older adults, documented ~~highlighted~~ by the present findings, strongly emphasize the importance of an active life style in old age, avoiding or minimizing periods of inactivity particularly when these are due to hospitalization and BR.

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Legends to figures

Figure 1: Changes in muscle volume and contractile function in relation to bed rest and rehabilitation. Changes in quadriceps volume (QVOL), force in isometric maximal voluntary contraction (QF) and explosive power (QP) of leg extensor muscles during bed rest and rehabilitation are shown in panel A, B and C, respectively. BDC baseline data collection at the beginning of bed rest; BR14 values determined after 14-day bed rest; R+14 values determined on 14^{th} day of recovery; MVC maximal voluntary contraction. Data are shown as mean and standard deviation. $ denotes significant difference between groups at BDC with $P < 0.05$; a,b,c denote significant difference from BDC at BR14 or R+14 with $P < 0.05$ (a), $P < 0.01$ (b), $P < 0.001$ (c); # denotes significant difference between groups at BR14 or R+14 after adjusting BDC differences with $P < 0.05$.

Figure 2: Changes in aerobic power and gait stride length in relation to bed rest and rehabilitation. Changes in peak aerobic power normalized to body mass are shown in panel A, while changes in gait stride length are shown in panel B. BDC baseline data collection at the beginning of bed rest; BR14 values determined after 14-day bed rest; R+14 values determined on 14^{th} day of recovery; MVC maximal voluntary contraction. Data are shown as mean and standard deviation. $ denotes significant difference between groups at BDC with $P < 0.05$; a,b,c denote significant difference from BDC at BR14 or R+14 with $P < 0.05$ (a), $P < 0.01$ (b), $P < 0.001$ (c); # denotes significant difference between groups at BR14 or R+14 after adjusting BDC differences with $P <0.05$.

Figure 3: Changes in single muscle fibre cross sectional area and isometric force in relation to bed rest and rehabilitation. The variations in average cross sectional area (CSA) of single muscle fibres dissected from a biopsy sample taken from vastus lateralis are shown in panel A, while the changes in isometric force developed by the same fibers during maximal activations are shown in panel B. BDC baseline data collection at the beginning of bed rest; BR14 values determined after 14-day bed rest; R+14 values determined on 14^{th} day of recovery; MVC maximal voluntary contraction. Data are shown as mean and standard deviation. $ denotes significant difference between groups at BDC with $P < 0.05$; a,b,c denote significant difference from BDC at BR14 or R+14 with $P < 0.05$ (a), $P < 0.01$ (b), $P < 0.001$ (c); # denotes significant difference between groups at BR14 or R+14 after adjusting BDC differences with $P < 0.05$.

Figure 4: Changes in body composition, postprandial insulin sensitivity, postprandial lipaemia and plasma homocysteine concentration in relation to bed rest and rehabilitation. The variations in body composition, expressed as % fat abundance as determined with bio-impedance measurement are shown in panel A. Insulin response, as determined by Matsuda test in a postprandial phase is shown in panel B. Area under the curve (AUC) of plasma triglycerides variations in postprandial phase is shown in panel C. Plasma levels of homocysteine are shown in panel D. BDC baseline data collection at the beginning of bed rest; BR14 values determined after 14-day bed rest; R+14 values determined on 14^{th} day of recovery; MVC maximal voluntary contraction. Data are shown as mean and standard deviation. $ denotes significant difference between groups at BDC with $P<.05$; a,b,c denote significant difference from BDC at BR14 or R+14 with $P < 0.05$ (a), $P < 0.01$ (b), $P < 0.001$ (c); # denotes significant difference between groups at BR14 or R+14 after adjusting BDC differences with $P < 0.05$.

Table 1. Baseline anthropometric parameters for the two groups of participants.

	Young	Old	P
N	7	16	
Age (years)	23.1 ± 2.9	59.6 ± 3.4	< 0.001
Stature (m)	1.77 ± 0.07	1.73 ± 0.05	0.192
Body mass (kg)	74.8 ± 8.8	79.9 ± 12.3	0.336
Body mass index (kg/m^2)	24.0 ± 2.4	26.6 ± 4.4	0.142