

# The effects of bed-rest and countermeasure exercise on the endocrine system in male adults: Evidence for immobilization-induced reduction in sex hormone-binding globulin levels

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**ABSTRACT.** *Background and aim:* There is limited data on the effects of inactivity (prolonged bed-rest) on parameters of endocrine and metabolic function; we therefore aimed to examine changes in these systems during and after prolonged (56-day) bed-rest in male adults. *Subjects and methods:* Twenty healthy male subjects underwent 8 weeks of strict bed-rest and 12 months of follow-up as part of the Berlin Bed Rest Study. Subjects were randomized to an inactive group or a group that performed resistive vibration exercise (RVE) during bed-rest. All outcome parameters were measured before, during and after bed-rest. These included body composition (by whole body dual X-ray absorptiometry), SHBG, testosterone (T), estradiol (E<sub>2</sub>), PRL, cortisol (C), TSH and free T<sub>3</sub> (FT<sub>3</sub>).

*Results:* Serum SHBG levels decreased in inactive subjects but remained unchanged in the RVE group ( $p < 0.001$ ). Serum T concentrations increased during the first 3 weeks of bed-rest in both groups ( $p < 0.0001$ ), while E<sub>2</sub> levels sharply rose with re-mobilization ( $p < 0.0001$ ). Serum PRL decreased in the control group but increased in the RVE group ( $p = 0.021$ ). C levels did not change over time ( $p \geq 0.10$ ). TSH increased whilst FT<sub>3</sub> decreased during bed-rest ( $p$  all  $\leq 0.0013$ ). *Conclusions:* Prolonged bed-rest has significant effects on parameters of endocrine and metabolic function, some of which are related to, or counteracted by physical activity.

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## INTRODUCTION

Experimental bed-rest in healthy subjects, typically used as a model for spaceflight simulation (1), results in profound changes in a number of body systems. The most evident amongst these changes are a loss of muscle and bone mass, particularly in the load-bearing skeleton (2) as well as the development of orthostatic intolerance (3). Some studies have investigated the effect of prolonged bed-rest on endocrine regulation, although most of these have focused on glucocorticoid-related changes (4-16). In contrast, few studies have investigated the effects of prolonged bed-rest on multiple and interacting endocrine and metabolic parameters. In particular, sex hormones, their binding proteins and prolactin have received comparatively little attention in the spaceflight and bed-rest literature. Some studies have included these indices in short ( $\leq 7$ -days) (8, 14, 17) and longer term (15- to 42-days) (12, 16, 18, 19) bed-rest studies, but the results of these investigations remain controversial. Thus, some reports have found testosterone or prolactin levels to either remain unchanged (12, 17-19) or to decrease (8, 14, 16, 20). Furthermore, epidemiological studies (21-23)

have reported conflicting results in examining the relationship between sex steroid hormones and physical activity. Also, we have been unable to identify any studies considering changes in SHBG levels during bed-rest or spaceflight.

This lack of reliable data is somewhat surprising given the potential of endocrine regulation of sex and other centrally controlled hormones to impact upon musculoskeletal health. In the present study, we explored the effects of prolonged (56 days) bed-rest on clinically relevant parameters of endocrine regulation in young male adults, with an emphasis on sex hormones. To aid in the understanding of potential changes in the regulation of these indices, serum levels of cortisol, creatine kinase, TSH and free T<sub>3</sub> (FT<sub>3</sub>), as well as changes in body composition (by whole body dual X-ray absorptiometry) were measured.

## METHODS

### *Bed-rest protocol*

The Berlin Bed Rest Study was undertaken at the Charité Campus Benjamin Franklin Hospital in Berlin, Germany, from February 2003 to June 2005. Twenty male subjects underwent 8-weeks of strict horizontal bed-rest with a subsequent 12-month follow-up recovery period. The bed-rest protocol, as well as inclusion and exclusion criteria, is discussed in detail elsewhere (24). In brief, however, subjects were randomly allocated to either a group that remained inactive (control, CTRL group) or a group that underwent a whole body resistive vibration exercise countermeasure programme (RVE group) using the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany).

*Key-words:* Spaceflight, microgravity, inactivity, Berlin Bed Rest Study.

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Table 1 - Baseline (BDC-2) endocrinological and metabolic marker levels.

Parameter	Subject group	
	CTRL	RVE
no.	10	10
Age (yr)	33.4 (6.6)	32.6 (4.8)
Height (cm)	185 (7)	183 (9)
Weight (kg)	79.4 (9.7)	81.7 (14.4)
Body mass index (kg/m <sup>2</sup> )	23.3 (1.7)	24.2 (2.6)
Lean mass (kg)	57.8 (4.9)	56.4 (6.5)
Fat mass (kg)	13.1 (5.5)	17.0 (7.5)
Testosterone (µg/l; 0.020)	5.9 (1.2)	5.5 (1.1)
SHBG (nmol/l; 0.35)	40.1 (8.4)	32.3 (12.2)
PRL (µg/l; 0.47)	19.4 (10.4)	14.7 (8.3)
Estradiol (ng/l; 5.0)	28.2 (9.5)	27.9 (7.3)
Cortisol (µg/l; 0.18)	185.3 (37.0)	155.4 (43.7)
Creatine kinase (U/l; 7)	124.3 (53.1)	202.0 (152.7)
TSH (mIU/l; 0.005)	1.3 (0.7)	1.4 (0.7)
Free T <sub>3</sub> (pmol/l; 0.4)	5.0 (0.6)	5.2 (0.7)

Values are mean (SD). No differences between groups at baseline ( $F_{1,18} < 2.6$ ,  $p > 0.11$ ). Values given after units of concentration of each parameter represent the "lower limit of detection" which is capable of being differentiated from a concentration of zero. The reader will note that the concentrations found in the current study are well above these limits. CTRL: control group; RVE: resistive vibration exercise group.

Mainly for practical reasons but also to avoid seasonal effects impacting upon the findings of the study, five campaigns were conducted of four subjects (two control, two countermeasure exercise) each over the course of 14 months. Baseline anthropometric characteristics are given in Table 1. The Ethics Committee of the Charité University Medicine approved this study and subjects gave their informed written consent.

#### Countermeasure exercise

RVE was performed using a dedicated prototype (Galileo Space) of a commercially available vibration platform (Novotec Medical, Pforzheim, Germany). The countermeasure exercise protocol is described in detail elsewhere (24). In brief, the subjects were placed in supine position (Fig. 1), an axial force between 1.0 and 1.8 times body weight was placed through the subjects' trunk and spine via elastic shoulder straps and whole-body vibration was applied at the feet. Six morning and five afternoon exercise sessions of 30 min duration each week were performed. For each morning session, the following exercises were performed: squatting, heel raises, toe raises, explosive kicks. In the afternoon session, only one exercise was performed and subjects retained their feet on the platform in nearly extended position without movement. Further details of the training protocol have been published elsewhere (25).

#### Nutrition

The diet during bed-rest was balanced with regards to caloric intake, using the Harris-Benedict equation (26) with an adjustment by an activity factor of 1.2 for bed-rest. Calcium input was set at 1000 mg/day. Daily diet plans were prepared, using the nutrition-software EBISpro (Dr. Erhardt, University of Hohenheim, Germany). All meal components were weighed, and their nutritional contents [energy (kJ), water (g), protein (g), fat (g), carbohydrates (g), vitamin D (g), sodium (mg), potassium (mg),

calcium (mg), magnesium (mg), iron (mg)] were taken from prepared meal charts. Every second Sunday, higher caloric intakes (i.e. pizza or pasta meal of own choice) were allowed to keep up motivation. Resting energy expenditure was regularly assessed to correct the caloric intake if necessary.

#### Whole body composition

Whole body dual X-ray absorptiometry (DXA; Delphi W; Hologic, Waltham, MA) was performed following standard procedures at baseline (baseline data collection, BDC), 3 days prior to the start of bed-rest (BDC-3), on bed-rest (BR) days BR2, BR17, BR31, BR45 and BR55 and on post-bed-rest recovery (R+) days R+14, R+28, R+90, R+180 and R+360. Due to the height of the subjects and the technical requirements of the scanner, it was not possible to include the head in a number of the subjects during measurement. Sub-total (excluding head) bone, lean and fat tissue mass (kg) were calculated. All scanning and analyses were performed by the same operator to ensure consistency and standard quality control procedures were followed. The sub-total bone mass data has been published elsewhere (25), but is used here for correlation analyses.

#### Blood drawings

Venous blood samples were taken between 07:00 and 08:00 h after 12 h in fasting state during baseline collection at two days before the start of bed-rest (BDC-2), on BR1, BR3, BR5, BR12, BR19, BR26, BR33, BR40, BR47 and BR56 and on R+1, R+3, R+7, R+28, R+90 and R+180. Samples were centrifuged at 3500 rpm for 10 min 30 min after drawing, serum obtained and stored at -80 C until analysis.

#### Serum analyses

TSH, FT<sub>3</sub>, cortisol, SHBG, total testosterone, prolactin and estradiol (Roche Diagnostics, Penzberg, Germany) were measured by means of an automated electrochemiluminescence immunoassay (ECLIA). The MODULAR ANALYTICS® E170 immunoanalyzer (Roche Diagnostics, Penzberg, Germany) is using ruthenium electrochemiluminescence and biotinylated components, together with streptavidin coated microparticle technol-



Fig. 1 - Resistive vibration exercise countermeasure during bed-rest. Subjects were required to perform leg exercises against a resistive force transmitted via belts at the waist and shoulders and via hand-grips. Vibratory stimuli in the legs are generated by rotation of the suspended platform around a vertically oriented axis. Axial loading of the spine occurs via the shoulder straps.

ogy resulting in fast assays (either sandwich or competitive assay formats) with high sensitivity, low imprecision and a broad measuring range. Total imprecision data of the various assays have been retrieved from our internal quality assurance database and are given according to the concentration range of the measured hormone values in the healthy male subjects listed in Table 1. The coefficient of variation was 3.9% for testosterone, 3.4% for SHBG, 4.1% for prolactin, 4.6% for estradiol and 2.4% for cortisol, respectively. Creatine kinase (NAC-act) was measured by means of an automated clinical chemistry analyzer MODULAR ANALYTICS®P-Module (Roche Diagnostics, Penzberg, Germany). The coefficient of variation was 2.5% for creatine kinase. All analytical platforms and assays were used in the laboratory for routine testing. They were run strictly in accordance with the guidelines given by the manufacturers and were subject to continuous maintenance and service according to the laboratories standard operating procedures for good laboratory practice. Samples were shipped on dry ice to the laboratory (Labor Limach, Heidelberg, Germany) and stored at  $-80^{\circ}\text{C}$  until analysis. Serum samples were thawed at the day of analysis at ambient temperature, homogenized on a head-over-head mixer and centrifuged before measurement. To further reduce imprecision of measurement, all serum samples were analyzed in one batch utilizing one reagent lot.

### Statistical analysis

Linear mixed-effects models were employed in statistical analysis (27). For all variables separate statistical models were built for the bed-rest and recovery phases. In analysis of variance (ANOVA) for each parameter, fixed effects of subject group (CTRL, RVE) and study date, as well as a 2-way interaction between these variables were considered. Baseline data were included as a linear covariate in analyses. The "R" statistical environment (version 2.4.1, www.r-project.org) was used to implement these analyses. An  $\alpha$  of 0.05 was taken for statistical significance. The normality of distribution of residuals from model fitting was checked via diagnostic plots.

Partial correlation analyses (controlling for study date, subject-group and their interaction) were performed between the serum markers and body-composition variables. SPSS for Windows (version 10.0.1, www.SPSS.com) was used in these analyses. An  $\alpha$  of 0.001 was taken for statistical significance.

## RESULTS

No baseline differences existed between groups for sub-total (without head) fat or lean mass ( $F < 1.63$ ,  $p > 0.21$ ) (Table 1) or for any of the serum markers examined ( $F < 2.6$ ,  $p > 0.11$ ) (Table 1). For recovery examinations 2 weeks after bed-rest (R+14) and beyond, data were not available from all subjects (Table 2). On the remaining testing days before, during and in the first week after bed-rest data were available from all subjects.

### Whole body composition (dual X-ray absorptiometry)

Significant changes in fat mass occurred during both bed-rest [study date (BDC to BR55):  $F = 19.6$ ,  $p < 0.0001$ ] and recovery [study date (BR55 to R+360):  $F = 2.8$ ,  $p = 0.039$ ], with the CTRL group showing increases in sub-total body fat over the course of bed-rest which persisted through the recovery phase ( $p = 0.09$  at R+360) (Table 3). The increase in sub-total body fat was less pronounced in the

Table 2 - Number of subjects available for analysis at each study-date in recovery.

Study-date	Serum parameters		DXA		
	CTRL	RVE	CTRL	RVE	Study-date
			9	9	R+14
R+28	10	9	10	9	R+28
R+90	10	9	10	9	R+90
R+180	9*	10*	9	10	R+180
			9	9	R+360

\*For TSH and free  $T_3$  at R+180 there were samples available from 7 subjects in the control (CTRL) group and 8 subjects in the resistive vibration exercise (RVE) group. On all other testing dates, data/samples from 10 subjects in each group were available. R+: day of recovery; DXA: whole body dual X-ray absorptiometry.

RVE group [on average between BR31 and BR55 RVE increased 6.2(3.5)%,  $p = 0.094$  and CTRL increased 9.7(3.6)%,  $p = 0.015$ ], but this difference was not statistically significant (group $\times$ study date:  $F$  both  $\leq 1.8$ ,  $p$  both  $> 0.11$ ).

ANOVA showed only a trend for changes in sub-total body lean mass during bed-rest [study date (BDC to BR55):  $F = 2.3$ ,  $p = 0.061$ ] (Table 3). The CTRL group showed marginal (on BR31 and BR55  $p = 0.06$ ) decreases in lean body mass during bed-rest (Table 3) and although the RVE group did not, these marginal differences between groups were not significant [group $\times$ study date BDC to BR55):  $F = 0.5$ ,  $p = 0.72$ ]. In the recovery phase there was evidence for a different response in the two groups [study date (BR55 to R+360):  $F = 6.1$ ,  $p = 0.0001$ : group $\times$ study date (BR55 to R+360):  $F = 3.1$ ,  $p = 0.014$ ] with the CTRL group showing increases in lean mass above pre-bed-rest levels and the RVE group not deviating significantly from pre-bed-rest levels (Table 3).

### Endocrine and metabolic parameters

With the exception of cortisol, ANOVA suggested significant changes in all parameters (SHBG, prolactin,

Table 3 - Body composition from dual X-ray absorptiometry.

Study-date	Lean mass (kg)		Fat mass (kg)	
	CTRL	RVE	CTRL	RVE
Baseline	57.8 (1.6)	56.4 (2.1)	13.1 (1.9)	17.0 (2.4)
BR18	57.6 (1.6)	56.4 (2.1)	13.0 (1.9)	16.8 (2.4)
BR31	57.0 (1.6)	56.0 (2.1)	14.1 (1.9)‡	17.5 (2.4)
BR44	57.3 (1.7)	56.9 (2.1)	14.3 (1.9)‡	17.1 (2.5)
BR55	57.0 (1.6)	55.7 (2.2)	15.2 (1.9)‡	18.6 (2.5) <sup>o</sup>
R+14	58.5 (1.6)	57.3 (2.1)	15.7 (1.9)‡	18.5 (2.5) <sup>o</sup>
R+28	58.6 (1.6)	57.3 (2.1)	15.7 (2.0)‡	18.3 (2.5)*
R+90	58.7 (1.6)*	56.9 (2.2)	15.4 (2.1) <sup>o</sup>	16.8 (2.5)
R+180	59.1 (1.6) <sup>o</sup>	56.0 (2.1)	15.2 (2.1)*	16.3 (2.5)
R+360	58.8 (1.6)*	56.1 (2.2)	14.9 (2.2)	16.0 (2.6)

Values are mean (SEM). \* $p < 0.05$ ; <sup>o</sup> $p < 0.01$ ; <sup>‡</sup> $p < 0.001$  and indicate significance of difference to baseline value (combined data from BDC-3 and BR2). BR: day of bed-rest; R+: day of recovery; CTRL: control group; RVE: resistive vibration exercise group. There was little evidence on analysis of variance for a group difference on changes in body composition during bed-rest ( $p > 0.11$ ). After bed-rest, evidence for a different response in the two groups was present for lean-mass only ( $p = 0.014$ ). See text for further details.

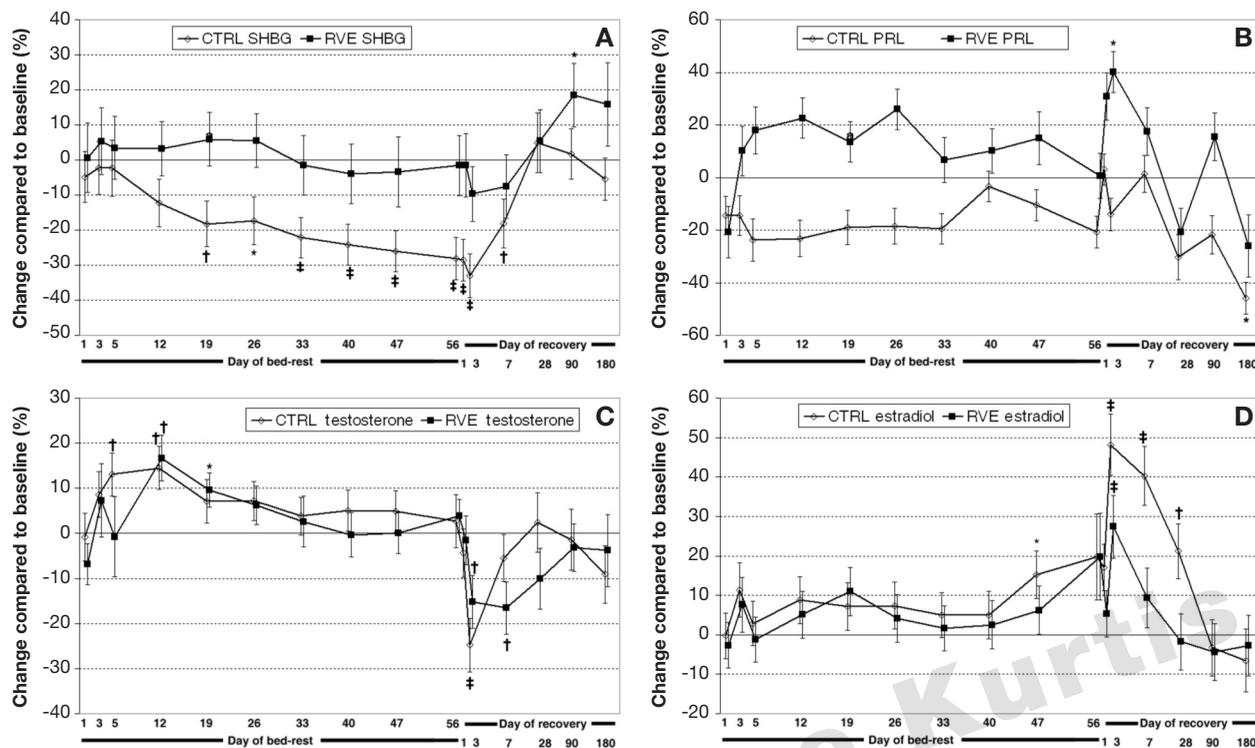


Fig. 2 - Effect of bed-rest and countermeasure exercise on sex hormones. A) SHBG; B) PRL; C) total testosterone; D) estradiol. Values are mean (SEM) percentage change compared to baseline. Time axis is to scale up until R+7. Control (CTRL) and resistive vibration exercise (RVE) groups have been offset slightly. Significance of difference to baseline indicated by: \* $p < 0.05$ ; † $p < 0.01$ ; ‡ $p < 0.001$ . Increase of estradiol at R+1 in the CTRL group is significant ( $p = 0.0036$ ). R+1 blood-drawing occurred prior to subjects re-ambulating.

testosterone, estradiol, TSH,  $FT_3$ , creatine kinase) in both the bed-rest ( $F \geq 2.6$ ,  $p \leq 0.0035$ ) and recovery phases ( $F \geq 5.4$ ,  $p \leq 0.0002$ ). Serum SHBG concentrations continuously decreased in the CTRL group during bed-rest and this reduction persisted for up to a week after remobilization (Fig. 2A). By day 28 post recovery, serum SHBG levels had reached baseline levels. In the RVE group, in contrast, serum SHBG levels remained unchanged during bed-rest, but increased significantly during recovery. The differences between the CTRL and RVE groups in SHBG were significant in bed-rest ( $F = 9.6$ ,  $p < 0.0001$ ) and recovery ( $F = 48.2$ ,  $p < 0.0001$ ).

Although no significant changes compared to baseline occurred for serum prolactin within each group during bed-rest (Fig. 2B), prolactin decreased on average during bed-rest by 15.0% in the CTRL group (BR3-R+1) and increased by 15.3% in the RVE group. The difference between the groups during bed-rest was significant ( $F = 2.1$ ,  $p = 0.021$ ). After re-ambulation (R+3 and beyond), prolactin decreased similarly in both groups. Total testosterone concentrations increased early during bed-rest up until day 19 in both groups (Fig. 2C) by which time they returned to baseline until re-ambulation. During recovery, testosterone concentrations dropped significantly and remained decreased up until R+7. The responses were similar in both subject groups ( $F \leq 1.9$ ,  $p \geq 0.10$ ). Serum estradiol concentrations increased towards the end of bed-rest (Fig. 2D), but countermeasure exercise did not appear to impact upon these

changes ( $F = 0.63$ ,  $p = 0.80$ ). Serum estradiol levels increased sharply following re-ambulation, and these changes were more pronounced in the CTRL group than in the RVE group ( $F = 3.3$ ,  $p = 0.0090$ ).

Serum TSH was increased during bed-rest and also during recovery (Fig. 3A), with no evidence for a different response between the two groups ( $F \leq 1.7$ ,  $p \geq 0.15$ ). Serum  $FT_3$  concentrations decreased within the first few days of bed-rest, but then recovered to baseline levels within the first week after re-ambulation (by R+7) (Fig. 3B), with both subject groups showing a similar response ( $F \leq 1.4$ ,  $p \geq 0.25$ ). Cortisol levels were generally decreased in the CTRL group ( $p < 0.022$  at BR5, BR12 and BR33) with no changes in the RVE group, and after re-ambulation, cortisol levels tended to increase in the RVE group ( $p < 0.015$  at R+3 and R+90) and return to baseline in the CTRL group (Fig. 3C). Despite this, little evidence existed overall from ANOVA supporting the hypothesis of changes in cortisol levels during bed-rest or for different response in the two groups in either phase of the study ( $F \leq 1.6$ ,  $p \geq 0.10$ ). Serum creatine kinase levels decreased to a similar extent in both groups during bed-rest ( $F = 1.3$ ,  $p = 0.21$ ) (Fig. 3D). Increases in serum creatine kinase were seen after bed-rest up until 28 days later and whilst the increase in creatine kinase in the CTRL group was significant at R+3, but not in the RVE group (Fig. 3D), ANOVA did not provide evidence for a different response of the two groups after bed-rest ( $F = 0.7$ ,  $p = 0.65$ ).

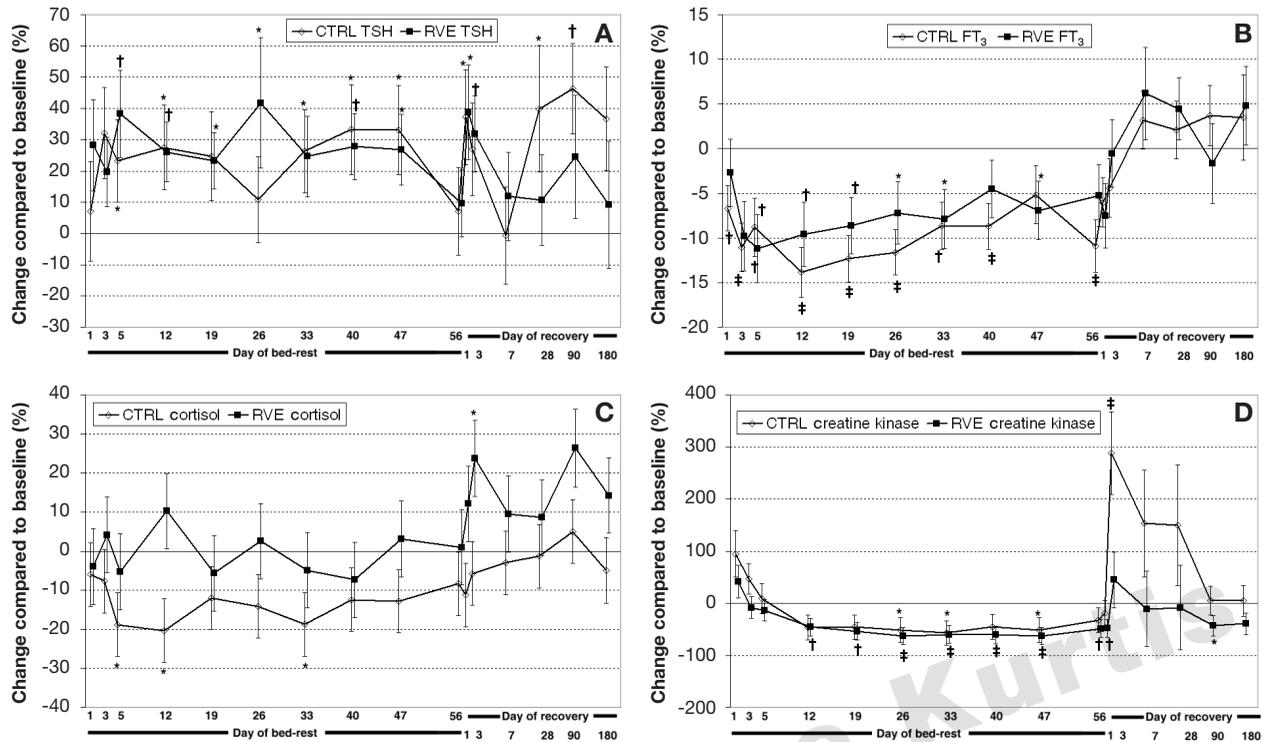


Fig. 3 - Effect of bed-rest and countermeasure exercise on thyroid hormones, cortisol and creatine kinase. A) TSH; B) free  $T_3$  ( $FT_3$ ); C) cortisol; D) creatine kinase. Values are mean (SEM) percentage change compared to baseline. Time axis is to scale up until R+7. Control (CTRL) and resistive vibration exercise (RVE) groups have been offset slightly. Significance of difference to baseline indicated by: \* $p < 0.05$ ; † $p < 0.01$ ; ‡ $p < 0.001$ .  $FT_3$  is significantly lower than at baseline on R+1 ( $p < 0.05$ ). R+1 blood-drawing occurred prior to subjects re-ambulating.

### Partial correlation analyses

The aim of these partial correlation analyses was to help evaluate potential mechanisms underlying the changes seen during bed-rest and as a result of countermeasure exercise. By (statistically) controlling for the effect of bed-rest and/or exercise, it is possible to see if there is any covariation in movement of parameter values. This helps to indicate whether there could be a link between changes in various parameters without the confounding factor of bed-rest and/or exercise. Partial correlation analyses showed, after controlling for the main effects of study date, training group and their interaction, no relationship between changes in bone or lean mass and changes in the endocrine or metabolic parameters (Table 4). However, increases in fat mass were associated with decreases in SHBG. Partial correlation analyses between the individual endocrine and metabolic parameters (Table 5) showed that increases in prolactin were associated with increases in cortisol and that increases in testosterone were associated with increases in both estradiol but decreases in TSH.

### DISCUSSION

The main findings of the current study with respect to sex steroids were a reduction of SHBG in the inactive (control) subjects during 56 days of bed-rest, but stable levels of SHBG in the exercise subjects. Furthermore, total testosterone levels increased in the first 3 weeks of

Table 4 - Partial correlations between body-composition and endocrinological/metabolic markers.

	Body composition (DXA)		
	Bone mass	Fat mass	Lean mass
Cortisol	-0.09	0.13	0.07
Testosterone	0.12	-0.07	-0.11
SHBG	-0.15	-0.60†	-0.37
PRL	0.01	0.21	0.09
Estradiol	0.06	-0.04	-0.09
TSH	-0.12	-0.10	0.06
$FT_3$	-0.32	0.00	-0.15

Values are partial correlation coefficients controlling for study date, subject group and their interaction. Data from bed-rest phase only used. † $p < 0.001$ . DXA: dual X-ray absorptiometry;  $FT_3$ : free  $T_3$ .

bed-rest, and then returned to baseline in both groups, but decreased in the first week after re-ambulation. Prolactin levels decreased by 15.0% in the inactive subjects and increased by 15.3% in the countermeasure subjects, but the differences compared to baseline levels were not significant. Decreases in SHBG levels during bed-rest correlated moderately with increases in total body fat, but no significant correlation was seen between the other endocrine and metabolic parameters and changes in body composition.

To our knowledge, this is the first study to examine SHBG

Table 5 - Partial correlations between endocrinological and metabolic markers.

	Creatine kinase	Cortisol	Testosterone	SHBG	PRL	Estradiol	TSH
Cortisol	-0.02						
Testosterone	0.13	-0.20					
SHBG	-0.05	-0.17	0.34				
PRL	-0.11	0.51 <sup>‡</sup>	0.01	-0.29			
Estradiol	0.03	-0.04	0.55 <sup>‡</sup>	-0.04	0.30		
TSH	-0.23	0.36	-0.47 <sup>‡</sup>	-0.06	0.13	-0.31	
FT <sub>3</sub>	0.23	0.19	0.04	0.17	-0.13	-0.06	0.05

Values are partial correlation coefficients controlling for study date, subject group and their interaction. Data from bed-rest phase only used. <sup>‡</sup>p<0.001.

in spaceflight simulation. The different response of SHBG in the exercise and inactive control subjects during bed-rest is quite remarkable, and not wholly explained by changes in other hormones. T<sub>3</sub> is known to be important in stimulating SHBG secretion (28). Increased concentrations of testosterone early in bed-rest could also result in a reduction of SHBG levels (29). Whilst these findings may help to explain the changes in SHBG seen in the inactive subjects, they cannot explain the stable SHBG values seen in the countermeasure group: both groups showed similar patterns of FT<sub>3</sub> (decrease) and testosterone (increased up to week 3 of bed-rest). Body fat, in contrast, is associated with SHBG levels, with obese people usually showing lower levels (30) and anorexic females showing higher serum SHBG concentrations (31). Increased levels of insulin were seen in both subject groups (unpublished observations) and the changes in insulin were uncorrelated ( $r=-0.09$ ) with the changes in SHBG. Hence, changes in resting insulin levels are unlikely to be able to explain the changes observed in SHBG. Unfortunately, data on insulin resistance and carbohydrate intake are not available for these subjects. In the current study, however, a moderate inverse correlation was seen between changes in total body fat and changes in SHBG levels within subject during bed-rest. This may form part of the explanation for decreases in SHBG levels in the inactive subjects, but given that the exercise subjects also showed increased fat levels despite stable SHBG levels, this association is unlikely to completely explain the phenomenon.

Physical activity itself may help to explain the differential response of SHBG in the two subject groups. With the additional physical activity load imposed upon the exercise subjects, this may have been the key factor in preventing reductions in SHBG. Cross-sectional studies have suggested that reductions in physical activity influence serum levels of SHBG (21-23).

It is noteworthy that acute resistance exercise has been shown to increase SHBG levels (32, 33) and although no studies have yet examined the effect of whole-body vibration exercise on SHBG, whole-body vibration has been shown to impact upon growth hormone (34, 35), glucose metabolism (35) and catecholamine levels (35-37). However, given the limited relationship in the current study of changes in SHBG with other endocrinological

and metabolic markers during bed-rest, understanding the mechanisms of any relationship between physical activity and SHBG is difficult. Understanding this is, however, important as lower SHBG levels have been found predictive of the subsequent development of metabolic syndrome (38) and prior works have considered the potential link between SHBG and cardiovascular disease (39).

The increases in testosterone concentrations at the beginning of bed-rest and decreases upon re-ambulation are unlikely to be associated with changes in muscle or bone mass. The administration of testosterone in males is well-known to promote increases in muscle mass (40) and lower serum levels of free testosterone in men are found to correlate with lower levels of muscle mass (41). In contrast, in the current study, concentrations of total testosterone (and presumably free testosterone given changes in SHBG) are increased as muscle mass is being lost. Furthermore, the countermeasure exercise did not influence changes in testosterone and no correlation was apparent between changes in testosterone and changes in bone or muscle. Changes in hemoconcentration in the first few weeks of bed-rest with subsequent re-hydration after bed-rest may explain the 15.6% increase during bed-rest (BR12) and subsequent 20.0% decrease after bed-rest (R+3) of total testosterone concentration, but unfortunately data on hematocrit or hemoglobin levels in these subjects are not available. Prior studies have found hematocrit levels (an indicator of hemoconcentration) to be increased during bed-rest (42) and decreased afterwards (43). Thus, fluid loss and hemoconcentration may have played a role in the changes seen in testosterone in the current study, but this is unclear. Irrespective of the underlying cause, pharmacodynamic studies (44, 45) suggest the half-life of testosterone to be on the order of weeks, which fits well with the amount of time taken for testosterone concentrations to return to baseline levels (by day 33 of bed-rest). Prior works in bed-rest have shown conflicting results with decreases in testosterone levels in during bed-rest (14, 16) or no change (17, 18). Evidently, the effect of extreme changes of activity (such as bed-rest) on testosterone levels is not clear, but the correlation analyses in current study suggests that endogenous testosterone levels do not appear to influence losses of muscle or bone during bed-rest.

Estradiol levels were generally increased during bed-rest, though this effect was significant only late in bed-rest. The additional exercise during bed-rest in the countermeasure group did not appear to affect any influence on estradiol levels. Also, no relationship was apparent between changes in estradiol levels during bed-rest and changes in muscle or bone mass. A prior study examining the effect of bed-rest on estradiol in men (46) found that estradiol levels were increased by 9.1% on the third day of bed-rest, which is comparable to our results. After bed-rest, marked increases in estradiol levels were seen with the inactive subjects showing greater increases in estradiol than the countermeasure exercise subjects. Other authors have suggested estrogen to have a role in protecting against muscle tissue damage (47), with more recent animal studies supporting this assertion (48) and other works showing higher estrogen levels facilitates mus-

cle repair after damage (49). Furthermore, women (having higher estrogen levels) show a more limited inflammatory response than men in response to eccentric exercise (50) and animal studies show the treatment of spinal-cord injured rats with estradiol reduces edema and inflammation (51). Upon re-ambulation, due to greater muscular deconditioning (52, 53), the inactive subjects likely experience more muscle damage, which is supported by the findings of a 290% increase in creatine kinase 3 days after bed-rest in the inactive subjects, but only a 45% increase in the exercise subjects. Whilst evidence suggests that estrogen can act to reduce muscle damage in response to overloading, we have not identified any works examining whether estrogen increases in response to muscle damage and the role of estrogen in muscle damage remains controversial (54). It remains possible, however, that the increases of estradiol in the current study after bed-rest may be part of an endocrine response to muscle damage after muscle reconditioning and re-loading for protection and/or repair of muscle tissue.

The responses of prolactin and cortisol to bed-rest were limited, through the effects seen may be associated with stress responses. Prior investigations in bed-rest and spaceflight (4-16) have not shown a consistent pattern of change in glucocorticoids, though cortisol has been shown to increase in response to acute stress in bed-rest (55). Prolactin has been studied little with one 4-day bed-rest study finding no change (8) and another 42-day study finding an increase in prolactin in the first 5 weeks with subsequent return to baseline levels prior to the end of bed-rest (12). Prolactin is also known to be released in response to stress (56) and we observed a moderate correlation between the changes in prolactin and cortisol levels. It may be that the inactive subjects experienced lower levels of stress during bed-rest (or higher levels prior to bed-rest) than the exercise subjects. It is also worth noting that although the administration of glucocorticoids can have a catabolic effect on muscle and bone, there is little evidence, both from the current or other studies for a consistent impact of the glucocorticoids on bone or muscle loss during bed-rest. Indeed, prior work has suggested that increases in glucocorticoid levels within normal physiological ranges do not influence muscle protein breakdown (57). Furthermore, we observed no correlation between within-subject changes in cortisol and bone or muscle mass. Overall, these results suggest that endogenous cortisol has little influence upon muscle or bone loss during bed-rest.

It is important to mention some specific limitations of the current study. Firstly, the small sample size could have resulted in certain comparisons not reaching statistical significance. Hence, some caution should be applied when interpreting the non-significant findings from the current study. Furthermore, some other parameters, such as C-reactive protein, and hormone receptor density, could not be measured which could help to shed further light on some findings. A number of parameters (e.g. cortisol) have a typical 24-h variation in their concentration and we performed all of our measurements at the same time of day (08:00 h) in order to avoid the influence of intra-day variation on our results. It remains a possibility

however, that measurements performed at other times of the day may have then shown different effects of bed-rest. Also, in the current work, we report total testosterone and estradiol concentrations as calculations of "free" or "bioavailable" hormones has never been clearly proven to be of any clinical relevance: the effects of all (sex) hormones depend on tissue metabolism, receptor density and a number of other factors that cannot easily be measured and hence we feel attempting to calculate free or bioavailable levels of certain hormones would not aid to enlighten us further as to the potential effects of bed-rest on the endocrinological system. Analyses of receptor density would however improve our ability to understand the physiological implications of the changes seen and could be considered in future work.

In conclusion, the current study found that SHBG levels are reduced significantly during bed-rest in inactive subjects but remained stable in subjects undergoing a RVE program during bed-rest. Increases of estradiol levels were seen after bed-rest and are in response to muscle damage upon re-ambulation. The alteration of thyroid function (TSH, FT<sub>3</sub>) can largely be explained by decreased energy expenditure (13, 58). Prolactin showed little change during or after bed-rest. Changes in the sex hormones, cortisol, TSH and FT<sub>3</sub> appear unrelated to losses of muscle or bone in men during bed-rest. Due to the limited number of subjects, caution should be applied in interpreting these negative findings.

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## Disclosure statement

Dieter Felsenberg and Jörn Rittweger are acting as consultants to the European Space Agency and Novotec Medical for the exploitation of this study's results. All other authors have no conflicts of interest.

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