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ORIGINAL ARTICLE

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Does resistance exercise prevent body fluid changes after a 90-day bed rest?

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Abstract Although various exercise regimens are commonly used as countermeasures to reduce the cardiovascular deconditioning induced by microgravity, the underlying mechanisms are not well understood. In this study we aimed to test whether lower limb resistance exercise with flywheel technology can prevent the fluid homeostasis alterations induced by 90-day head-down tilt bed-rest (HDT), and thus improve orthostatic tolerance. Total body water (TBW, measured by isotope dilution) and plasma volume (PV, calculated from the haemoglobin and the haematocrit) were measured in a control group (Co, $n=9$) and a countermeasure group (CM, $n=9$). Simultaneously, plasma atrial natriuretic peptide (ANP), renin (AR), and aldosterone (Aldo), as well as urinary anti-diuretic hormone (ADH), were measured. Orthostatic tolerance was evaluated with a 10 min $+80°$ tilt-test the first day of recovery. After HDT, both groups showed a comparable decrease in orthostatic tolerance [8.2 (0.9) min, Co; 8.0 (0.7) min, CM], PV [−4.7 (1.8)%, Co; –6.2 (2.5)%, CM, $P<0.05$] and TBW [−6.3 (5.4)%, Co; –3.7 (2.1)%, CM, $P<0.05$]. AR [97.4 (22.0)%, Co; 117.3 (26.4)%, CM] and Aldo [111.3 (58.4)%, Co; 100.6 (52.0)%, CM] increased significantly in both groups but the countermeasures produced no noticeable effects [data are expressed as mean (SE)]. The drop in ANP was also similar in both groups [−42.0 (15.2)%, Co; –51.1 (27.7)% for the CM]. Surprisingly, urinary ADH declined similarly in both groups during the basal data control period [−25.3 (5.2)%, Co; −26.1 (9.6)%, CM) and was sustained at this level during the 90-day HDT. These results show that, under the conditions described, the flywheel exercise device failed to improve characteristic manifestations of cardiovascular deconditioning and suggest that more frequent and powerful exercise, associated with another device (e.g. LBNP) might be a better countermeasure.

Keywords 90-Day head-down tilt bed rest · Cardiovascular hormones · Flywheel exercise device · Plasma volume · Total body water

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Introduction

Although man is preparing to fly to Mars, the cardiovascular deconditioning induced by exposure to microgravity is still a clinical problem (or source of concern) as 50% of astronauts exhibit symptoms of orthostatic intolerance (OI) when returning to Earth's gravity (Convertino 1996). The physiopathogenesis of OI is not well understood but is likely to be multi-factorial (Convertino 1996; Pavy-Le Traon et al. 1999). Of the factors involved, alterations in fluid homeostasis have been suggested to be important (Gharib et al. 1992; Greenleaf 1997; Pavy-Le Traon et al. 1999). A loss of total body water (TBW) occurs initially in flight due to (1) a decrease in intake favoured by space motion

sickness (Leach-Huntoon et al. 1998; Leach at al. 1996) and (2) a loss of plasma volume (PV) triggered by the activation of the Henry-Gauer reflex and disturbed secretion of PV-regulating hormones (Fortney et al. 1996; Gharib et al. 1992; Maillet et al. 1994, 2000). Later on in the mission, TBW decreases further, accompanied by the loss of lean tissue (Blanc et al. 1998; Lane and Feeback 2002; Parsons et al. 2000) and a diminished sensation of thirst (Grigoriev et al. 1994; Leach-Huntoon et al. 1998; Leach et al. 1996). To prevent these perturbations in fluid homeostasis, several countermeasures have been tested during actual and simulated weightlessness. Many of these, such as lower body negative pressure (LBNP) (Gharib et al. 1992) and "thigh-cuff" devices (Millet et al. 2000), as well as various exercise regimens (Convertino 1996; Engelke et al. 1996; Greenleaf et al. 1977, 1989; Maillet et al. 1996), tend to have a beneficial effect on PV and thus on the orthostatic tolerance. Nevertheless, even though a normal PV is required to prevent orthostatic intolerance it should be associated with greater circulating levels of norepinephrine, efficient vasoconstrictor mechanisms and the baroreflex (Engelke et al. 1996). All these countermeasure programs are, however, time consuming and are not easily compatible with the busy timetable of the cosmonaut. As a result, new countermeasures which can be performed in as little time as possible, and which effectively prevent cardiovascular deconditioning, muscle atrophy and bone demineralization, need to be developed.

For these reasons, a new lower limb resistance-training device, the flywheel exercise device (FED), has been developed and adapted for the International Space Station (Alkner and Tesch 2002; Alkner et al. 2003; Elmann-Larsen and Schmitt 2003). This study investigates whether this new resistance exercise countermeasure can prevent the alterations in body fluid induced by spaceflight and thus mitigate orthostatic intolerance.

Methods

Subjects

Eighteen healthy young males were selected on the basis of medical history, a physical examination, blood chemistry and haematology analysis and an orthostatic tolerance test (10 min +80% head-up tilt-test) for voluntary participation in a 90-day head-down bed rest study (HDT). At the time of the study, the subjects were not taking medication and all subjects were non-smokers. After being fully informed of the nature of the scientific protocols and potential risks of participation, each subject provided written informed consent for the protocol approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Midi-Pyrénées I (France). Once selected, the subjects were randomly divided into two homogeneous groups (without differences for their anthropomorphic data): nine control subjects [Co, age 33.8 (1.1) years; weight 71.4 (1.7) kg; height 1.73 (0.01) m; BMI 23.8 (0.5)] and nine subjects who performed resistance exercise every 3 days [countermeasure group: CM, age 37.7 (1.7) years; weight 70.6 (1.8) kg; height 1.75 (0.02) m; BMI 22.9 (0.5)].

Experimental procedure

The subjects remained in the medical facility of the MEDES (Toulouse, France) for a total of 120 days, including 15 days of ambulatory baseline control days (BDC), 90 days in the –6° HDT position without interruption (all testing, showering, voiding and defecation functions were performed in the head-down position) and 15 days of recovery (R). The subjects were given an energy intake that matched the requirements given in previously published data (Blanc et al. 1998), i.e. 1.5 times the resting metabolic rate (RMR) measured by indirect calorimetry during the BDC period and 1.2 times the RMR during the HDT period. Sodium intake was 3 g/day. The water intake was limited to 3 l/day. The fluid and energy losses induced by training were compensated with energetic (≈600 kcal) and fluid complements (+1 l) given on the day of training. The subjects were supervised and monitored 24 h/day and room lighting was on between 07:00 and 23:00 h daily. All studies were performed in a quiet room at a temperature of ≈ 24°C.

Exercise protocol

The resistance exercise was performed in the –6° HDT position with a device using flywheel technology; the FED has been described in detail in previous studies (Alkner and Tesch 2002; Alkner et al. 2003) and was designed for the International Space Station (Fig. 1). The exercise regimen chosen for this study was the same as that of the SFINCSS-99 study (Alkner et al. 2003),

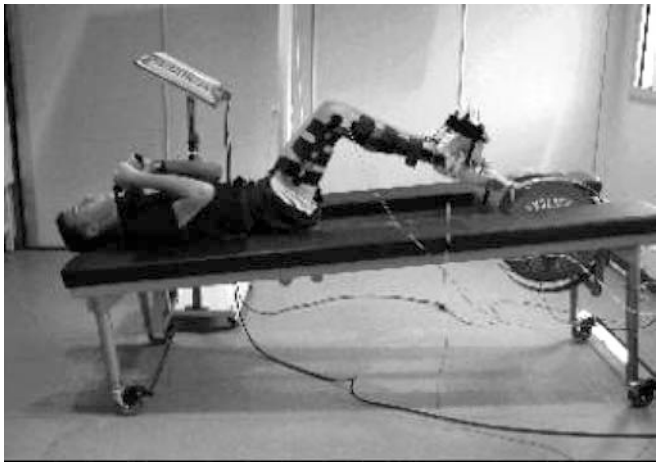

Fig. 1 Subject performing the exercise training with flywheel exercise device

for which the exercises were designed by the NASA's exercise program. This program stipulates that it is compulsory for the astronauts of the ISS to perform calf presses, squats and back extensions. Emphasis on these exercises is vital because the postural muscles which are involved in executing these tasks show the most severe atrophy after spaceflight (LeBlanc et al. 1992). Training sessions consisted of a warm-up at submaximal levels followed by two types of exercise: the squat and the calf press. The squat emphasizes the knee and hip extensor muscle groups and the calf press uses the ankle plantar flexors. The supine squat was performed to stress the quadriceps muscles, but it also involves the gluteus maximus, hamstrings and adductor muscles. The calf press involves the triceps surae muscle group. Training was composed of 29 sessions and was performed every 3 days, starting on day 5 of HDT. Progressive warm-ups preceded four sets of seven maximal concentric and eccentric repetitions of the squat followed by four sets of 14 repetitions of the calf press. Two minutes of rest were allowed between sets, and 5 min between exercises. Subjects pushed with maximal concentric force (80–100% of the maximal effort) until almost full extension, paused for a moment just after the turning point and then attempted to stop the action (eccentric force). The stop occurred on average at the 50° knee joint angle (squat) and 65° ankle joint angle (calf press). The calf press was performed at about 160–180° knee angle. Visual feedback of the work produced was provided on a computer display facing the subjects. The total time of maximal muscle action averaged about 35 min.

Body mass, blood pressure

Each morning, on waking, before breakfast and after the first void, body mass was measured in the supine position (0° during the BDC period and –6° during HDT) using a HDT weighing machine (Arjo 232300, Elsov, Sweden). At the same time blood pressure and heart rate were measured with an automated sphygmomanometer (Dinamap-Criticon).

Plasma volume, haematological data, and total body water

PV variations were determined according to Dill and Costill's equation (1974): $\Delta PV(\%) = 100\ [Hb_{BDC\text{-}1}(1\text{-}Hct_{HDT90}\times10^{-2})]/[Hb_{HDT90}(1\text{-}Hct_{BDC\text{-}1}\times10^{-2})]\text{-}100$, where Hb and Hct are the haemoglobin and haematocrit values measured at BDC-1 and HDT-90, respectively. Hct was measured after centrifugation for 6 min at 11,500 rpm (Ttettich haematocrit centrifuge, Tullingen, Germany). Hb concentrations were determined on total blood using a β-haemoglobin photometer (HemoCue AB, Angelhom, Sweden). Mean corpuscular volume (MCV) and erythrocyte concentration were determined with a Coulter Gen-S Haematology analyser (Beckman, Minn., USA). TBW was measured at BDC-1, HDT-45, and HDT-90 by isotope dilution of $H_2{}^{18}O$. The dose was 0.4/kg estimated TBW of $H_2{}^{18}O$ (10%) (C.I.L, USA). Mass spectrometry and calculations have been described in detail in another study (Blanc et al. 2002).

Blood assays

For 2 days during the control period (BDC-12, BDC-1), 3 days during HDT (HDT-3, HDT-45 and HDT-90) and 2 days during the recovery period (R-3, R-9), while still in the supine position for the basal and recovery periods, just after the subjects awoke and before breakfast, an intravenous catheter was inserted into an antecubital vein for blood sampling. At least 20 min later, an 8 ml blood sample was collected into heparinized tubes. Hct and Hb were immediately determined on total blood. The samples were then kept on ice until centrifugation (4°C, 20 min, 3,000 rpm) and plasma was stored at –80°C until analysis. Plasma active renin (AR), aldosterone (Aldo), proteins, Na^+, K^+ and creatinine levels, as well as osmolality, were determined. All hormone assays were analyzed using radio-immunological methods. The sensitivity of the Aldo (Immunotech Kit) assay was 6 pg/ml. Intra-assay variability was 8% and inter-assay variability 7%. For AR assays were done using an ERIA Pasteur diagnostic kit with sensitivity of 1.5 pg/ml, with an intra-assay variability of 5% and inter-assay variability of 6%. Na^+ and K^+ were determined by flame photometry (Delhomme, Milan, Italy). Proteins were determined using the Biuret reaction. Plasma and urine osmolality were measured by freezing point depression (Fisk One-Ten Osmometer, Needham Heights, Mass., USA). Creatinine was determined using the Jaffe method.

Urine assays

The 24 h urinary volumes were collected daily. Urinary anti-diuretic hormone (ADH) was determined with a technique developed in the laboratory (Allevard et al. 1979). The sensitivity of the assay was 1.25 pg/ml. The intra-assay variability was 10% and the inter-assay variability 11%. The glomerular filtration rate (GFR) was estimated by creatinine clearance.

Orthostatic test

A standard 10 min tilt-test ($+80°$), part of the integrated test regimen (ITR) defined by the space agencies, was performed at BDC-15 and R-0 in order to identify the intolerant subjects (non-finishers). During the orthostatic test, the subjects stood up for the first time. In order to identify their level of tolerance, the subjects were monitored with a Portapres (beat-by-beat measurement) and with an oscillometric device (Dynamap,

Criticon). According to the ITR criteria of orthostatic intolerance, the subjects were classified as non-finishers if SBP decreased by ≥ 30 mm Hg below the initial value, heart rate increased suddenly by more than 15 beats/min, and if the subjects showed signs of presyncope such as nausea, pallor, sweating, dizziness, visual disturbances, or on the request of the subject because of discomfort.

Statistical analysis

Statistical comparisons of the two groups of subjects were made using the non parametric Mann-Whitney test. The different periods of bed rest were compared to the last day of the basal data control period (BDC-1) using the Wilcoxon test. Data are expressed as mean ± SE. Statistical significance was set at $P \leq 0.05$.

Results

Effects of bed rest on the control group

During the R-0 tilt-test, four of the nine subjects in the Co group were classified as non-finishers (Table 1). The body mass of the Co group decreased progressively throughout the bed rest and was significantly different from the BDC-1 value from HDT-58 to HDT-90 ($P < 0.05$) (Fig. 2). At the end of the 90-day HDT, a decrease in PV [−4.7 (1.8%), $P < 0.05$] and a drop in TBW [−6.3 (5.4)%, $P < 0.05$] (Fig. 4) were observed. A significant decrease in MCV and an increase in Hb (Table 2) were also consequences of the HDT. Concerning the hormone concentrations, HDT induced a significant decrease in atrial natriuretic peptide (ANP) on HDT-45 (Fig. 5A), an increase in AR from BDC-1 to HDT-90 [+109.0 (15.4)%, $P < 0.05$], (Fig. 5B) and a progressive and sustained increase in Aldo concentration [+87.2 (38.9)%, $P < 0.05$] (Fig. 5C). Plasma protein concentration (Fig. 6A) and osmolality (Fig. 6B) were not altered by the HDT. From HDT-45, plasma Na^+ increased significantly compared to the BDC-1 value (Fig. 6C) whereas no K^+ changes were seen (Fig. 6D).

Daily urinary volume, which was not affected by the 90-day HDT, increased significantly during the first days of recovery (R-2 to R-5), (Fig. 7). During the BDC period, urinary ADH concentrations decreased significantly [18.8 (6.5)%] and were maintained at this level throughout the HDT period (Fig. 8). Urinary Na^+ was not affected by the bed rest whereas urinary K^+ and osmolality increased transiently at the HDT(1–3) and HDT(42–46) periods respectively (Table 3). The GFR [56.6 (3.9) ml/min per /m^2 of body surface] and daily arterial pressures (Fig. 3) were not affected by the 90-day HDT.

Countermeasure effects

From the orthostatic tolerance test results, we observed that the CM group did not have a better capacity to

Table 1 Tolerance time in minutes for each subject during the tilt-test

	Control group							Counter-measure group										
Subject	E1	F1	G1	I1	J1	C2	D2	E2	F2	A1	B1	C1	D1	G2	H2	I2	J2	K2
BDC-15	10	10	10	10	10	10	10	10	10	10	10	10	10	10	8*	10	10	9*
R-0	8	5	8	10	10	10	10	3	10	10	10	10	5	7	10	5	7	8

*The subject was tolerant at the selection tilt-test

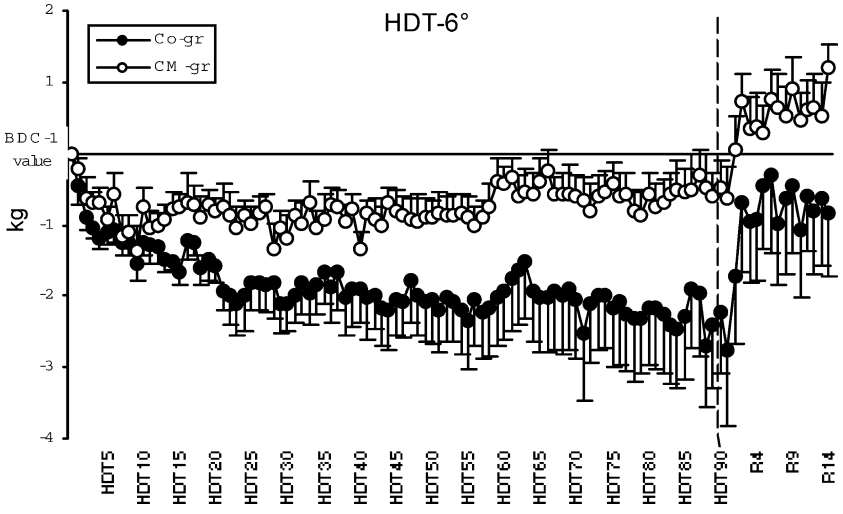

Fig. 2 Body weight variations over time, versus BDC-1 values, in the control (*Co-gr*) and exercise (*CM-gr*) groups

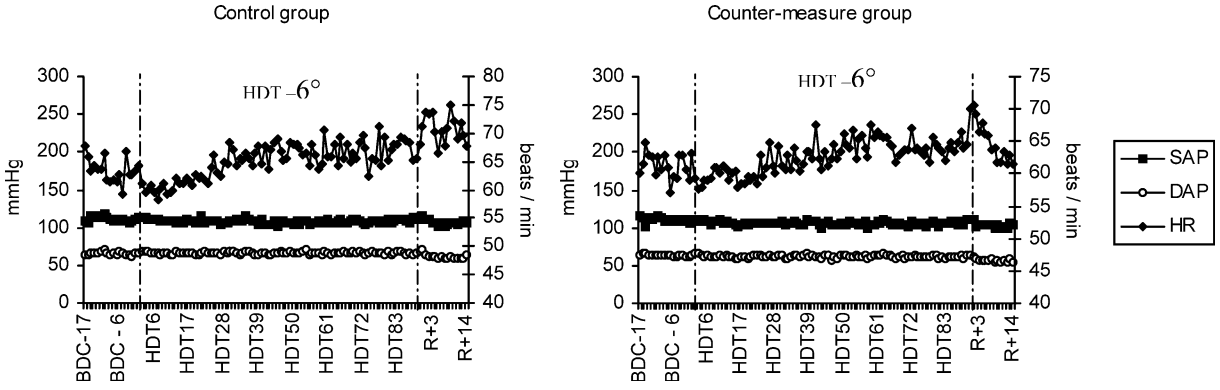

Fig. 3 Daily morning systolic (*SAP*), diastolic (*DAP*), arterial pressure and heart rate (*HR*) in the control and exercise group

withstand the 10 min tilt-test than the Co group. This was revealed by the fact that five of the nine subjects were non-finishers (Table 1). Contrary to the Co group, the CM group showed no decrease in body mass during the bed rest (Fig. 2). Although the TBW did not differ in a statistically significant manner between the groups, TBW in the CM group tended to decrease less than in the Co group [−6.3 (5.4)%, Co vs –3.7 (2.1)%, CM] (Fig. 4). PV was not maintained in the CM group and decreased in similar proportions to the Co group [−4.7 (1.8)%, Co vs –6.2 (2.5)%, CM; $P < 0.05$]. The FED also had no effect on the haematological data, which followed the same pattern as the Co group: a significant decrease in MCV, and an increase in Hb (Table 2).

For the plasma hormones (ANP, AR, Aldo), electrolyte concentrations (Na^+, K^+, PP), and the osmolality, the variations in the CM group were similar to that of the Co group (Fig. 5 and 6). The differences between the groups were indistinguishable. The only difference regarding the urinary data observed in the CM group was an increase in K^+ concentrations during the HDT.

Discussion

The purpose of this study was to test whether resistance exercise training with the FED limits the changes in body fluid induced by 90 days of simulated microgravity. The results showed that this countermeasure failed to prevent the decrease in PV and TBW that may contribute to the lack of improvement in the orthostatic tolerance. Furthermore, the FED had no effect on the changes in plasma and urinary hormone concentrations induced by long-term HDT.

Body mass, total body water and plasma volume

As previously reported during spaceflight (Lane and Feeback 2002; Leach et al. 1996) or simulated microgravity (Blanc et al. 1998; Sigaudo et al. 1998), the subjects in our control group showed a progressive decrease in body mass of 1–3 kg [2.8 (0.8) kg]. Body mass changes, dependent on lean body mass (LBM) and fatty mass, could be explained by the variation of one or both of these variables. In fact, muscle atrophy, highlighted by a reduction in the calf cross-sectional area [18 (4)%, $P < 0.05$], was observed during this 90-day HDT study (Alkner and Tesch 2002). Although this result is included in the fluid shift induced by HDT, it reflects the modifications of the whole body, and tends to explain the LBM reduction and consequently the body mass changes. Since changes in TBW are closely linked to LBM changes, the muscle atrophy induced by the 90-day HDT study seems to be a good argument for explaining the drop in body mass on the one hand, and the reduction in TBW on the other. From the known hydration coefficient of LBM (73.2%), unchanged

Table 2 Haematological data (*MCV* mean cellular volume, *Hb* haemoglobin concentration, *Eryth* erythrocyte concentration) of the control group (*Co*) and the exercise group (*CM*)

	MCV (fl)		Hb (g/dl)		Eryth ($\times 10^5$ /µl)	
	Co	CM	Co	CM	Co	CM
BDC-14	88.9 (1.1)	89.5 (0.6)	14.9 (0.2)	14.7 (0.2)	49.9 (0.7)	48.7 (0.7)
HDT14	87.4 (1.1)#	87.9 (0.5)#	15.5 (0.3)#	15.2 (0.3)	51.7 (0.7)#	50.7 (0.9)
HDT30	86.8 (1.1)#	87.0 (0.4)#	15.5 (0.3)#	15.2 (0.4)	52.0 (0.8)#	50.6 (1.1)*
HDT60	86.3 (0.9)#	87.2 (0.5)#	15.3 (0.3)	14.7 (0.3)*	51.5 (0.9)#	49.6 (0.9)*
HDT90	86.1 (1.1)#	87.1 (0.4)#	14.6 (0.3)	14.7 (0.4)	49.5 (1.0)	49.2 (1.1)
R13	88.7 (1.0)	89.0 (0.6)	13.5 (0.3)	13.7 (0.4)	45.5 (1.0)#	45.7 (1.1)#

* Significant difference between the groups ($P \leq 0.05$)
\# Significant difference with BDC-14 ($P \leq 0.05$)

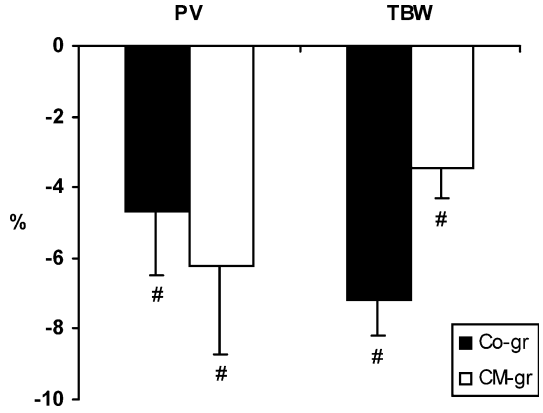

Fig. 4 Percentage changes in plasma volume (*PV*), and total body water (*TBW*) versus BDC-1 values, in the control group (*Co-gr*) and in the countermeasure group (*CM-gr*). # represents a significant difference ($P \leq 0.05$) versus the BDC-1 value in each group

during HDT (Blanc et al. 1998), we concluded that TBW loss of 2.6 (0.7) kg corresponds to a loss of LBM of about 3.5 kg. However, the subjects presented only a 2.8 (0.8) kg loss of body mass. This means that fatty mass increased and partly offset the loss in total body mass. From our results it could be deduced that the subjects presented an increase in fatty mass of about 0.7 kg. Such an increase is supported by the results from previous spaceflight (Lane and Feeback 2002) or bed rest (Parsons et al. 2000).

Changes in TBW can be partly explained by variations in body composition, but are also influenced by water balance. As reported by most ground-based models of simulated spaceflight (Grigoriev et al. 1994; Lane and Feeback 2002) a negative water balance was observed after initiation of the HDT bed rest. This phenomenon, essentially due to the decrease in water intake, was observed on the first day of HDT and argues for the decrease in TBW. The decrease in the thirst sensation mentioned previously (Grigoriev et al. 1994) tends to explain this, but, as the water balance is maintained at a steady level during the following days of HDT, it cannot explain the progressive decrease in TBW. This result suggests that the drop in TBW is induced by the negative water balance following fluid redistribution and is sustained by the progressive decrease in LBM. This probably explains why the decrease in TBW is linked to the duration of exposure to microgravity (Blanc et al. 1998; Fortney et al. 1996; Millet et al. 2000).

Since TBW includes PV, this latter variable also plays a part in TBW regulation. In this study, PV changes were assessed indirectly using Dill and Costill's equation (1974), which is more frequently used to assess instantaneous plasma volume changes (Greenleaf et al. 1979). Its utilization during long term bed rest studies was validated by comparing the results obtained with this technique to those obtained with the Evans blue dilution during a 42-day HDT study (Johansen et al. 1997). The PV variations observed in this study were equivalent to

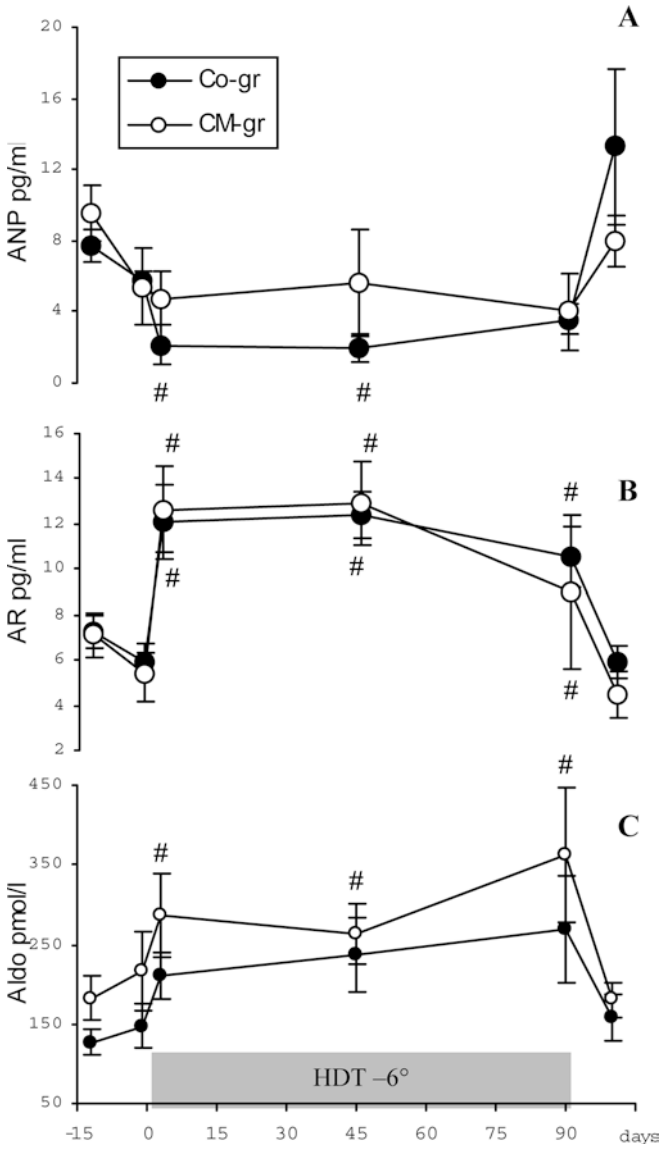

Fig. 5 Evolution over time of plasma ANP (**A**), renin (**B**), and aldosterone (**C**) concentrations in the control (*Co-gr*) and countermeasure groups (*CM-gr*). # represents a significant difference ($P \leq 0.05$) versus the BDC-1 value in each group

those observed during previous bed rests of different durations (4, 7, 28, or 42 days) (Pavy-Le Traon et al. 1999). This confirms that length of exposure to real or simulated microgravity has no effect on the decrease in plasma volume and invalidates the time-related equation established by Greenleaf et al. (1977). In addition, this decrease in PV is also an argument for explaining the reduction in TBW induced by the bed rest.

Haematological data

As previously observed during a 10-day HDT study (Lampe et al. 1992), alterations in blood volume also include an increase in erythrocyte concentrations, in the Hb concentration and a decrease in the MCV. These results indicated the occurrence of a haemoconcentration.

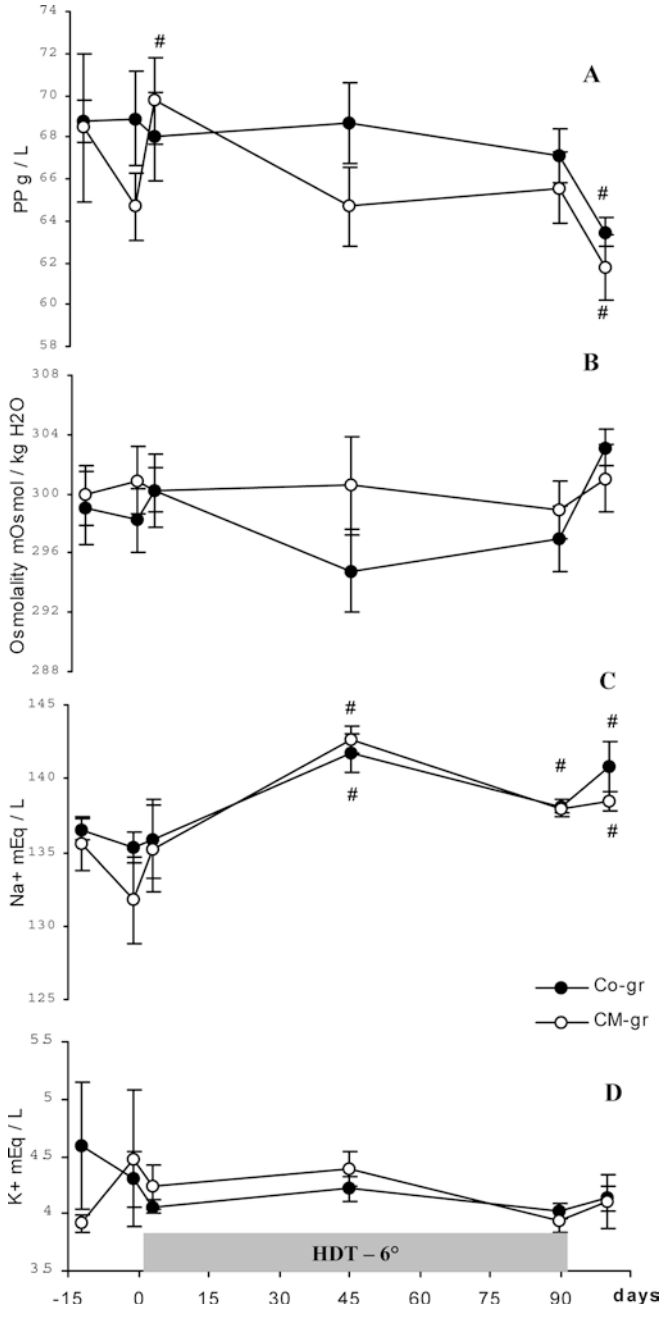

Fig. 6 Evolution over time of plasma protein (PP) (**A**), osmolality (**B**), Na^+ (**C**) and K^+ (**D**) concentrations in the control (*Co-gr*) and countermeasure groups (*CM-gr*). # represents a significant difference ($P \leq 0.05$) versus the BDC-1 value in each group

In contrast to Lampe et al. (1992) the 3.2% decrease in MCV observed in our study was statistically significant. This could partly explain the haemoconcentration, and may be due to the preferential destruction of younger erythrocytes, which are larger than older ones (Watenpaugh 2001). If confirmed, this hypothesis would be in agreement with the lack of release of new red blood cells from bone narrow observed during spaceflight (Alfrey et al. 1996a,1996b). During the first days of exposure to microgravity, a decrease in PV could partially explain the increase in erythrocyte concentration. However, the maintenance of this volume at a steady level throughout the study could not be responsible for the increase in the erythrocyte concentration. This increase seems to be in agreement with many long-term spaceflights, during which an increase in abnormally shaped red cells with a dot at their centre has been observed (Gunga et al.1996). Thus, these results were not consistent with the reduction in erythropoietin observed during previous spaceflights (Alfrey et al 1996a,1996b) or HDT studies (Gunga et al. 1996).

Hormones and electrolytes

During our 90-day HDT study, high AR and Aldo concentrations were observed. Such hormonal modifications induced plasma Na^+ retention and K^+ excretion, which did not support the loss in TBW and PV. The same conclusion can be drawn from the reduction in ANP. Theoretically, these hormonal and electrolyte changes, previously observed during simulated microgravity studies (Maillet et al. 2000; Sigaudo et al. 1998), are a consequence of hypovolemia. The decrease in PV is perceived by the atrial and kidney receptors and triggers hormone secretions and activation of the sympathetic component of the autonomic nervous system. Theoretically, when combined, these actions should act to increase the PV. No activation of the sympathetic component was observed however (Sigaudo et al. 1998), and the increase in AR, Aldo and plasma Na^+ failed to enhance the PV but did maintain it after the initial reduction.

As reflected by the decrease in urinary hormone concentrations, the water retention mechanism mediated by ADH was not activated. Although high ADH concentrations have been observed on the first day of spaceflight (Leach et al. 1996) or under simulated microgravity (Maillet et al. 1994), the decrease, without variation in plasma osmolality, has already been observed during a 120-day HDT study (Maillet et al. 2000) and spaceflight (Grigoriev et al. 1994; Lane and Feeback 2002; Leach-Huntoon 1998). Several factors, such as PV, atrial distension, osmolality and intracellular fluid volume, are involved in ADH regulation. Consequently, all these factors should be taken into account to understand the ADH and fluid modifications observed. Given the decrease in PV that we observed, we expected to find an increase, rather than a decrease, in ADH concentration. As suggested by Vokes and Robertson (1984), plasma osmolality seems to play a key role in ADH regulation and thus is potentially more important than PV. This would allow us to explain the reduced ADH release in the presence of a decrease in PV, whereas during this 90-day HDT and another 120-day HDT study (Maillet et al. 2000), plasma osmolality tended to decrease. As a slight decrease in plasma osmolality (1–2%) induced a reduction in ADH release, our non-significant decrease in plasma osmolality may certainly explain the ADH variations that we have

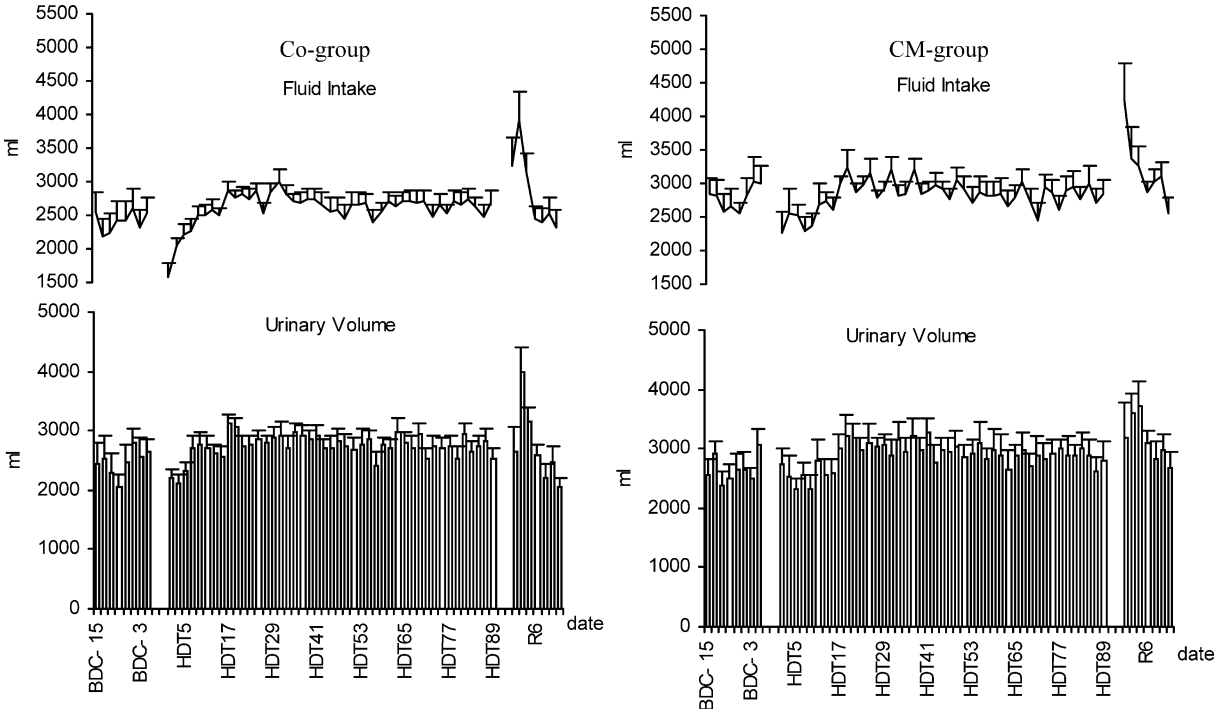

Fig. 7 Daily fluid intake and urinary volume (ml) values, represented 1 out of every 2 days, in the control (*Co*) and countermeasure (*CM*) groups

observed. Although not commonly admitted (Fortney et al. 1996; Gharib et al 1992), an increase in intracellular fluid volume has been suggested (but not demonstrated) during spaceflights (Leach et al. 1996; Watenpaugh 2001) or simulations (Greenleaf et al. 1977; Leach-Huntoon et al. 1998). If confirmed, this argument would explain the low urinary ADH concentration and would suggest that exposure to microgravity induces a deregulation in the atrial receptors and the central osmoreceptors. Furthermore, during spaceflight, a reduced urinary ADH concentration has been associated with a decrease in renal sensitivity to ADH (Grigoriev et al. 1994; Leach-Huntoon et al. 1998). Decreased renal sensitivity might explain the lack of fluid retention. However, these renal property changes should theoretically be associated with a decrease in urinary osmolality and ADH concentration in order to compensate for the decreased sensitivity. This was not, however, what was observed. Urine osmolality is linked to two factors: Na^+ and urea. Urea is the end product of protein catabolism. Protein synthesis decreases during bed-rest whereas breakdown is unchanged, resulting in a net negative nitrogen balance. This could explain the elevation of urine osmolality without significant modifications in plasma osmolality.

Countermeasure efficiency

Contrary to actual or simulated microgravity studies testing isotonic, isokinetic and endurance exercise, thigh-cuffs or LBNP, using the FED (Convertino 1996; Engelke et al. 1996; Gharib et al. 1992; Greenleaf et al. 1989, 1992; Maillet et al. 2000; Millet et al. 2000) did not lessen the hypovolemia. The frequency of the exercise regimen

Table 3 Mean value of Na^+ and K^+ urinary concentrations and urinary osmolality of the control (*Co*) and countermeasure (*CM*) group for three consecutive days

	Na^+ (mol/D)		K^+ (mol/D)		Osmo (mosmol/D)	
	Co	CM	Co	CM	Co	CM
BDC (−15 to −12)	136.04 (18.85)	144.11 (23.66)	62.99 (9.53)	69.90 (18.73)	884.47 (21.34)	862.73 (30.98)
BDC (−7 to −1)	155.25 (6.33)	148.54 (6.29)	61.80 (2.35)	63.40 (3.20)	879.10 (40.53)	871.44 (23.03)
HDT (1–3)	145.72 (5.03)	162.71 (6.60)	68.64 (2.64)	76.45 (2.67)#	889.82 (25.06)	945.14 (33.02)
HDT (6–9)	145.73 (6.45)#	132.81 (6.12)	66.48 (3.07)	64.04 (4.28)	906.38 (28.78)	891.32 (36.15)
HDT (13–16)	152.68 (7.98)	173.06 (5.48)#	70.48 (3.72)	69.87 (4.08)	958.06 (37.33)	965.88 (31.36)#
HDT (42–46)	163.97 (6.47)	163.85 (5.12)	69.06 (3.70)	80.11 (4.71)#	945.58 (34.47)#	932.82 (32.41)
HDT (67–70)	161.01 (13.11)	162.10 (9.88)	68.92 (4.12)	81.51 (5.08)#	949.75 (36.63)	945.99 (30.59)
HDT (75–78)	145.53 (11.32)	145.67 (11.24)	63.61 (2.61)	77.21 (5.11)#	923.75 (40.19)	921.12 (29.40)
HDT (79–86)	139.49 (5.33)	137.23 (9.52)	66.93 (5.81)	74.05 (7.27)	919.98 (39.97)	896.35 (23.27)
HDT (87–90)	141.36 (6.81)	143.42 (10.70)	63.62 (3.20)	71.45 (4.52)	874.22 (25.09)	884.58 (44.17)
R (0–3)	135.20 (12.31)	140.49 (16.31)	61.01 (2.62)	78.68 (6.20)#	745.22 (48.24)#	876.52 (48.82)
R (4–9)	151.89 (9.16)	142.52 (11.91)	68.70 (3.46)	75.56 (5.62)	848.35 (32.97)	836.48 (40.58)

Significant difference ($P \leq 0.05$) with the BDC-1 value

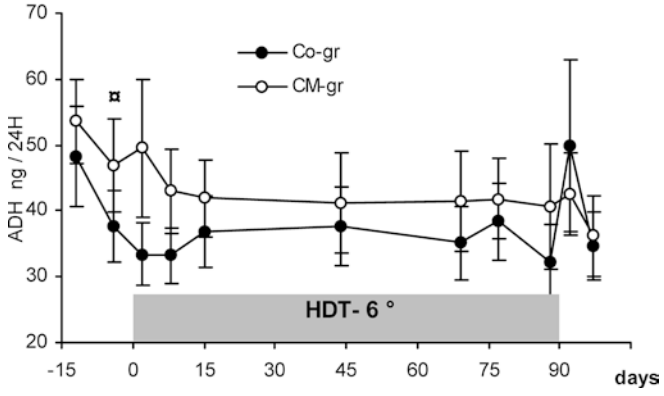

Fig. 8 Evolution over time of urinary ADH excretion in the control (*Co-gr*) and countermeasure groups (*CM-gr*). ¤ represents a significant difference ($P \leq 0.05$) versus the BDC-12 value in each group

and the exertion it required might be responsible for this lack of effect. Indeed, the PV loss was mitigated either with daily isotonic leg exercise training of 1 h per day, 5 days per week (Greenleaf et al. 1992) or with a multistage graded exercise bout to exhaustion, using a Quinton supine cycle ergometer applied 24 h before the end of a 16-day exposure to HDT (Engelke et al. 1996). In the present study, exercise sessions were performed for only 35 min every 3 days. Compared to the studies cited, our results suggest that either the frequency of the training or the exertion of the exercise was not sufficient. This seemed to be confirmed by the lack of difference between the groups for all PV regulating hormones, electrolytes and osmolality. Although the FED failed to prevent fluid loss, it has previously been reported that this countermeasure prevents calf volume decrease (Alkner and Tesch 2002; Elmann-Larsen and Schmitt 2003). This efficiency in maintaining muscle mass may explain the lack of body mass variation and the lower decrease in TBW observed in the CM group. However, the decrease in TBW in the CM group was 3.7 (2.1)%, which corresponds to a variation of about 2.1 (2.2) kg of LBM. As this group showed no modification in body mass, this means that in many subjects in the CM group the fatty mass probably increased to offset the decrease in LBM. Although further investigations need to be conducted to obtain more accurate data on these variations in body composition, our results tend to confirm that our countermeasure protocol was inefficient in the prevention of body changes.

Orthostatic tolerance

Regardless of fluid and hormonal volume, neither of the two groups presented better orthostatic tolerance than the other at the end of the 90-day HDT. The inefficiency of this countermeasure could not be attributed solely to its lack of effect on PV. Efficient counter-measures known to prevent PV loss have previously been shown to either improve orthostatic tolerance (Engelke et al. 1996) or have little effect (Greenleaf et al. 1992). To efficiently prevent post-HDT orthostatic intolerance, PV improvement should be associated with other improvements in vasoconstrictive capacity, baroreflex sensitivity, venous compliance, vestibular system alterations and cerebral perfusion.

Conclusion

The present study suggests that: (1) as a countermeasure the FED did not prevent the decrease in PV and TBW or the hormonal changes induced by the 90-day bed rest; and (2) this resistance exercise did not improve orthostatic tolerance. These results do not preclude any beneficial effects of resistance training on other physiological functions such as bone and muscle loss, but do show a failure to prevent cardiovascular deconditioning.

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