Changes in peak oxygen uptake and plasma volume in fit and unfit subjects following exposure to a simulation of microgravity

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To test the hypothesis that the magnitude of reduction in plasma volume and work capacity following simulated microgravity is dependent on the initial level of aerobic fitness, maximal oxygen uptake (VO2max) was measured in a group of physically fit subjects and compared to VO2max in a group of relatively unfit subjects before and after 10 days of continuous 6° head down tilt (HDT). Ten fit subjects (40 ± 2 yr) with mean ± SE VO2max = 48.5 ± 1.9 ml/kg/min were matched for age, height, and lean body weight with 10 unfit subjects (VO2max = 38.2 ± 1.8 ml/kg/min). Before and after HDT, plasma, blood, and red cell volumes and body composition were measured and all subjects underwent a graded supine cycle ergometer test to determine VO2max. The reduction in VO2max in fit subjects (-16.2%, P < 0.05) was greater (P < 0.05) than that of unfit subjects (-6.1%, P < 0.05). Similarly, reductions in plasma volume (-18.5%, P < 0.05) and blood volume (-16.0%, P < 0.05) in fit subjects were larger (P < 0.05) than those of unfit subjects (blood volume = -6.6%, P < 0.05; plasma volume = -6.6%, P < 0.05). Reduction in plasma volume was associated with greater (P < 0.05) negative body fluid balance during the initial 24 h of HDT in the fit group (912 ± 154 ml) compared to unfit subjects (453 ± 200 ml). Red cell volume was unchanged in the unfit subjects but decreased by 12.2% (P < 0.05) in the fit subjects. Percent change for VO2max correlated with percent change in plasma volume (r = +0.79, P < 0.05). Following exposure to simulated microgravity, fit subjects demonstrated a larger reduction in VO2max than unfit subjects which was associated with larger reductions in plasma and blood volume.

Bed rest, blood volume, fitness, maximal oxygen uptake, microgravity, fluid intake, urine output
Changes in peak oxygen uptake and plasma volume in fit and unfit subjects following exposure to a simulation of microgravity

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ABSTRACT

To test the hypothesis that the magnitude of reduction in plasma volume and work capacity following exposure to simulated microgravity is dependent on the initial level of aerobic fitness, peak oxygen uptake ($V_o_{2peak}$) was measured in a group of physically fit subjects and compared with $V_o_{2peak}$ in a group of relatively unfit subjects before and after 10 days of continuous 6 head-down tilt (HDT). Ten fit subjects (40 ± 2 year) with mean ± SE $V_o_{2peak} = 48.9 ± 1.7 \text{ mL kg}^{-1} \text{ min}^{-1}$ were matched for age, height, and lean body weight with 10 unfit subjects ($V_o_{2peak} = 37.7 ± 1.6 \text{ mL kg}^{-1} \text{ min}^{-1}$). Before and after HDT, plasma, blood, and red cell volumes and body composition were measured and all subjects underwent a graded supine cycle ergometer test to determine $V_o_{2peak}$. Reduced $V_o_{2peak}$ in fit subjects (−16.2%) was greater than that of unfit subjects (−6.1%). Similarly, reductions in plasma (−18.3%) and blood volumes (−16.0%) in fit subjects were larger than those of unfit subjects (blood volume = −5.6%; plasma volume = −6.6%). Reduced plasma volume was associated with greater negative body fluid balance during the initial 24 h of HDT in the fit group (912 ± 154 mL) compared with unfit subjects (453 ± 200 mL). Per cent change for $V_o_{2peak}$ correlated with percentage change in plasma volume ($r = +0.79$). Following exposure to simulated microgravity, fit subjects demonstrated larger reductions in $V_o_{2peak}$ than unfit subjects which was associated with larger reductions in plasma and blood volume. These data suggest that the magnitude of physical deconditioning induced by exposure to microgravity without intervention of countermeasures was influenced by the initial fitness of the subjects.

Keywords bedrest, blood volume, fitness, fluid intake, microgravity, peak oxygen uptake, urine output.

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The reduction in maximal or peak oxygen uptake ($V_o_{2peak}$) following exposure to spaceflight (Levine et al. 1996) and ground simulations of low gravity (Convertino 1995) is well recognized. However, the contribution of physical fitness to the magnitude of loss in aerobic power induced by microgravity is unclear. The observation that the magnitude of increase in $V_o_{2peak}$ with endurance exercise training is limited by pre-training $V_o_{2peak}$ (a so-called 'ceiling effect') (Saltin et al. 1969) leads to the logical hypothesis that individuals with high $V_o_{2peak}$ may experience greater reductions in $V_o_{2peak}$ during exposure to reduced physical activity compared with their less aerobically fit counterparts. This hypothesis has important implications for development and application of exercise management for individuals confined to clinical and low gravity environments. The notion that greater baseline aerobic fitness might be associated with larger reduction in $V_o_{2peak}$ was supported by the early observation that a 22% reduction in $V_o_{2peak}$ was measured following 28 days of bedrest in a subject with initial $V_o_{2peak}$ of 4.15 L min$^{-1}$ compared with 13% reduction observed in a subject with initial $V_o_{2peak}$ of 3.54 L min$^{-1}$ (Taylor et al. 1949). This relationship was further corroborated by the observations that four endurance-trained runners who underwent 6 h of water immersion reduced their $V_o_{2peak}$ by 19% compared with only 10% in four untrained subjects (Stegemann et al. 1969) and that pre-bedrest $V_o_{2peak}$ was significantly correlated with percentage reduction in $V_o_{2peak}$ in 15 subjects after
14 days bedrest (Convertino et al. 1986). However, analysis of data from other investigations (Chase et al. 1966, Saltin et al. 1968) did not support a close relationship between initial $\dot{V}O_{2peak}$ and the magnitude of reduction in $\dot{V}O_{2peak}$ during low gravity analogues, a finding which may have been compounded by high experimental variability and low sample sizes (Greenleaf & Kozlowski 1982).

In addition to high $\dot{V}O_{2peak}$ cross-sectional comparisons have demonstrated that athletes and fit individuals have larger plasma and blood volumes compared with sedentary populations (Convertino 1991), a relationship that is closely associated with $\dot{V}O_{2peak}$. As exposure to low gravity environments and their analogues cause hypovolemia (Fischer et al. 1967, Johnson et al. 1975, Greenleaf et al. 1989, Convertino 1990, 1995, 1996, 1997), it seems reasonable to expect that larger reductions in $\dot{V}O_{2peak}$ following exposure to microgravity may be related to larger reductions in plasma volume.

The relationship between fitness level and magnitude of reductions in $\dot{V}O_{2peak}$ and plasma volume has not been systematically examined. Therefore, $\dot{V}O_{2peak}$, plasma volume, and body fluid balance were measured in a group of aerobically fit subjects before and after exposure to 10 days of head-down tilt (HDT) and compared with changes in an unfit group to test the hypothesis that greater aerobic capacity would be associated with larger reduction in $\dot{V}O_{2peak}$ and circulating vascular volume.

METHODS

Subjects

Twenty healthy men, with a mean age ± SE of 41 ± 1 year (range 35–51), a mean height of 178 ± 1 cm (range 168–185), a mean weight of 77.2 ± 1.9 kg (range 64.0–92.9), and a mean relative body fat of 19.9 ± 1.2% (range 9.8–28.9) were selected to participate in this study. After selection, the subjects signed a written consent form indicating that they understood the purpose of the study, nature of all tests, foreseeable inconvenience, discomforts, risks, restrictions and controls. All experimental procedures and protocols were approved by the Human Research Review Boards of the National Aeronautics and Space Administration (NASA) at Ames Research Centre. Selection of subjects was based on results of a screening evaluation comprising a detailed medical history, physical examination, blood chemistry analysis, urinalysis, chest X-ray, and electrocardiogram. Subjects were familiarized with all laboratory personnel, procedures, protocols, mechanics of cycle ergometry in the supine position, and underwent a graded treadmill test to determine their maximal oxygen uptake ($\dot{V}O_{2peak}$) during a 5-day orientation period conducted prior to the study.

General protocol

Subjects were divided into fit and unfit groups ($N = 10$ subjects each) according to the 10 highest and 10 lowest treadmill maximal oxygen uptake ($\dot{V}O_{2peak}$) values. This procedure produced two groups that were matched for age, height, and lean body mass (Table 1). The overall experimental protocol consisted of a 7-day ambulatory baseline period (pre-HDT) followed by 10 days of continuous bedrest in the anterioorthostatic 6 HDT position. During HDT, subjects remained in the head-down position continuously for all activities including excretory functions, showering, and eating. During this 17-day experimental period, subjects were housed 24 h day$^{-1}$ in the Human Research Facility at NASA–Ames Research Centre and followed a controlled diet. The average daily caloric intake was 2500–2800 kcal (∼45% carbohydrate, 38% fat, 17% protein). Dietary sodium and potassium were held constant at ∼120 and 60–80 mEq day$^{-1}$, respectively. Fluid intake was ad libitum. The photoperiod was 16 h light to 8 h dark with lights on at 0700 h. The 6 HDT was chosen because actual changes in cardiovascular responses induced by spaceflight have been closely simulated by this ground-based analogue (Convertino 1995). Each subject underwent measurement of their plasma volume and supine ergometer $\dot{V}O_{2peak}$ on day 7 of the pre-HDT and on the final day of HDT (post-HDT).

<table>
<thead>
<tr>
<th></th>
<th>Unfit ($N = 10$)</th>
<th>Fit ($N = 10$)</th>
<th>t value</th>
<th>P value</th>
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</thead>
<tbody>
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<td>Age, year</td>
<td>40 ± 2</td>
<td>42 ± 2</td>
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<td>Height, cm</td>
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<td>Lean mass, kg</td>
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<tr>
<td>Body fat, %</td>
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<td>13.8 ± 1.2</td>
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</tr>
<tr>
<td>Blood volume, mL kg$^{-1}$</td>
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<td>79.1 ± 1.6</td>
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</tr>
<tr>
<td>$\dot{V}O_{2peak}$, mL kg$^{-1}$ min$^{-1}$</td>
<td>37.7 ± 1.6</td>
<td>48.9 ± 1.7</td>
<td>4.882</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± 1SE

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Measurements of maximal oxygen uptake

Each subject performed a maximal exercise tolerance test in the supine position on a Collins electronic cycle ergometer. The exercise test used to determine $\dot{V}O_2\text{peak}$ began with a 4-min warm-up period of no resistance pedalling and increased 15 W each minute until volitional exhaustion. The supine position was chosen to minimize the orthostatic factor which contributes to the reduction in work tolerance following HDT (Convertino 1995). Oxygen uptake and heart rate were measured during each 30 s of the exercise test.

The subjects used an Otis–McKerrow respiratory valve and the volume of expired gas was measured by a Parkinson–Cowan high-velocity, low-resistance meter. A potentiometer at the gas meter dial transmitted electrical outputs to a two-channel recorder (MRE Model M22). In this way, expired ventilation flow rate could be recorded. The expired gas was continuously extracted via a pump (Scientific Products) from a 5-L mixing chamber (R-Pel) which was placed between the subject and the gas meter to a 2-L gas bag (Ohio Medical) attached to a Costill–Wilmore valve. The ventilation flow rate was corrected by adding the pump withdrawal volume rate of 2.5 L min$^{-1}$ to the recorded volume of expired air. The composition of expired gas was analysed for the fraction of mixed expired oxygen and carbon dioxide using mass spectrometry (Perkin–Elmer). The gas analysers were calibrated with known oxygen and carbon dioxide concentrations before and after each test.

Measurements of plasma volume

Plasma volume was determined using a modified Evans blue dye dilution technique (Greenleaf et al. 1979, Convertino et al. 1996). After each subject was stabilized in the supine position for 30 min, an intravenous injection of 11.5 mg of dye diluted with isotonic saline solution (2.5 mL) was administered. The dye from a 10-min post-injection blood sample, recovered from the plasma with a wood-cellulose powder (Sokka-Floc SW-40 A) chromatographic column, was compared with a standard dye solution at 615 nm with a spectrophotometer. Peripheral venous blood for duplicate microhematocrit (Hct) determinations was drawn into capillary tubes and centrifuged for 10 min. Total circulating blood volume (BV) and red cell volume were calculated from the PV and Hct. Total blood volume was calculated from the plasma volume and hematocrit measurements. Using these procedures in our laboratory, test–retest correlation coefficient for blood volume was 0.969 ($N = 12$) and the average change was 82 mL (average $\%\Delta = 1.5\%$, $N = 17$), 75 mL (average $\%\Delta = 1.5\%$, $N = 19$), and 56 mL (average $\%\Delta = 1.1\%$, $N = 23$) when measurements were determined 4, 8, and 15 days apart, respectively (Greenleaf et al. 1979), and was 0.881 ($N = 7$) with average change of 25 mL (average $\%\Delta = 0.7\%$) when measurements were determined 11 months apart (Convertino et al. 1996).

Assessment of 24-h fluid intake and urine output

Fluid intakes and urine outputs were measured daily for total volume to the nearest 1 mL. During HDT, subjects remained in the head-down posture to urinate into a sterile urinal. All 24-h volumes were calculated from the sum of all volumes from samples collected between the start of the second urine void of each morning through and including the first urine void of the following day. The group mean for 24-h intake and output volumes during pre-HDT was determined from the average volumes collected during the final 6 days of the baseline ambulatory period while a value calculated to represent the mean 24-h intake and output volumes during HDT was determined by averaging individual 24-h values over the days of HDT.

Data were presented as mean ± 1 standard error (±SE). Differences between pre-HDT and post-HDT alterations in $\dot{V}O_2\text{peak}$, plasma, blood and red cell volumes, fluid intake, urine output, and net body fluid balance were analysed by Student’s $t$-test statistics for independent groups.

RESULTS

Body composition

Although group matching produced no statistically discernable differences in age, height, and lean body mass, fit subjects were characterized by less fat and body weight and greater blood volume (Table 1). Body weight and lean body mass remained essentially unchanged ($P = 0.3935$) in both the fit (0.2 ± 0.5% and $-0.08 ± 0.4\%$, respectively) and the unfit (0.3 ± 0.4%) groups after HDT. HDT increased percentage body fat ($P < 0.05$) in fit ($\%\Delta = 4.2 ± 1.9\%$) and unfit ($\%\Delta = 3.8 ± 1.9\%$) subjects.

$\dot{V}O_2\text{peak}$

According to the experimental design, average baseline $\dot{V}O_2\text{peak}$ was 23% higher in fit than unfit subjects (Table 1). HDT decreased $\dot{V}O_2\text{peak}$ from 2.87 ± 0.17 to 2.41 ± 0.13 L min$^{-1}$ and from 2.40 ± 0.15 to 2.24 ± 0.13 L min$^{-1}$ in the fit and unfit subjects, respectively. The percentage reduction in $\dot{V}O_2\text{peak}$ in the fit group was more than double ($t = 4.597, P = 0.0002$) that of the unfit group (Fig. 1).

Plasma volume

HDT decreased baseline blood volume in both groups primarily as a result of a mean reduction in plasma
Figure 1: Percent reductions in peak oxygen uptake (V\textsubscript{O\textsubscript{peak}}), total blood volume (TBV), red blood cell volume (RBCV), and plasma volume (PV) after 10 days of HDT in unfit () and fit (■) subjects. Lines represent ± 1SE and * indicate P < 0.05 for fit vs. unfit groups.

The plasma volume reduction of 18.3% in the fit subjects from 47.9 ± 1.5 mL kg\textsuperscript{-1} before HDT to 39.1 ± 1.2 mL kg\textsuperscript{-1} after HDT was greater (t = 6.140, P < 0.0001) than the 6.6% reduction measured in the unfit group (from 43.4 ± 1.3 mL kg\textsuperscript{-1} before HDT to 38.7 ± 1.1 mL kg\textsuperscript{-1} after HDT). As a result of plasma volume reductions, blood volume was decreased proportionately more (t = 7.074, P < 0.0001) in the fit subjects compared with their unfit counterparts (Fig. 1). A reduction in red blood cell volume from 2268 ± 100 mL before HDT to 2013 ± 92 mL after HDT in the fit subjects was greater (t = 3.307, P = 0.004) than the statistically unchanged RBC volume measured in the unfit group (from 2214 ± 83 mL before HDT to 2131 ± 61 mL after HDT).

**Fluid intake and urine output**

Measurements for average 24-h fluid intakes and urine outputs during the pre-HDT baseline period and 10 days of HDT are presented in Fig. 2. HDT induced a transient diuresis during the initial 24 h of exposure, increasing urine flow from an average 6-day pre-HDT baseline level of 2070 ± 230 to 2631 ± 267 mL the first day of HDT in the fit group (+561 ± 136 mL) that was greater (t = 2.208, P = 0.040) than the increase in urine output from 2308 ± 275 to 2456 ± 274 mL (+148 ± 128 mL) in the unfit group. HDT decreased fluid intake in a similar magnitude (t = 0.048, P = 0.962) in both groups, from an average 6-day pre-HDT baseline level of 2107 ± 226 to

Figure 2: Average 24-h urine outputs (a), fluid intakes (b), and net fluid balance (c) before (Baseline) and during 10 days of 6° head-down tilt (6° HDT) in unfit () and fit (■) subjects. Lines represent ± 1SE.

1619 ± 49 mL during HDT in the fit group (−388 ± 63 mL) compared with a decrease from 2399 ± 270 to 2123 ± 37 mL (−396 ± 159 mL) in the unfit group. As a result of increased urine output and
Figure 3. Relationship between percentage changes (%Δ) in plasma volume and \( \dot{V}O_{2peak} \) after 10 days of HDT in 10 fit (•) and 10 unfit (○) subjects.

Reduced fluid intake, a net negative fluid balance of 912 ± 154 mL induced by the initial 24 h of HDT in the fit group was twice as great (t = 1.819, P = 0.086) as the net negative fluid balance of 453 ± 200 mL in the unfit group (Fig. 2).

Relationship between %Δ\( \dot{V}O_{2peak} \) and %Δ plasma volume

Figure 3, generated from the individual data sets of the 10 fit and 10 unfit subjects, illustrates the relationship between percentage changes in plasma volume and \( \dot{V}O_{2peak} \) as a result of HDT. This analysis produced a regression equation where %Δ\( \dot{V}O_{2peak} \) = 0.76 [%Δ plasma volume] & minus;1.7, with a correlation coefficient of 0.787.

DISCUSSION

Investigations limited by variabilities from small sample sizes and cross-study comparisons in experimental protocols have provided anecdotal evidence that both support (Taylor et al. 1949, Stegemann et al. 1969, Convertino et al. 1986) or reject (Chase et al. 1966, Saltin et al. 1968, Greenleaf and Kozlowski 1982) the hypothesis that a larger baseline aerobic fitness is associated with greater reduction in \( \dot{V}O_{2peak} \) following exposure to a ground-based analogue of low gravity. A strength of the present study was the use of an experimental protocol specifically designed to test this hypothesis with a sample size that provided adequate statistical power. The major finding from the present study was consistent with the concept that individuals with higher aerobic capacity have greater reduction in \( \dot{V}O_{2peak} \) during adaptation to microgravity than less fit individuals as the reduction in \( \dot{V}O_{2peak} \) of a group of aerobically fit subjects was more than two times that of a group of aerobically unfit subjects. These results also suggest that levels of physical conditioning may influence the degree of adaptation that occurs with prolonged exposure to microgravity.

The mechanisms of reduction in \( \dot{V}O_{2peak} \) during exposure to low gravity conditions is clearly multi-faceted. One of the most consistent and rapid adaptations to microgravity is the reduction in blood volume (Fischer et al. 1967, Johnson et al. 1975, Convertino 1990, 1995, 1996). The significant correlation of 0.79 between %Δ in plasma volume and %Δ in \( \dot{V}O_{2peak} \) observed in the present study suggests that reduction in circulating vascular volume was a major contributing factor to the magnitude of loss in aerobic capacity in fit and unfit subjects. This coefficient was similar to the correlation of 0.84 between %Δ in plasma volume and %Δ in \( \dot{V}O_{2peak} \) generated from a cross-sectional compilation of data from 12 independent investigations (Convertino 1995). High correlation coefficients between the relative changes in plasma volume and \( \dot{V}O_{2peak} \) suggest that microgravity-induced hypovolemia probably contributes importantly to the reduction in the capacity of the body to transport and utilize oxygen during exercise.

It is well established that the reduction in \( \dot{V}O_{2peak} \) following exposure to actual or ground-based analogues of microgravity is primarily the result of reduced stroke volume (Saltin et al. 1968, Levine et al. 1996). As high \( \dot{V}O_{2peak} \) is associated with large blood volumes (Convertino 1991) and deconditioning-induced hypovolemia is associated with reduced maximal stroke volume and \( \dot{V}O_{2peak} \) (Coyle et al. 1986), it is not surprising that the fit subjects in the present study who demonstrated greater reduction in their \( \dot{V}O_{2peak} \) also had proportionately larger hypovolemia compared with the unfit subjects (Fig. 1). The adverse impact of greater hypovolemia on stroke volume and \( \dot{V}O_{2peak} \) following exposure to microgravity may be further exaggerated by a steeper Frank–Starling curve that has been reported in aerobically fit subjects (Levine 1993). A causal relationship between the magnitude of hypovolemia and reduction in \( \dot{V}O_{2peak} \) is further supported by the observation that reduction in \( \dot{V}O_{2peak} \) after HDT has been minimal when hypovolemia was ameliorated (Greenleaf et al. 1989). Although several mechanisms may contribute, it is clear that the greater reduction in \( \dot{V}O_{2peak} \) in fit individuals may be attributed to the association between their larger decreases in blood volume and left ventricular filling.

Reductions in plasma volume during HDT in the present investigation reflected alterations in balance between fluid intake and urine output induced by...
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exposure to this ground-based analogue of microgravity. Generally, there was a negative fluid balance during the initial 24 h of HDT in all subjects resulting from a diuresis and attenuated fluid intake (Fig. 3). After the initial 24 h of HDT, average fluid intake and urine output were attenuated proportionately throughout the remaining 9 days of HDT, maintaining fluid balance within pre-HDT levels. While the attenuation of fluid intake during HDT was similar between fit and unfit subjects, greater reduction in plasma volume observed in the fit subjects was associated with their nearly fourfold greater diuresis during the initial 24 h of HDT. Greater urine flow in fit compared with unfit subjects in the present study was similar to findings of a previous investigation that demonstrated a 22% greater diuresis in trained runners compared with sedentary subjects during 5 h of head-out water immersion (Convertino et al. 1993). Mechanisms underlying greater hypovolemia and diuresis during HDT in fit compared with unfit subjects may include relatively larger central blood volume shifts associated with greater baseline blood volume in aerobically fit individuals, resulting in greater glomerular filtration and inhibition of anti-diuretic hormone and aldosterone (Convertino et al. 1993).

In addition to the contribution of reduced plasma volume to the impact of vascular hypovolemia on the reduction in $\dot{V}O_2_{peak}$ after exposure to HDT, the fit subjects demonstrated a significant loss in red blood cell volume that was not observed in the unfit group. Decreased red blood cell volume might have contributed to the greater reduction in $\dot{V}O_2_{peak}$ observed in the fit subjects by lowering oxygen carrying capacity of the blood. Other investigators have verified that red cell mass can decrease from 5 to 25% during prolonged bedrest (Convertino 1995, Branch et al. 1997) or spaceflight (Fischer et al. 1967, Johnson et al. 1975). The findings of the present study might suggest that the stimulus for red blood cell production/degradation may be more sensitive in individuals with high aerobic capacity. However, the relationship between changes in red cell mass and changes in $\dot{V}O_2_{peak}$ is tenuous because significant reductions in $\dot{V}O_2_{peak}$ following bedrest have occurred without change in red cell mass (Convertino et al. 1986, Williams and Convertino 1988, Convertino 1995). It therefore appears that the primary effect of microgravity-induced hypovolemia on reducing $\dot{V}O_2_{peak}$ is a result of the contraction of the plasma volume with a more sedentary individual with small $\dot{V}O_2_{peak}$ reserve. This relationship suggests that greater levels of physical activity may be required during spaceflight or rehabilitation following inactivity in aerobically fit individuals to minimize detrimental effects associated with deconditioning. However, these results should not be misinterpreted to suggest that it is detrimental to select astronauts who are aerobically fit. On the contrary, fit individuals remain at a physical and physiological advantage because their aerobic power remains greater than their unfit counterparts after adaptation to microgravity and this represents greater reserve to meet the challenge to perform a given absolute work task.

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