Modulation of endothelial and smooth muscle function by bed rest and hypoenergetic, low-fat nutrition

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Hesse, Christiane, Heike Siedler, Steffen P. Luntz, Bianca M. Arendt, Roland Goerlich, Ruth Fricker, Martina Heer, and Walter E. Haefeli. Modulation of endothelial and smooth muscle function by bed rest and hypoenergetic, low-fat nutrition. J Appl Physiol 99: 2196–2203, 2005. First published August 11, 2005; doi:10.1152/japplphysiol.00888.2005.—Prolonged microgravity alters the regulation of the peripheral vasculature. The influence of reduced food intake, as often observed in astronauts, on vascular function is unclear. In a randomized, four-phase, crossover study, the effect of simulated microgravity (13 days of bed rest), energetic restriction (~25%, fat reduced), and their combination on endothelium-dependent and -independent vasodilation was compared with ambulatory control conditions. Using venous occlusion plethysmography, cumulative intra-arterial dose-response curves to endothelium-dependent (acetylcholine) and -independent (sodium nitroprusside) vasodilators were constructed in 10 healthy male volunteers before and on day 13 of each of the four intervention periods. Bed rest combined with normoenergetic nutrition impaired the dose-response to acetylcholine (ANOVA, P = 0.004) but not to sodium nitroprusside, whereas hypoenergetic diet under ambulatory conditions improved responses to acetylcholine (P = 0.044) and sodium nitroprusside (P < 0.001). When bed rest was combined with hypoenergetic diet, acetylcholine responses did not change. Similarly, under control conditions, no change was observed. Individual changes in the total cholesterol-to-HDL ratio were correlated with changes in endothelial and vascular smooth muscle relaxation. In conclusion, short-term bed rest impairs endothelium-dependent arterial relaxation in humans. A hypoenergetic, low-fat diet modulates serum lipids, improves endothelium-dependent and -independent relaxation, and may antagonize the unfavorable effects of simulated microgravity on endothelial function.

Human endothelium; nitric oxide; vasodilation; simulated microgravity

ENDOTHELIAL DYSFUNCTION is an early essential step in the development of atherosclerosis and vascular diseases in humans (12). Regular physical activity improves endothelium-dependent vasodilation in the forearm (17) and coronary circulation (14) and prevents cardiovascular mortality and morbidity (29, 35), whereas physical inactivity (sedentary state) is a risk factor for cardiovascular disease (5).

The positive effect of exercise on endothelial function may be explained by the increase in vascular shear stress, which enhances the expression of the vascular endothelial nitric oxide (NO) synthase (NOS III) gene, leading to an increase in NO production and bioavailability (14). Conversely, the transition from a state of regular physical activity to sustained rest may be expected to reduce many of the factors promoting the expression of NOS III. Indeed, in hindlimb-unloaded rats, an animal model for simulation of microgravity effects and bed-rest deconditioning (39), reduced physical activity and chronic reduction of soleus blood flow resulted in a decrease of shear stress associated with reduced expression of NOS III mRNA and protein and attenuated endothelium-dependent vasodilation (19). In humans, it is well known from astronauts during spaceflight and ground-based bed-rest studies that microgravity alters the regulation of the peripheral vascular tone and increases peripheral resistance (30, 37), and these changes may also be expected in patients with illnesses acutely requiring bed rest. The contribution of altered endothelial vasodilator function to increased peripheral resistance is uncertain.

However, studies in microgravity have often been performed in astronauts who voluntarily ate less than they should (15), making it difficult to distinguish between vascular changes induced by microgravity and those affected by reduced food intake. The effect of caloric restriction on endothelial function in healthy, lean individuals is unknown, but has been studied extensively in obese patients (4, 33, 34). These studies suggest beneficial effects of weight loss on a number of pathways (changes in lipids, blood pressure, oxidative stress) that modulate vascular responsiveness. However, a clear differentiation of underlying mechanisms is not possible, because the methods to achieve weight loss included exercise (34), a confounder that has well-recognized effects on endothelial function, or pharmacological intervention (4) with potential direct effects of the medication. Furthermore, only obese patients were included, who often had other cardiovascular risk factors, such as diabetes (4) or hypertension (33).

The purpose of this study was to investigate the effects of bed rest, a hypoenergetic, low-fat diet, and their combination on endothelium-dependent vasodilation in healthy humans without cardiovascular risk factors in a strictly controlled setting.

EXPERIMENTAL PROCEDURES

Participants. This was a prospective study in 10 healthy male Caucasians. After approval of the study by the responsible Ethics

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Committees of the Medical Faculty of the University of Heidelberg and of the State Chamber of Physicians of North Rhine-Westphalia, Düsseldorf, the study was conducted in accordance with the Declaration of Helsinki and its current amendments. The individuals were enrolled after giving written, informed consent, if they met all of the following inclusion criteria: physical examination, ECG, urinalysis and routine laboratory without clinically relevant findings, total cholesterol ≤200 mg/dl, LDL ≤130 mg/dl, HDL ≥35 mg/dl, and fasting glucose ≤106 mg/dl. Exclusion criteria were a history of allergies, known conditions causing endothelial dysfunction, such as diabetes, hyperlipidemia, and arterial hypertension, regular medication and/or treatment with drugs within the last 6 wk, acute or chronic illness, smoking within the 12-mo period preceding the study, and drug and/or alcohol abuse.

Study design. The study was performed in a randomized crossover design as part of a multidisciplinary project evaluating the effects of bed rest and hypoenergetic nutrition on bone metabolism and cardiovascular function. The subjects participated in four study phases that were separated by at least 4 mo to allow complete recovery of the participants. Each study phase was divided into a 9-day adaptation period and a 14-day intervention period; in each of the four intervention periods, the participants were exposed to either bed rest or ambulatory control conditions, while receiving either a tailored normoenergetic or hypoenergetic diet (see Fig. 1). Endothelium-dependent and-independent vasodilator responses were investigated twice in each phase: on the last day of the adaptation period and on day 13 of the intervention period.

All four study phases were identical with respect to environmental conditions and study protocol; only the variables body position/physical activity and energy intake were changed. Nine volunteers participated in all four study phases. One participant dropped out after completion of his first study phase (normoenergetic, ambulatory conditions) and was replaced by a volunteer who participated in the remaining three study phases.

Ambulatory and bed-rest conditions. The participants stayed in the metabolic ward [of the Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany] all of the time, i.e., also during the ambulatory study periods. In this facility, room temperature (24°C) and relative humidity (50%) were kept constant. During the bed-rest phases, participants were exposed to 6° head-down-tilt bed rest for 24 h/day and were not allowed to elevate their heads >30° from horizontal. All activities, including food intake, using the toilet, showering, and weighing, were carried out in the recumbent position. Six-degree head-down tilt was chosen because it is a validated model for simulation of microgravity (27). Whereas the induced cardiovascular changes occur more rapidly, their nature and extent are very similar to those observed in the supine position (13, 18). During the ambulatory control phases, the participants were in the normal upright position during the day, were allowed to walk around in the ward, and performed light muscular workload (including bicycle ergometry for 10 min three times a day).

Diet. During all adaptation and recovery periods as well as in the normoenergetic, ambulatory intervention period, the participants received a normoenergetic standard diet. Energy requirements were calculated for each individual, according to the World Health Organization equations (38): participants received a specifically prepared diet containing 1.4 times their basal metabolic rate; 10% of the total calories were added to account for dietary-induced thermogenesis. The average caloric intake was 11.4 ± 1.3 MJ/day. The diet consisted of 1 g protein·kg body wt⁻¹·day⁻¹, 50 ml water·kg body wt⁻¹·day⁻¹, 2.5 mmol sodium·kg body wt⁻¹·day⁻¹, 1,000 mg calcium/day, and 400 IU vitamin D/day administered as fixed-dose tablets (Dekristol; Jenapharm, Jena, Germany). Dietary protein, fat, and carbohydrate intakes were calculated according to dietary reference intake values (40) (i.e., 10–15% of the daily energy intake were administered as protein, 30% as fat, and 55–60% as carbohydrates). All other nutrients without experiment-specific requirements were matching the dietary reference intake levels of the German Nutrition Society (10). The diet did not include caffeine, other methylxanthines, or alcohol. For the calculation of the nutrient content, as well as for the definition of the individual menu of each volunteer, PRODI 4.2 software (Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany) was used. The volunteers received and ate the exact amount of food that was predefined in their individual menu.

During the intervention periods, the energy content of the diet was modified as follows:

1. Hypoenergetic, ambulatory: ~25% decrease in calories compared with standard diet. The average caloric intake was 9.0 ± 1.1 MJ/day.

2. Normoenergetic, bed rest: energy content was reduced compared with the standard diet to adjust to the reduced physical workload. Participants received a diet containing 1.1 (instead of 1.4) times their basal metabolic rate. The average caloric intake was 9.4 ± 1.4 MJ/day.

3. Hypoenergetic, bed rest: ~25% decrease in calories compared with the respective normoenergetic, bed-rest phase. The average caloric intake was 7.7 ± 0.9 MJ/day.

Reduction in energy intake was mainly achieved by reduction of fat intake to a minimum level of 60 g/day to keep the recommended level of essential fatty acids. In case the reduction in energy intake could not be met by decreasing fat intake, the remaining energy intake reduction was derived from carbohydrates. Except for fat and carbohydrates, nutrient composition of each experiment day was identical to the normoenergetic study periods.

Assessment of endothelium-dependent and -independent vasodilator responses. Endothelium-dependent and-independent vasodilator responses were assessed in a quiet room in the supine position. For adaptation of drug dosage, forearm volume (FAV) was determined by the water displacement method. A 22-gauge catheter was inserted into the brachial artery under local anesthesia (Lidocaine·HCl 2%; Braun, Melsungen, Germany) and connected to a pressure transducer for continuous determination of arterial blood pressure. As a rule, the brachial artery of the same arm was punctured in the two corresponding investigations of one phase, and the contralateral arm was used during the next phase. After a period of at least 50 min to allow resting blood flow to stabilize, baseline forearm blood flow (FBF) was measured by means of forearm venous occlusion plethysmography (2, 3). Briefly, strain gauges were placed around both forearms and connected to a plethysmograph (Filtrass 2001, Domed, Munich, Germany). Before FBF was measured, wrist cuffs were inflated to...
suprasystolic values to exclude the circulation to the hand. Then venous outflow of the arm was blocked by rapid inflation of congestion cuffs on the upper arm to 40 mmHg.

When resting FBF values had been established, the endothelium-independent vasodilator sodium nitroprusside (SNP; Nipruss, Schwarz Pharma, Monheim, Germany), diluted in 5% glucose, was infused intra-arterially in six increasing dose rates (0.006 to 0.03 to 0.075 to 0.15 to 0.3 to 0.6 µg·min⁻¹·100 ml FAV⁻¹). After a washout period of at least 45 min, six increasing dose rates (0.01 to 0.05 to 0.2 to 1 to 4 to 16 µg·min⁻¹·100 ml FAV⁻¹) of the endothelium-dependent dilator ACh (Clinalfa/Merck Biosciences, Läufelfingen, Switzerland), diluted in 0.9% saline, were infused. Each dose rate was administered for a period of 7 min to allow sufficient time to equilibrate. FBF was measured at both baseline periods immediately before drug administration and during the last 2 min of the infusion of each dose rate. The mean of the final five measurements of each recording period was used for analysis.

Data analyses. FBF responses to vasodilators were evaluated by investigators blinded for participants’ body position and dietary status. FBF responses to vasodilators were calculated as the absolute change of FBF during drug infusion from baseline FBF, assessed immediately before infusion of the first dose of the respective vasodilator, and were expressed as milliliters per minute per 100 ml tissue. Area under the dose-response curves (AUC) were calculated from individual dose-response curves and were expressed as (log µg·min⁻¹·100 FAV⁻¹)·(ml·min⁻¹·100 ml FAV⁻¹).

Arterial blood pressure and ECG were monitored with the Surveyor II (Mortara Instrument, Essen, Germany) monitoring system. The ECG was sampled at 500 Hz, and heart rate was recorded from leads II, V₁, and V₅. Each intra-arterial pressure wave was sampled at 250 Hz and integrated in real time to obtain mean arterial pressure; diastolic and systolic pressure values were taken as the maximum and minimum values of the pressure wave. Beat-to-beat pressures and heart rates were averaged (16 beat moving average), and the resulting values were stored every 15 s during the experiment.

Laboratory methods. Venous blood samples were drawn in fasting state on the morning of the respective study days. Total cholesterol, HDL, LDL, and triglycerides were measured by using enzymatic colorimetric methods. As a biomarker of oxidative stress (25), DNA isolated lymphocytes (9), and the electric field was 1 V/cm for single-cell gel electrophoresis (comet assay) following the standard protocol of Bauch et al. (1), but whole blood was used instead of isolated lymphocytes (9), and the electric field was 1 V/cm for electrophoresis. Comet images were analyzed with an image processing software (Comet Assay II, Perceptive Instruments), and tail moment (TM) was calculated as tail length multiplied by percentage of DNA in tail (24).

Statistics. Data are expressed as mean values ± SE. Although one individual had to be replaced after the first session, all participants were included into the final data analysis, because the results of the intervention period were always evaluated compared with the intra-individual findings in the respective adaptation period. Local hemodynamic responses to infusion of study drugs (adaptation vs. intervention period) were compared using two-factor, repeated-measures analysis of variance. Differences in anthropometric and baseline hemodynamic parameters as well as lipid values (adaptation vs. intervention period) were assessed with Wilcoxon signed-rank test. Data of the four adaptation periods were compared by using repeated-measures analysis of variance.

Spearman rank-order correlation was performed to analyze the influence of selected factors on vasodilator responses. A P value of <0.05 was considered significant.

RESULTS

Demographic characteristics. At screening, the clinical characteristics of the study population were as follows: age 24 ± 1 yr, body wt 75.8 ± 2.3 kg, height 182 ± 2 cm, body mass index 22.9 ± 0.8 kg/m², total cholesterol 161 ± 7 mg/dl, HDL 51 ± 3 mg/dl, LDL 97 ± 5 mg/dl, resting systolic blood pressure 123 ± 2 mmHg, and resting diastolic blood pressure 78 ± 3 mmHg.

Body weight and FAV. Thirteen days of hypoenergetic diet induced a weight loss of 2.75 ± 0.19 kg during bed rest (P = 0.002) and 1.54 ± 0.22 kg under ambulatory conditions (P = 0.002). Bed rest with normoenergetic nutrition induced a weight loss of 1.58 ± 0.28 kg (P = 0.002), 59% of which was achieved on the first day of intervention (weight loss on day 1: 0.93 ± 0.07 kg). No significant changes were observed during normoenergetic, ambulatory conditions (P = 0.32). FAV did not change during any of the intervention periods (Table 1).

Baseline hemodynamic measurements. Baseline hemodynamic parameters on day 9 of the adaptation period and on day 13 of the intervention period are listed in Table 1. No statistically significant changes in resting FBF and forearm vascular conductance were observed between adaptation and intervention, but mean values of resting FBF and forearm vascular conductance tended to be higher after normoenergetic, ambu-

Table 1. Effects of bed rest and nutrition on anthropometric, hemodynamic, and DNA strand break variables in 10 healthy participants

<table>
<thead>
<tr>
<th></th>
<th>Ambulatory + Normoenergetic</th>
<th>Bed Rest + Normoenergetic</th>
<th>Ambulatory + Hypoenergetic</th>
<th>Bed Rest + Hypoenergetic</th>
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<tbody>
<tr>
<td></td>
<td>Adaptation</td>
<td>Intervention</td>
<td>Adaptation</td>
<td>Intervention</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.1 ± 0.4</td>
<td>75.8 ± 0.6</td>
<td>76.9 ± 0.7</td>
<td>75.4 ± 0.9</td>
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<td>BMI, kg/m²</td>
<td>22.8 ± 3.2</td>
<td>22.7 ± 3.3</td>
<td>23.1 ± 2.9</td>
<td>22.7 ± 3.0</td>
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<tr>
<td>Mean BP, mmHg</td>
<td>71 ± 3¹</td>
<td>71 ± 2</td>
<td>68 ± 3</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65 ± 2²</td>
<td>61 ± 2</td>
<td>63 ± 2</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>FAV, ml</td>
<td>1.465 ± 68</td>
<td>1.455 ± 65</td>
<td>1.480 ± 68</td>
<td>1.465 ± 70</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
<td>3.0 ± 0.5</td>
<td>4.0 ± 0.7</td>
<td>4.0 ± 0.7</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>AUCₐChS₉, units</td>
<td>14.2 ± 1.8</td>
<td>14.2 ± 1.4</td>
<td>15.5 ± 1.5</td>
<td>14.6 ± 2.1</td>
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<tr>
<td>AUCₐChC₉, units</td>
<td>22.9 ± 3.2</td>
<td>24.9 ± 3.3</td>
<td>25.5 ± 4.8</td>
<td>19.3 ± 3.6</td>
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<tr>
<td>AUCₐCh/AUCₐChS₉</td>
<td>1.64 ± 0.18</td>
<td>1.82 ± 0.23</td>
<td>1.63 ± 0.29</td>
<td>1.18 ± 0.34</td>
</tr>
<tr>
<td>TM, µm</td>
<td>2.30 ± 0.18</td>
<td>2.31 ± 0.19</td>
<td>2.16 ± 0.34</td>
<td>2.39 ± 0.22</td>
</tr>
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</table>

Values are means ± SE from 10 participants, with the following exceptions: n₉ = 9 and n₈ = 8 participants (missing data due to hard disk failure), n₈ = 8 subjects (missing data due to gel damage during storage). Data are from day 9 of the adaptation and from day 13 of the intervention period. BMI, body mass index; BP, blood pressure; FAV, forearm volume; FBF, forearm blood flow; TM, tail moment; AUCₐChS and AUCₐChC₉, area under the dose-response curves of SNP and ACh, respectively. *P < 0.05, **P < 0.01 for comparison intervention vs. respective adaptation period. Data of the four adaptation phases were not significantly different.

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latory conditions and to be lower after all other three interventions.

**SNP-induced vasodilation.** Local infusion of SNP or ACh into the brachial artery had no local or systemic adverse effects. Figure 2 illustrates the FBF response to infusion of the endothelium-independent vasodilator SNP. Increasing concentrations of SNP dose-dependently increased FBF, e.g., in the adaptation period before the normoenergetic, ambulatory phase, change in (Δ) FBF gradually increased up to 15.6 ± 1.7 ml·min⁻¹·100 ml FAV⁻¹, and on day 13 of the intervention the vasodilator response was unchanged (P = 0.98). Similarly, during normoenergetic bed rest, the dose-response curve to SNP was unchanged (P = 0.53). Under hypoenergetic, ambulatory conditions, the vasodilator responses to SNP were augmented (P < 0.001), whereas, during hypoenergetic bed rest, relaxation was unchanged (P = 0.08).

**ACh-induced vasodilation.** Figure 3 illustrates the FBF response to infusion of the endothelium-dependent vasodilator ACh. During normoenergetic, ambulatory conditions, the dose-dependent increase in FBF was unchanged compared with preintervention (P = 0.94). However, normoenergetic bed rest shifted the dose-response relationship to the right toward higher ACh doses (P = 0.004). In contrast, during hypoenergetic, ambulatory conditions, vasodilator responses to ACh were augmented (P = 0.044), whereas, during the combined effect of bed rest and hypoenergetic, low-fat diet, ACh responses were unchanged (P = 0.21).

**Ratio of ACh-induced to SNP-induced vasodilation.** To consider concurrent changes in vascular smooth muscle responsiveness (as observed during hypoenergetic diet) in the assessment of endothelial function, the ratio of the AUCs of the ACh and SNP dose-response curves was calculated for each participant (Table 1). This ratio increased under hypoenergetic, ambulatory conditions (P = 0.049) and was unchanged during hypoenergetic bed rest (P = 0.28).

**Serum lipids, oxidative stress, and vasodilator responses.** Serum lipids decreased during the hypoenergetic study sessions (Table 2). The correlations between individual changes in serum lipids in these sessions and the respective changes in oxidative DNA damage and vasodilator function are listed in Table 3. Individual changes in LDL were correlated with the respective changes in DNA strand breaks (expressed as TM), and changes in both parameters were correlated with changes in the AUCs to SNP. Individual changes in total cholesterol-to-HDL ratio were correlated with changes in the AUCs to SNP as well as to changes in the AUCs to ACh (Fig. 4). During normoenergetic bed rest, individual changes in DNA strand breaks were correlated with respective changes in the

![Fig. 2. Effect of bed rest and/or hypoenergetic diet on vascular smooth muscle relaxation. Forearm blood flow (FBF) response to sodium nitroprusside (SNP) is shown before (○) and after 13 days (●) of intervention. The intervention consisted of normoenergetic nutrition under ambulatory conditions (A) or bed rest (B), or hypoenergetic diet under ambulatory conditions (C) or bed rest (D). FAV, forearm volume; n.s., not significant. Data are expressed as absolute change (Δ) from baseline FBF. Values are means ± SE; n = 10 participants.](http://jap.physiology.org/)
AUCs to SNP (Spearman correlation coefficient = −0.738, \( P = 0.029 \)).

**DISCUSSION**

The aim of the present study was to determine the effects of bed rest, a hypoenergetic, low-fat diet, and their combination on peripheral vascular function in healthy volunteers under strictly controlled conditions to avoid any confounding factors.

**Effect of bed rest.** The first major finding of the present study was that endothelium-dependent vasodilator responsiveness of forearm resistance vessels to intra-arterial infusion of ACh was impaired after short-term bed rest and normoenergetic nutrition.

**Fig. 3. Effect of bed rest and/or hypoenergetic diet on endothelium-dependent relaxation.** FBF response to acetylcholine (ACh) is shown before (○) and after 13 days (●) of intervention. The intervention consisted of normoenergetic nutrition under ambulatory conditions (A) or bed rest (B), or hypoenergetic diet under ambulatory conditions (C) or bed rest (D). Data are expressed as absolute change (Δ) from baseline FBF. Values are means \( \pm SE; n = 10 \) participants.

| Table 2. Relative changes in serum lipids after 13 days of intervention |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
|                          | Ambulatory + Normoenergetic | Bed Rest + Normoenergetic | Ambulatory + Hypoenergetic | Bed Rest + Hypoenergetic |
| ΔTriglycerides, %         | −4±7             | +4±13            | −10±5            | −4±4            |
| ΔTotal cholesterol, %     | −5±2             | −9±6             | −12±3†           | −12±3*          |
| ΔHDL, %                   | −9±2†            | −14±4*           | −15±3†           | −17±3†          |
| ΔLDL, %                   | −4±4             | −2±13            | −13±4*           | −8±5            |
| ΔTotal cholesterol/HDL, % | +5±2             | +7±9             | +5±4             | +7±3            |

Values are means \( \pm SE \) from 10 subjects. Δ Change. Relative changes in serum lipids are shown from day 9 of the adaptation to day 13 of the intervention period. \( *P < 0.05, †P < 0.01 \) for comparison intervention vs. corresponding adaptation period.

| Table 3. Correlation between changes in serum lipids, oxidative DNA damage, and vascular responsiveness in 10 healthy participants |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
|                          | \( \Delta \text{AUC}_{\text{SNP}} \) | \( \Delta \text{AUC}_{\text{ACh}} \) | \( \Delta \text{TM}, \% \) |
|                          | \( P \text{ value} \) | \( r_s \) | \( P \text{ value} \) | \( r_s \) | \( P \text{ value} \) | \( r_s \) |
| ΔTotal cholesterol, %     | 0.01             | −0.559          | NS              | NS              |
| ΔHDL, %                   | <0.001           | 0.693           | NS              | NS              |
| ΔLDL, %                   | 0.008            | −0.573          | 0.023           | −0.504          | NS              |
| ΔTotal cholesterol/HDL, % | 0.003            | −0.711          | NS              | NS              |

Correlation between percent changes in lipids, percent changes in DNA strand breaks [tail moment (TM)], and unit changes in the area under the dose-response curves (AUC) of sodium nitroprusside (SNP) and acetylcholine (ACh) during the hypoenergetic study phases. \( r_s \), Spearman correlation coefficient; NS, not significant.
Bed rest, hypoenergetic diet, and endothelial function

Endothelial dysfunction with a diminished bioavailability of NO has been associated with impaired endothelium-dependent vasodilation (19). Concurrently, this procedure also reduces the expression of superoxide dismutase-1 (39) and may thus contribute to blunted endothelium-dependent vasodilation by decreasing the half-life of NO. Hence, impaired endothelial function observed in bed rest and normoenergetic nutrition in the present study might be explained by downregulation of NOS III and/or superoxide dismutase induced by alteration of shear stress, resulting in decreased NO bioavailability.

Bed rest may reduce shear stress via two factors: physical inactivity and hypovolemia. The acute change from an upright to a horizontal body position induces a central fluid shift with a rapid and transient increase in central venous pressure (27). Hemodynamic adaptation occurs quickly and includes increased urine flow and reduction in plasma volume (27). Fluid loss in our participants is mirrored in the weight loss of nearly 1 kg observed within the first 24 h of bed rest. Within 10–14 days of both horizontal and 6° head-down-tilt bed rest, hypovolemia results in a 9–15% reduction of plasma volume (13, 30, 31), a 16–20% reduction in left ventricular end-diastolic volume (18, 31), and a 10–12% reduction in cardiac output (30, 31). Thus hypovolemia induced by bed rest alters preload and cardiac output similarly in horizontal and head-down-tilt bed rest and is likely to contribute to reduced shear stress (and endothelial dysfunction).

Earlier studies suggest that bed rest also modulates circulating vasoactive compounds interacting with the NO-cGMP-pathway (e.g., endothelin, natriuretic peptides) (7, 11). However, the expected changes during bed rest (endothelin-1 increase, atrial natriuretic peptide decrease) would augment vascular tone and thus impair SNP responses, which was not the case. This finding suggests that the changes in NO availability and cardiac output is caused by changes in NO bioavailability.

The enzyme responsible for generation of NO in the vascular smooth muscle function. Because ACh responses in the human forearm are almost exclusively mediated by NO release through activation of NOS III (22) and only to a small extent through prostaglandins (21), our findings suggest that bed rest induces endothelial dysfunction with a diminished bioavailability of NO, i.e., with a reduced amount of NO available locally at the site of action in active form. The potential impairment of the NO pathway is supported by the findings of a recent study (20), in which a 52% increase in leg vascular resistance after 14 days of bed rest was accompanied by a 46% decrease of plasma nitrate/nitrite (Nox) concentrations, indirectly suggesting that NO release from the endothelium was reduced. In contrast, another study (6) showed an enhanced flow-dependent vasodilatation in the brachial artery after 7 days of bed rest, indicating that large arteries might react differently to immobilization than resistance arteries.

The enzyme responsible for generation of NO in the vasculature is NOS III. NOS III mRNA, protein, and enzyme activity have previously been shown to be regulated by changes in flow or shear stress (26). Hindlimb unloading in rats results in chronic reduction of soleus blood flow, which is associated with reduced expression of NOS III mRNA and protein, reduced NO release, and subsequently attenuated endothelium-dependent vasodilation (19).

Effect of a hypoenergetic, low-fat diet. The second major finding of the present study was that a short-term, low-fat, hypoenergetic diet improved vascular smooth muscle function (Fig. 2C), as well as endothelial function (Fig. 3C), in ambulatory healthy volunteers in the absence of cardiovascular risk factors.

As expected, the low-fat diet induced significant reductions in serum lipids (Table 2). The individual changes in LDL were correlated with individual changes in oxidative stress and vascular smooth muscle relaxation (Table 3). However, presumably because of the small number of participants, a significant correlation with individual changes in endothelial function was only found for the parameter cholesterol-to-HDL ratio (Fig. 4).

To our knowledge, no study has so far investigated dietary approaches to lower serum lipids and their effects on endothelial function in healthy, lean individuals. In a recent study in hypercholesterolemic patients, monounsaturated fat was substituted by walnuts. Similar to our study, endothelium-dependent vasodilation improved, with changes in vasodilation being inversely correlated with changes in cholesterol-to-HDL ratios (32). The effect of the walnut diet may be mediated in part through the improved lipid profile, but special components of walnuts, such as α-linolenic acid, l-arginine, and antioxidants, might have contributed to the beneficial effects (32). Another study in hypercholesterolemic patients (36) investigated the effects of LDL apheresis on vascular function. A single session of LDL apheresis decreases LDL and augments endothelial function and NOx production. Endothelium-independent vaso-
dilation after LDL-apheresis was unchanged (36). However, this is not in contrast with our data, because the highest SNP dose infused in this earlier study corresponds to the third dose used in our study. Differences in SNP response were only evident with higher doses and thus may have been missed in this earlier trial. Our finding of augmented endothelium-independent vasodilator responses is consistent with a study investigating the relationship between cardiovascular risk factors and vascular drug responses (8) that found that a lower cholesterol-to-HDL ratio is associated with enhanced responses to both NO donors and ACh. It is possible that decreased endothelin-1 may contribute to improved vasodilator function. However, endothelin-1 increases after acute LDL lowering (36) and thus would augment vascular tone and impair SNP responses, which was not the case.

The strong correlations between changes in ACh responses after LDL apheresis and respective changes in LDL, oxidized LDL, and NOx production (36), as well as the finding that LDL increases vascular production of superoxide anion, which can inactivate NO rapidly (28), suggest that improvement of endothelial function after LDL lowering may be caused by augmented NO bioavailability due to reduction in oxidative stress (36). Indeed, the individual decrease in LDL values in our study was correlated with the reduction of oxidative stress as assessed by DNA strand breaks (expressed as TM). A recent study in rabbits has confirmed that this parameter is not only associated with cholesterol-induced atherosclerotic plaque formation but also decreases quickly after cholesterol withdrawal (24).

Epidemiological and angiographic studies have firmly established a causal relation between elevated serum cholesterol levels and the development of atherosclerosis and ischemic heart disease (23). Furthermore, the results of primary- and secondary-prevention trials provide compelling evidence that cholesterol reduction leads to a significant improvement of endothelial vasomotor function and a decrease in the rate of cardiovascular events (23). Our study expands this knowledge in so far that, even in healthy humans without any cardiovascular risk factors, only 13 days of a moderate hypoenergetic, low-fat diet, independent of confounding factors such as co-medication or physical activity, result in a substantial increase in vasodilator function along with the expected decrease of serum lipids.

**Combined effect of bed rest and hypoenergetic diet.** When bed rest was combined with hypoenergetic diet, endothelium-dependent vasodilation was still lower at all ACh dose rates (Fig. 3D), but it was less pronounced than the effect of normoenergetic bed rest and did not reach statistical significance. These data may, therefore, indicate that a hypoenergetic, low-fat diet may antagonize the unfavorable effects of bed rest on endothelial function, possibly by partially restoring NO bioavailability.

Unfortunately, our study design does not allow quantification of the extent of improvement properly. Although the standardization within a study session (comparison before and after intervention) was high, comparison between study sessions is less exact due to relatively long periods between study sessions, and the change of infusion arm between sessions. In summary, strict bed rest for 13 days impairs endothelium-dependent arterial relaxation in healthy men. This effect was offset by a concurrent low-fat diet, which modulates serum lipids and oxidative stress, and improves endothelium-dependent and -independent vasodilation. Because endothelial dysfunction predicts the occurrence of cardiovascular disease (16) and is independently related to future cardiovascular events (12), protective effects of a low-fat diet (or other nutritional or pharmacological interventions) on endothelial function are worth being investigated in astronauts and bedridden patients in future studies.

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