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Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers

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Navasiolava NM, Dignat-George F, Sabatier F, Larina IM, Demiot C, Fortrat JO, Gauquelin-Koch G, Kozlovskaya IB, Custaud MA. Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. *Am J Physiol Heart Circ Physiol* 299: H248–H256, 2010. First published May 14, 2010; doi:10.1152/ajpheart.00152.2010.—A sedentary lifestyle has adverse effects on the cardiovascular system, including impaired endothelial functions. Subjecting healthy men to 7 days of dry immersion (DI) presented a unique opportunity to analyze the specific effects of enhanced inactivity on the endothelium. We investigated endothelial properties before, during, and after 7 days of DI involving eight subjects. Microcirculatory functions were assessed with laser Doppler in the skin of the calf. We studied basal blood flow and endothelium-dependent and -independent vasodilation. We also measured plasma levels of microparticles, a sign of cellular dysfunction, and soluble endothelial factors, reflecting the endothelial state. Basal flow and endothelium-dependent vasodilation were reduced by DI (22 ± 4 vs. 15 ± 2 arbitrary units and $29 \pm 6\%$ vs. $12 \pm 6\%$, respectively, $P < 0.05$), and this was accompanied by an increase in circulating endothelial microparticles (EMPs), which was significant on *day 3* (42 ± 8 vs. 65 ± 10 EMPs/μl, $P < 0.05$), whereas microparticles from other cell origins remained unchanged. Plasma soluble VEGF decreased significantly during DI, whereas VEGF receptor 1 and soluble CD62E were unchanged, indicating that the increase in EMPs was associated with a change in antiapoptotic tone rather than endothelial activation. Our study showed that extreme physical inactivity in humans induced by 7 days of DI causes microvascular impairment with a disturbance of endothelial functions, associated with a selective increase in EMPs. Microcirculatory endothelial dysfunction might contribute to cardiovascular deconditioning as well as to hypodynamia-associated pathologies. In conclusion, the endothelium should be the focus of special care in situations of acute limitation of physical activity.

dry immersion; weightlessness; microcirculation; endothelial dysfunction; soluble endothelial markers

THE VASCULAR ENDOTHELIUM is a key element in the control of local blood flow (12, 46). It is now widely recognized that alterations of endothelial integrity and physiological functions represent pivotal mechanisms in the initiation and development of vascular diseases. A decrease in endothelial vasomotor function (endothelium-dependent vasodilation), which correlates with a risk of cardiovascular events, has been demonstrated in patients with atherosclerosis, hypertension, and peripheral vascular diseases (49). Physical inactivity and a sedentary lifestyle are known to cause cardiovascular deconditioning and increase the risk of cardiovascular disease (5). Endothelial dysfunction seems to be intimately linked to this deconditioning. A chronic decrease in shear stress forces during physical inactivity, especially significant in small vessels of the microcirculatory bed (8), may impair endothelial functions.

Experimental physical inactivity of varying duration and intensity in healthy humans can be achieved through confinement (altitude chambers), partial immobilization, bedrest, and "dry" water immersion (33, 34). Dry immersion (DI) induces extreme physical inactivity in short-term studies. Supportlessness and the extreme degree of physical unloading are immediately reflected by a deep decrease in muscle tone and motor activity level (25). This model was also developed to simulate conditions of weightlessness. A series of reports about macrocirculatory alterations induced by physical inactivity of different degrees and duration involving bedrest (6), lower limb suspension (7), and patients with spinal cord injuries (17) have been recently published by De Groot and colleagues (16). However, only a few studies were devoted to microcirculatory dysfunction. We (18) recently showed that 2 mo of bedrest in women induces endothelial dysfunction. However, shorter bedrest periods of 5 and 21 days also seem sufficient to reduce endothelial vasodilatory capacity (26, 27).

Various markers serve to study the state of the endothelium, but their use is limited by a host of factors. Among these markers, functional testing represents the gold standard; however, this remains difficult to apply systematically for a large population. It is relatively easy to quantify plasma endothelial markers reflecting endothelial changes, such as soluble adhesion molecules or angiogenic factors. However, these markers have been rather disappointing due to the lack of relevant clinical significance at the individual level, although they may provide information on given pathological mechanisms associated with endothelial dysfunction. More recently, attention has been focused on such biomarkers of endothelial injury as circulating endothelial cells (CECs) and endothelial microparticles (EMPs). We (18) have already shown that 2 mo of

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bedrest in women induces a significant increase in CECs. EMPs are microvesicles (0.1–1.5 μm) released from the membrane of activated or apoptotic endothelial cells. Besides being relevant and specific markers of endothelial integrity, they also behave as biological vectors able to disseminate various pathogenic signals amplifying endothelial dysfunction (13). Convergent data from different studies have demonstrated that the EMP level correlates with the severity of endothelial dysfunction assessed by flow-mediated dilation in patients with end-stage renal disease (4) or coronary artery disease (11). To our knowledge, no studies have investigated the influence of physical inactivity on EMP levels in healthy subjects.

The aim of this work was to study the influence of enhanced physical inactivity in healthy volunteers on endothelial integrity. We hypothesized that 7 days of physical inactivity induced by DI would be enough to cause endothelial alterations, as assessed by functional testing combined with the measurement of EMP levels.

MATERIALS AND METHODS

Subjects

This study was carried out at the Dry Immersion Facility "Khoroshevskaya," which is affiliated with the Institute of Biomedical Problems (State Scientific Center of Russia, Moscow, Russia). Eight healthy nonathletic Russian men participated in the study. Their age, body height, usual weight, and level of regular physical activity (including daily walking) were 23 ± 0.5 yr old, 175 ± 3 cm, 76 ± 4 kg, and 6 ± 1.7 h/wk (means ± SE), respectively. Eligible subjects were enrolled after a medical interview and thorough examination. They were all without any cardiovascular, pulmonary, or kidney disease and were non-smokers. The volunteers were informed about the procedures and gave their informed written consent. The experimental protocol was approved by the Russian National Committee on Bioethics of the Russian Academy of Sciences and conformed with the standards set by the Declaration of Helsinki.

DI Method

DI involves immersing the subject into thermoneutral water covered with a special elastic waterproof fabric film (30, 33, 43) (Fig. 1). The subject is kept dry, since the film separates the subject from the water. The subject thus appears to be "freely suspended" in the water mass. The film is thin and large enough to ensure that the water's hydrostatic pressure is equally distributed throughout the surface of the body, providing conditions similar to full supportlessness. It must be emphasized that the conditions created by immersion are best for achieving maximal hypokinesia and hypodynamia, not only due to the acute limitation of usual motor activity (which can be achieved in bedrest) but also because the antigravity component of muscle work is compensated by the buoyancy force (40). Furthermore, muscle tone and muscle tension drop due to the absence of support stimuli (25, 36), whereas in bedrest–even in prolonged bedrest–they are always partly preserved (35).

Dry Immersion Protocol

All subjects remained at the Dry Immersion Facility for 11 days, including 2 ambulatory control days before immersion [baseline *days −2* and *−1* (*days B-2* and *B-1*)], 7 days of DI [DI *days 1–7* (*days DI 1–DI 7*)], and 2 days of recovery [recovery *days +1* and *+2* (*days R+1* and *R+2*)]. In addition, subjects were called in for blood sampling [on baseline *day −7* (*day B-7*) and recovery *day +4* (*day R+4*)]. Subjects were dipped one after another; each subject underwent DI for 7 days.

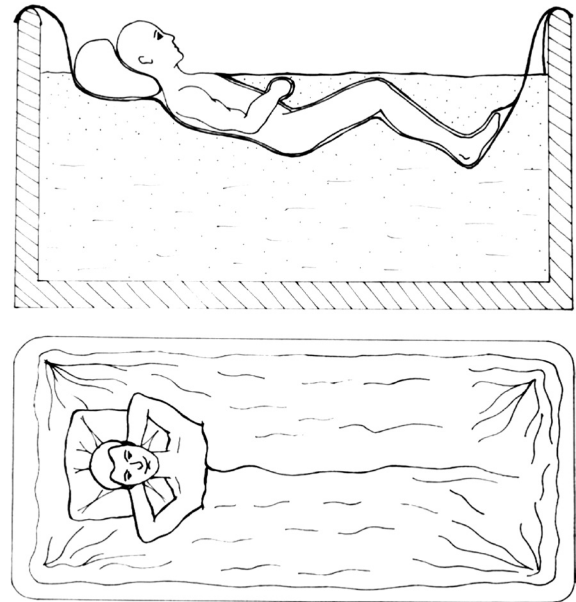

Fig. 1. Dry immersion (DI) method. The subject is immersed up to the armpits and separated from the water by waterproof fabric. *Top*: side view; *bottom*: view from above.

During immersion, subjects remained in a supine position continuously for all activities except for two 15-min periods for weighing (in a standing position) and hygiene procedures (at 9 AM and 9 PM). The water temperature was set between 32 and 33°C. Subjects were continuously supervised by video observation. Body temperature (armpit level) was measured 2 times/day. The ambient temperature was 25.4 ± 1.2°C on *day B-1* and 24.5 ± 1.1°C on *day R+2*.

During immersion, the daily caloric intake was ~2,270 ± 50 kcal; before and after immersion, it was ~2,880 ± 60 kcal. Total water consumption was ~50 $ml \cdot kg^{-1} \cdot day^{-1}$ (~3.7 l/day) with direct water intake [including water, tea (without theine), or juice] of ~2.6 l/day. Dietary sodium was set to 2.8 $mmol \cdot kg^{-1} \cdot day^{-1}$, daily calcium intake was adjusted to 1,000–1,150 mg, and protein intake was 1.15 $g \cdot kg^{-1} \cdot day^{-1}$. In this immersion protocol, unlike the majority of DI studies with quantified ad libitum water intake, water and sodium consumption were strictly standardized.

Twenty-four-hour urine pools were collected daily throughout the study, and the partial water balance (defined as the difference between consumed water and urine volume) was calculated.

Functional Testing of the Endothelium by Iontophoresis

The functional properties of the skin microcirculation were evaluated in the morning, before immersion (*day B-1*), during immersion (*days DI 1*, *DI 3*, and *DI 7*), and in the recovery period (*day R+2*). On *day 1*, testing was performed 1 h after the beginning of the immersion. Pre- and postimmersion assessments were performed in a quiet room with controlled air temperature. Subjects were placed in a supine position and rested for 30 min before data were recorded.

During immersion, a thermoneutral water temperature was continuously maintained. The recording started after readings had stabilized.

Skin blood flow was assessed at the calf level, apart from the superficial veins. The calf was shaved 24 h before to avoid skin irritation on the day of the experiment.

The protocol included the assessment of basal skin blood flow, endothelium-dependent and endothelium-independent vasodilation, and maximal vasodilation.

Laser Doppler (LD) flowmetry is recognized as a validated and reproducible method of assessing and monitoring endothelial function

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(47). To evaluate skin perfusion, the LD flowmetry technique coupled with iontophoresis was applied.

Three LD multifiber probes (wavelength: 780 nm) connected to a LD flowmeter (Periflux PF4001, Perimed) were used. Two specially designed iontophoretic probes (PF 481-1, Perimed) were used for local temperature measurement, current application, local heating, and simultaneous skin blood flow recording. These active probes had circular chambers for placing the double-sided adhesive patches with a sponge measuring ~0.6 cm^2 (PF383, Perimed). The laser beams passed through the hole in the center of the sponge.

The active probes were also connected to temperature-regulated heating systems (Peritemp PF4005, Perimed) and to regulated current suppliers (Periiont PF 382, Perimed), allowing for the delivery of currents with regulated intensity and duration.

A third probe (PF408, Perimed) was used as a reference 5 cm from the iontophoretic sites. Skin temperature was maintained as thermoneutral between 33.5 and 34.5°C; this temperature has shown to cause neither constriction nor dilation of cutaneous vessels (13). The probes were placed with the same topography in all subjects, respectively, to the distal apex of patella. To assure the same placement of probes for subsequent repeated measurements, the position of the probes was marked on the skin.

Basal blood flow. A stable baseline was registered for 5 min before iontophoresis was performed. Basal calf skin blood flow was calculated as a mean of basal values from three probes (the reference probe and both active probes) and is presented in non-normalized values.

Endothelium-dependent and endothelium-independent vasodilation. Iontophoresis involves the use of a continuous monopolar current for the local transcutaneous delivery of small amounts of pharmacological agents. In this study, we measured the blood flow changes in response to iontophoresis of 2% ACh (a substance that induces endothelium-dependent vasodilation) and 1% sodium nitroprusside (SNP; which induces endothelium-independent vasodilation). These substances were diluted in deionized water.

In each experiment, the sponges were moistened with 0.2 ml of solution. The current application consisted of a 10-s, 0.1-mA anodal current for ACh and a 20-s, 0.1-mA cathodal current for SNP. Two disposable Ag/AgCl electrodes (Care 610, Kendall, Neustadt, Germany) were positioned immediately next to the active probes and served as the opposite poles in each experiment. With applied current intensity and duration, the drug delivery was local, which was confirmed by stable flow values at the level of the reference probe during iontophoresis.

After the iontophoretic period, the signals were recorded during 20 min to estimate the iontophoretic response. ACh induces a biphasic vascular response with the early (peak) phase mainly mediated by the nitric oxide pathway and the late (plateau) phase mainly related to prostaglandins (20). Both peak and plateau vasodilation were estimated for the ACh-induced response. The SNP-induced vascular response was measured at the plateau.

Maximal vasodilation. At the end of the experiment, the active probes were continuously warmed to 44°C for 20 min to cause maximal cutaneous vasodilation (15).

Analysis of LD Flow Data

For LD flow data analysis, we defined the following: baseline (the mean for the last minute recorded before the current application), peak (the mean for the 10-s interval of maximal values within the 5 min after current application), plateau (the mean for the last minute of recovery), and maximal vasodilation (the mean for the last minute of local heating). Responses to iontophoresis were expressed as percentages of the maximal vasodilatory response to local heating from the same active probe.

An example of the raw data is shown in Fig. 2.

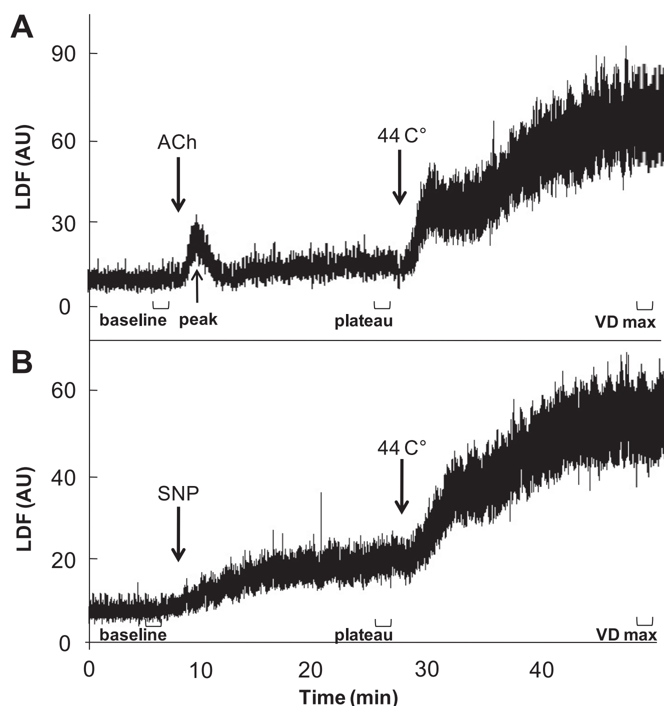

Fig. 2. Examples of laser-Doppler flow [LDF; in arbitrary units (AU)] recordings with the ACh probe (*A*) and sodium nitroprusside (SNP) probe (*B*) at baseline, responses to iontophoresis of ACh (peak and plateau phases) and SNP (plateau), and responses to local heating [maximal vasodilation (VD max)]. Arrows indicate the beginning of iontophoresis and beginning of heating, respectively.

Blood Analysis

Blood sampling. Antecubital venous blood samples were collected in citrate [for assessing microparticles (MPs) and endothelial factors], EDTA (for blood counts), and nonanticoagulant (for glucose and lipids determination). Vacutainers in the morning before breakfast were used.

Complete blood counts. Erythrocyte, Hb, and Hct levels were measured on *days B-2*, *DI 3*, *DI 7*, and *R+4*. Leukocyte and platelet levels were assessed on *days B-7* and *R+1*. Measurements were performed with a MEK 6318 hematological auto analyzer.

Calculation of plasma volume evolution. Percent changes in plasma volume (ΔPV) were calculated from changes in Hb and Hct using the Dill and Costill method (19) as follows: ΔPV (in %) $= 100 \times [Hb_{B\text{-}2}(1 - 0.01Hct_i)]/[Hb_i(1 - 0.01Hct_{B\text{-}2})] - 100$, where $Hb_{B\text{-}2}$ and $Hct_{B\text{-}2}$ are the Hb and Hct levels on *day B-2*, respectively, and Hb_i and Hct_i are Hb and Hct on *days DI 3*, *DI 7*, and *R+4*, respectively.

Blood biochemical analyses (glucose and lipids). Biochemical tests including measurements of total cholesterol, HDL-cholesterol, triglycerides, and glucose were carried out by enzymatic calorimetric methods using commercially available kits (DiaSys). LDL-cholesterol was calculated using the Friedewald formula.

Assessment of MPs

Plasma preparation and MP labeling. Blood samples were drawn into 0.129 mol/l sodium citrate tubes (Vacutainer, Becton Dickinson) between 8:00 and 8:30 AM and processed for MP analysis within 1 h postdrawing according to recommendations of the International Society of Thrombosis and Hemostasis (31). Briefly, samples were centrifuged at 1,500 *g* for 15 min to obtain platelet-free-plasma followed by a 2-min centrifugation at 13,000 *g* to remove residual platelets and cell debris. These platelet-free-plasma samples were then frozen at −80°C until used. Analysis of MP subpopulations was done

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using the following specific fluorochrome-labeled monoclonal antibodies: anti PECAM-1 and GpIIbIIIa for EMPs (PE-CD31, clone 1F11, Beckman Coulter, Marseille, France, and FITC-CD41, clone PL2-49, Biocytex, Marseille, France), anti-GpIIbIIIa for platelet MPs (PE-CD41, clone PL2–49), anti-glycophorin A for erythrocyte MPs (PE-CD235a, clone KC16, Beckman Coulter), and anti-CD11B/CD66 for leukocyte MPs (FITC-CD66b, clone 80H3, and FITC-CD11b, clone Bear-1, Beckman Coulter). Annexin V binding was used to numerate phosphatidylserine-expressing circulating MPs irrespective of their cellular origin (total MPs). After being thawed, 30 μl of plasma were incubated with specific monoclonal antibodies or corresponding isotype controls (FITC-IgG1 and PE-IgG1, Beckman Coulter Immunotech) or with FITC-annexin V (AbCys.SA, Paris, France). After a 30-min incubation at room temperature, samples were diluted in 0.5 ml of PBS (Dulbelcco's, Life Technologies, Paisley, UK) or binding buffer for annexin V labeling (AbCys.SA). Then, 30 μl of internal standard (Flow Count beads, Beckmann Coulter) were added to each sample to allow the expression of MP results as absolute numbers per microliter.

Flow cytometry. Samples were analyzed by flow cytometry (CXP FC 500, Beckmann Coulter). MPs presented in plasma were analyzed according to their size and fluorescence in a logFS-logSS dot plot as previously described (3, 41). Briefly, the MP gate was defined in a logFS-logSS dot plot using, as a boundary, fluorescent beads of 0.5 and 0.9 μm in diameter (Megamix, Biocytex, France). As the numerical ratio between beads is well established (2:1, 0.5-μm/0.9-μm beads, respectively), the threshold was switched to FS and PMT voltage settings adjusted to obtain 50% of 0.5-μm beads. Each population of MPs was then numerated in one- or two-color fluorescence plots. The flow count beads were counted in a third dot plot, logSS-logFL3. The EMP flow cytometric analysis did not include a lower size restriction to enhance detection sensitivity.

Assessment of Soluble Endothelial Markers

Platelet-free plasma obtained as described above was divided into 400-μl aliquots and stored at −80°C until analysis. Plasma levels of E-selectin, VEGF receptor 1 (VEGFR1), and VEGF were analyzed by ELISA using Quantikine ELISA kits (Human sE-Selectin/CD62E Quantikine ELISA kit, Human VEGF Quantikine ELISA kit, and Human sVEGF R1/Flt-1 Quantikine ELISA Kit, R&D Systems Europe, Lille, France). ELISA tests were performed according to the manufacturer's instructions, and absorbance measurements were conducted using an Infinite F200 spectrophotometer instrument (TECAN). The intra-assay variability of the ELISA test was <5% and the interassay variability was <8%.

Statistical Analysis

The data received during immersion and in the recovery period were compared with baseline data. All values are presented as means ± SE. For statistical analysis, a Friedman test was used first followed by a post hoc Wilcoxon signed-rank test if significant differences were obtained. These nonparametric tests are appropriate for matched pairs of data where the samples are related and a normal distribution is not assumed. Values of $P < 0.05$ were considered significant. Analyses were performed using SPSS 15.0 for Windows.

RESULTS

Clinical Events and Daily Monitoring of Physiological Data

DI was well tolerated; all the subjects, however, had a backache at the beginning, which disappeared by the third to fourth day of immersion. During DI, no major changes in body temperature were observed (Table 1). A significant decrease in weight persisted throughout the entire immersion period (Table 1). Heart rate and blood pressure remained within normal limits, with a significant decrease in diastolic blood pressure on *day DI 1* (Table 2). We observed a significant decrease in plasma volume versus baseline of −15 ± 4% on *day DI 3* and −16 ± 3% on *day DI 7* ($P < 0.05$).

Complete Blood Count

At the end of DI, we observed a significant increase in erythrocytes ($5 \pm 0.11 \times 10^{12}$ erythrocytes/l at baseline vs. $5.5 \pm 0.08 \times 10^{12}$ erythrocytes/l on *day DI 7*, $P = 0.012$), Hb (149 ± 3.7 g/l at baseline vs. 162 ± 3 g/l on *day DI 7*, $P = 0.012$), and Hct (42.9 ± 1% at baseline vs. 46.4 ± 0.9% on *day DI 7*, $P = 0.012$). In the recovery period (*days R+4* and *R+7*), all these parameters were restored. We did not observe any significant differences in the numbers of white blood cells and platelets before (*day B-7*) and after (*day R+1*) DI ($6.8 \pm 0.6 \times 10^9$ vs. $6.3 \pm 0.5 \times 10^9$ white blood cells/l and $252 \pm 19 \times 10^9$ vs. $232 \pm 21 \times 10^9$ platelets/l, respectively).

Blood Glucose and Lipids

Blood glucose, total cholesterol, and the LDL fraction were not changed significantly during the 7-day DI. Triglycerides were slightly increased on *day DI 7*, although within normal limits (Table 3).

Testing of Endothelial Function

Basal calf skin blood flow. Basal calf skin blood flow (Fig. 3*A*) was significantly decreased on the third and seventh days of DI [11 ± 1 arbitrary units (AU), $P = 0.018$, and 15 ± 2 AU, $P = 0.025$, respectively] compared with baseline (22 ± 4 AU). In the recovery period (*day R+2*), basal flow tended to be restored (19 ± 5 AU).

Maximal skin vasodilation in response to heating. Maximal skin vasodilation in response to heating, expressed in nonnormalized values (Fig. 3*B*), was similar before, during, and after DI.

Table 1. *Body temperature, body weight evolution, and water balance before, during, and after DI*

	Day B-1	*Day DI 1*	*Day DI 3*	*Day DI 7*	*Day R+2*
Morning temperature, °C	36.2 ± 0.1	36.2 ± 0.1	36.1 ± 0.1	36.1 ± 0.1	36.2 ± 0.2
Evening temperature, °C	36.3 ± 0.1	36.3 ± 0.1	36.5 ± 0.2	36.4 ± 0.1	36.4 ± 0.2
Weight evolution vs. *day B-1*, kg		−0.9 ± 0.2*	−2.1 ± 0.5*	−2.3 ± 0.4*	−1.3 ± 0.2*
Water balance, ml	1,870 ± 210	−120 ± 220*	920 ± 150*	770 ± 150*	2,150 ± 200
Evening weight, kg	76.1 ± 4.1	75.2 ± 4.2*	74.0 ± 4.0*	73.8 ± 4.0*	74.9 ± 4.2*

Values are means ± SE. Days are indicated as follows: baseline *day −1* (*day B-1*), dry immersion (DI) *days 1*, *3*, and *7* (*days DI 1*, *3*, and *7*), and recovery *day +2* (*day R+2*). *$P < 0.05$ vs. baseline.

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Table 2. *Systolic and diastolic pressure, heart rate, skin temperature, and ambient temperature at the beginning of iontophoretic tests before, during, and after DI*

	Day B-1	*Day DI 1*	*Day DI 3*	*Day DI 7*	*Day R+2*
Systolic blood pressure, mmHg	125 ± 2	121 ± 2	123 ± 4	124 ± 3	121 ± 3
Diastolic blood pressure, mmHg	66 ± 3	59 ± 2*	65 ± 3	66 ± 2	66 ± 1
Heart rate, beats/min	59 ± 3	60 ± 3	58 ± 3	60 ± 2	63 ± 4
Skin temperature, °C	33.8 ± 0.2	33.7 ± 0.1	33.5 ± 0.1	33.4 ± 0.1	34.0 ± 0.2
Air temperature, °C	25.4 ± 0.5				24.5 ± 0.4
Water temperature, °C		32.4 ± 0.2	32.2 ± 0.1	32.3 ± 0.1	

Values are means ± SE. $^*P < 0.05$ vs. baseline.

Endothelium-dependent vasodilation. Endothelium-dependent vasodilation (Fig. 3, *C* and *D*), estimated at plateau, was significantly decreased on the seventh day of DI (12 ± 6% of maximum vasodilation) versus baseline (29 ± 6% of maximum vasodilation, $P = 0.046$). Peak vasodilation tended to decrease on the third and seventh day of DI.

Endothelium-independent vasodilation. No significant changes were observed in endothelium-independent vasodilation (Fig. 3*E*), although there was a tendency for it to decrease on the seventh day of DI.

Assessment of MPs

Enumeration of circulating MPs from different cell origins indicated that EMPs, identified as MPs expressing CD31 but negative for CD41, were significantly increased on *day DI 3* (64.9 ± 9.9 vs. 41.9 ± 8.1 events/μl at baseline, $P = 0.04$; Fig. 4*B*), whereas erythrocyte, platelet, and leukocyte MPs were not significantly modified under DI (Fig. 4, *C–E*). The total number of MPs expressing phosphatidylserine, assumed to be MPs binding annexin V (Fig. 4*A*), tended to decrease during DI, but the changes did not reach statistical significance.

Assessment of Soluble Endothelial Factors

In an attempt to document EMP variations, we investigated the level of E-selectin (soluble CD62E), attesting to endothelial activation, and soluble VEGF and soluble VEGFR1, reflecting the alterations of signals controlling survival/apoptosis equilibrium in endothelial cells. A significant decrease in the plasma levels of soluble VEGF was observed on *days DI 3* and *DI 7* compared with baseline values (51 ± 14% of baseline on *day DI 3* and 73 ± 9% on *day DI 7*, $P < 0.05$). No significant changes were observed in the plasma levels of soluble CD62E and soluble VEGFR1 under DI (Fig. 5).

DISCUSSION

This present study shows the microvascular disturbance with biological and functional impairment of endothelial functions after acute and enhanced physical inactivity in humans without other cardiovascular risks. One week of DI appeared sufficient to impair endothelium-dependent vasodilation at the skin level and to increase, specifically, endothelium-derived MPs.

Physical Inactivity and Endothelial Properties

Bedrest and DI protocols offer the chance to study, specifically, the effect of physical inactivity on vascular functions. Since the Framingham Heart Study several years ago, several studies have shown a correlation between physical inactivity and cardiovascular disease (for a review, see Ref. 32). However, physical inactivity is often associated with other strong cardiovascular risk factors such as metabolic syndrome (2). In our work, we were able to specifically study the role of physical inactivity in endothelial damage. We observed both a decrease in ACh-induced vasodilation at the skin level and an increase in EMPs (considered as a biological marker of endothelial dysfunction).

On *day DI 3*, we found a significant increase in EMPs. The peak response to ACh tended to decrease on this day. These results suggest an endothelial dysfunction involving the nitric oxide pathway. The vasodilatory capacity of smooth muscle cells was preserved on *day DI 3*, as shown by the unchanged iontophoretic response to SNP.

On *day DI 7*, the significant decrease of the plateau response to ACh may indicate an endothelial dysfunction predominant for the prostaglandin pathway. The tendency to EMPs to increase on *day DI 7* is also indicative of endothelial changes. However, on *day DI 7*, the LD flow response to SNP tended to decrease, suggesting an impairment also at the smooth muscle cell level.

In a previous study (18) involving a 2-mo bedrest in women, we showed a decrease in ACh-induced vasodilation at the skin level, with a significant increase in CECs. Increased numbers of CECs indicate disruption of endothelial integrity and might indicate desquamated, damaged, or dysfunctional endothelial cells (37). However, it seems that a shorter period of enhanced physical inactivity, with 7 days of DI, is also sufficient to impair the endothelial vasodilatory capacity, as had already

Table 3. *Dynamics of blood glucose and blood lipids in the experiment with 7-day DI*

Parameter	Reference	*Day B-7*	*Day DI 3*	*Day DI 7*	*Day R+1*
Plasma glucose, mmol/l	4.2–6.4	4.83 ± 0.19	4.94 ± 0.12	4.87 ± 0.18	4.67 ± 0.15
Total plasma cholesterol, mmol/l	2.8–5.2	4.7 ± 0.42	5.32 ± 0.38	5.11 ± 0.39	5.19 ± 0.46
HDL-cholesterol, mmol/l	>0.91	1.42 ± 0.11	1.38 ± 0.09	1.35 ± 0.07	1.3 ± 0.09
LDL-cholesterol, mmol/l	<4.0	2.63 ± 0.25	3.08 ± 0.35	2.85 ± 0.35	3.05 ± 0.4
Triglycerides, mmol/l	0.55–2.30	1.12 ± 0.16	1.61 ± 0.17	1.78 ± 0.25*	1.83 ± 0.32

Values are means ± SE. Data are from Markin et al. (38). *Day B-7*, baseline *day −7*; *day R+1*, recovery *day +1*. $^*P < 0.05$ vs. baseline.

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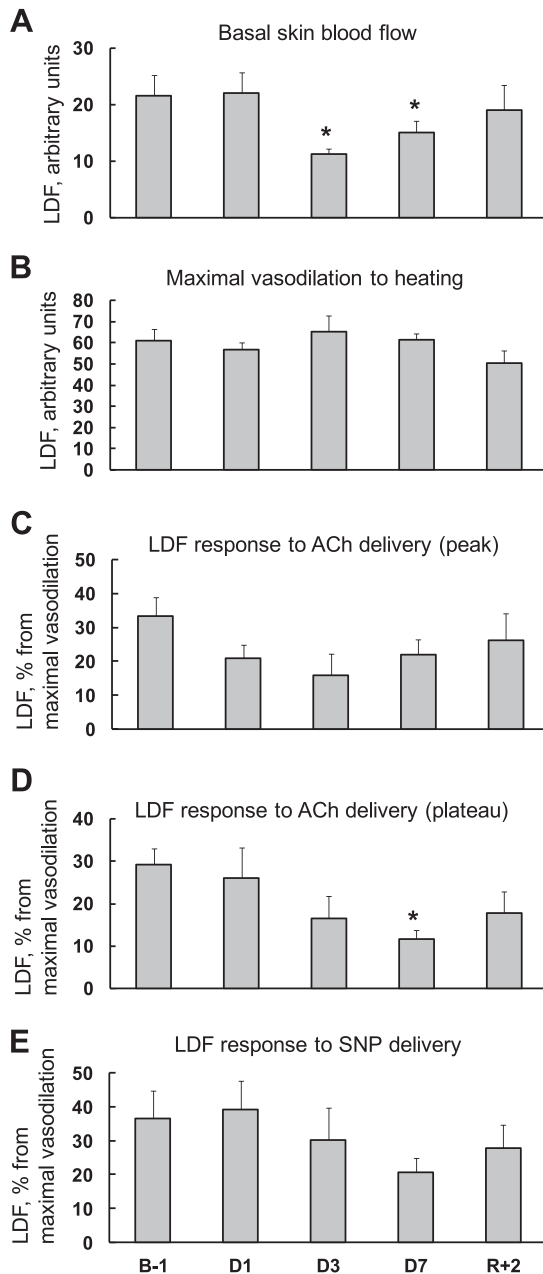

Fig. 3. Effect of physical inactivity induced by 7 days of DI on calf skin blood flow defined by the laser-Doppler technique. *A*: mean basal flow. *B*: flow stimulated by local heating up to 44°C. *C* and *D*: flow stimulated by iontophoresis of ACh on peak (*C*) and plateau (*D*) phases. *E*: flow stimulated by iontophoresis of SNP. Basal blood flow and maximal blood flow responses to heating are expressed as non-normalized values; blood flow responses to ACh and SNP delivery are expressed as percentages of skin maximal vasodilation to heating. Values are means ± SE. Protocol days are indicated as follows: baseline (B), DI (D), and recovery (R). *$P < 0.05$ vs. *day B-1*.

been observed after 5 days of bedrest (26) and after 21 days of bedrest (27). The increase in EMPs is in accordance with these previous results and provides a new mechanistic hypothesis.

Role of Decreased Shear Stress at the Microcirculatory Level

The essential condition of endothelial well-being is shear stress. It can be calculated as follows: shear stress = $4\ \mu Q/\pi r^3$, where r is the radius of the vessel, μ is the blood viscosity, and Q is the blood flow. Our DI model potentially acts on blood flow and viscosity.

Cardiac output/blood flow. DI provokes a diminution in stroke volume (29, 44, 48) and cardiac output (23, 44). Advanced physical inactivity in a water environment decreases metabolic tissue demands and the blood supply with a decrease in hemodynamics at the skin level and also at the whole body level. This is particularly true for muscle vessels, because DI induces a dramatic decrease in muscle tone and motor activity.

Whole blood viscosity. Blood viscosity depends mainly on Hct. Even modest decreases in Hct have been shown to impair flow-mediated dilation in large arteries (24). In a recent study (9) on the plasma level of EMPs and shear rate measured in large arteries in end-stage renal disease, Boulanger et al. showed that an increase in Hct increases shear stress and

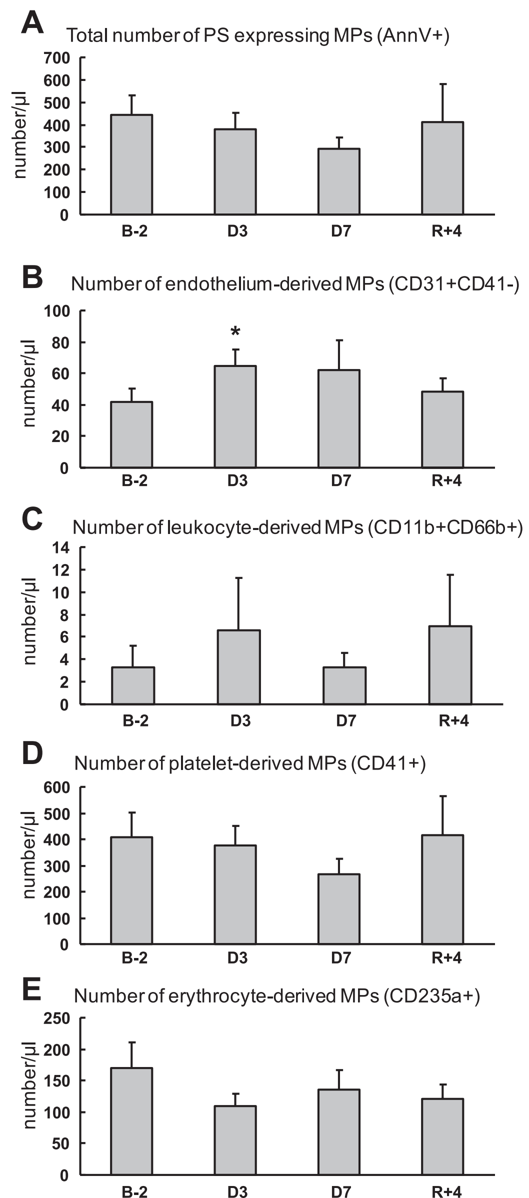

Fig. 4. Numbers of microparticles (MPs) as assessed by flow cytometry before (*day B-2*), during (*days D3* and *D7*), and after (*day R+4*) DI. Values are means ± SE. *$P < 0.05$ vs. *day B-2*.

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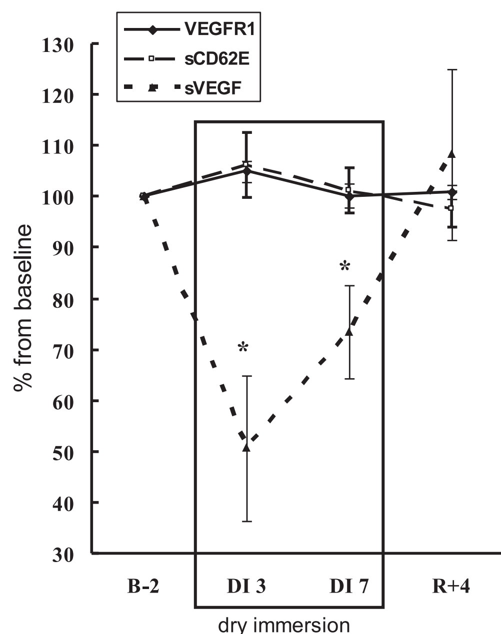

Fig. 5. Variations in soluble endothelial factors in plasma. VEGFR1, VEGF receptor 1; sCD62E, soluble CD62E; sVEGF, soluble VEGF. Values are means ± SE. *Significant difference vs. baseline ($P < 0.05$).

improves endothelial function. In our study, despite the moderate increase in Hct in DI, we observed an impairment of endothelial function. In this model, we have to take into account the deterioration in hemodynamic conditions as a major factor of endothelial dysfunction.

Potential role of the lipid profile and blood glucose. Nevertheless, other inactivity-associated factors might also participate in the observed microvascular dysfunction, for example, dyslipidemia or glucose intolerance. Physical inactivity associated with bedrest is known to increase rapidly insulin resistance, total cholesterol, and triglycerides (26). In our study, the lipid profile remained within normal values. Thus, dyslipidemia seems not to be involved in the endothelial impairment. We did not directly estimate glucose tolerance; however, in another 7-day DI protocol, a significant increase of fasting insulin was found (1), which favors the hypothesis of insulin resistance.

MPs, Mechanisms of Appearance, and Potential Role

We investigated the influence of DI-induced hypokinesia on the plasma levels of different MPs.

Mechanisms of appearance. MPs produced in vitro are generated by activating agents, such as TNF-α, or apoptotic stimuli, as the deprivation of growth factors. Several recent studies (10, 28) have supported the concept that plasma levels of EMPs represent a surrogate marker of endothelial cell damage. Our study is the first to show that enhanced physical inactivity specifically increases EMPs without effects on platelet- and leukocyte-derived MPs. Together with the alteration of endothelial vasodilatory capacity, these observations are consistent with a pathological process specifically targeting endothelial integrity. In various clinical situations, increased EMP levels have been shown to correlate with endothelial dysfunction assessed by flow-mediated vasodilation (11). Elevated EMP levels are also associated with most of the cardiovascular risk factors, such as obesity, hypertension, and diabetes, and appear indicative of a poor clinical outcome (42). Thus, EMPs appear as a potential new marker of the deleterious impact of physical inactivity on the endothelium and the related cardiovascular risk. The mechanisms underlying excessive endothelial vesiculation associated with DI remain to be investigated. However, the absence of a significant alteration in the soluble CD62E plasma levels suggests that the increase in EMPs occurred in the absence of marked endothelial inflammatory stimulation. Rather, endothelial vesiculation resulting from enhanced apoptosis can be postulated. Consistent with this hypothesis, we evidenced that DI induced a significant decrease in VEGF plasma levels. Such a decrease may contribute to the reduction of antiapoptotic tone for endothelial cells because of the acknowledged role of VEGF as a powerful survival signal (21, 45). VEGF transfers the signals of survival and proliferation toward endothelial cells, and the absence of this factor leads to apoptosis in vitro; thus, it characterizes the antiapoptotic tone of endothelial cells (22).

Potential role. MPs are not just "cell dust" waiting for phagocytosis; they are biologically functional and actively involved in cellular control mechanisms. Recent data have provided evidence that MPs, independently of their origin, can transfer biological information between cells, acting as veritable vectors of signal molecules (39). EMPs are not only a reflection of endothelial dysfunction but may also induce vascular dysfunction (10). It remains to be seen whether the release of EMPs is a cause or consequence of endothelial dysfunction or both. In the endothelial dysfunction induced by DI and bedrest, or in a more general way by enhanced physical inactivity, EMPs might quantify this endothelial dysfunction and spread this endothelial impairment within the vascular tree.

Conclusions

Our study showed that enhanced physical inactivity, induced by DI, brings about microcirculatory damage, predominantly at the endothelial level. The lower hemodynamics decrease shear stress at the microcirculatory level and are highly likely to contribute to this endothelial damage. The increase in endothelial MPs also indicates endothelial dysfunction, and the MPs might participate in a generalized endothelial dysfunction. Endothelial dysfunction at the microcirculatory level might contribute to several hypokinesia-induced pathologies, such as a decrease in exercise capacity, muscle atrophy, and cardiovascular deconditioning. The endothelial dysfunction at the microcirculatory level seems to be an early consequence of hypokinesia. Therefore, we believe that the endothelium could be a specific target for countermeasures designed to minimize or reverse the deleterious effects of physical inactivity on microvascular function in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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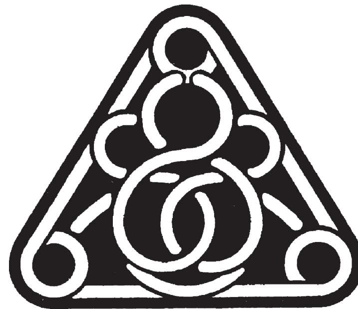

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