MECANISMES DE LA DYSFONCTION VASCULAIRE INDUITE PAR DES MODELES DE MICROGRAVITE

Nastassia Navasiolava

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A mes chers parents

Моим дорогим родителям

« Главная цель всякой науки, всякого знания - дать ответ на вопрос,

как сделать людей счастливыми. »

« L'objectif principal de toute science, de toute connaissance

c'est de répondre à la question, comment rendre les gens heureux. »

Constantin Tsiolkovski

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AVP…….Arginine-vasopressine

BNP…….Peptide natriurétique de type B

B-2 et B-1……...Jours de contrôle avant l'immersion sèche

CV……Cardio-vasculaire

DI…….Immersion sèche

EDHP……. Facteur hyperpolarisant dérivé de l'endothélium

EMPs……..Microparticules endothéliales circulantes

ET-1……Endothéline 1

HDBR……. Alitement antiorthostatique

HDT……. Inclinaison tête en bas

FC……Fréquence cardiaque

FMD ……...Vasodilatation induite par le flux

IL-6……..Interleukine 6

IBMP……Institut de Problèmes Biomédicaux

LBNP……...Mise en dépression de la partie inférieure du corps

LPS……..Lipopolysaccharide

NA ……..…. Noradrénaline

NASA..……..... Administration Nationale de l'Aéronautique et de l'Espace

NO…….....Monoxyde d'azote

NT-proBNP……. Prohormone N-terminal du peptide natriurétique de type B

PA……Pression artérielle

PAD…….Pression artérielle diastolique

PAS…….Pression artérielle systolique

PG……...Prostaglandines

PCO_2……...Pression partielle en dioxyde de carbone

PO_2……..Pression partielle en oxygène

RPT…….Résistances périphériques totales

R+1 et R+2…….Jours de récupération

SRA…….Système rénine-angiotensine

sVEGF…….Facteur soluble de croissance de l'endothélium vasculaire

ULLS……Suspension unilatérale des membres inférieurs

VC……Vasoconstriction

VD……Vasodilatation

$\dot{V}O_2$max……..Consommation maximale d'oxygène

TABLE DES MATIERES

Résumé……..…….**21**

Резюме……..……..**23**

Abstract……..**25**

Introduction……..….**27**

Objectifs de ce travail…….….**31**

Le contexte général…….….**33**

A. La microgravité**……...35**

1. Les paramètres environnementaux à prendre en compte dans l'espace**…….35**
2. Les modèles**…….…..….37**

B. Régulation de la pression artérielle et de la perfusion régionale**……...43**

C. Comment les paramètres environnementaux liés à l'espace peuvent modifier le fonctionnement vasculaire**……..……....45**

Travail expérimental chez l'homme : L'immersion sèche**……49**

A. L'immersion sèche: revue et perspectives**…….....51**

B. Etude des modifications cardio-vasculaires induites par l'immersion sèche**……..53**

1. Présentation de notre protocole**…….....53**
2. État de l'endothélium et des fonctions microcirculatoires**….....55**
3. Réponse cardio-vasculaire à l'orthostatisme**……57**
4. Métabolisme hydro-sodé et sa régulation hormonale**…….59**

Travail expérimental chez l'animal : Mécanismes de la dysfonction vasculaire, nouvelles hypothèses**…….61**

A. Nécessité des modèles animaux de microgravité**…….....63**
B. Liens avec la dépense énergétique**……..65**
C. Hypothèse des endotoxines**……...67**

D. Modifications vasculaires ultrastructurelles**……69**

Discussion et conclusion……..71

A. Discussion générale**……...73**

1. Est-ce que l'immersion sèche est un bon modèle de microgravité ?**.....73**
2. Déconditionnement cardio-vasculaire en immersion sèche, considérations méthodologiques**……..…75**
3. Quels seraient les mécanismes de l'atteinte vasculaire induite par la microgravité?.....**77**
4. Les atteintes vasculaires et le déconditionnement cardio-vasculaire général**…...80**

B. Conclusions et perspectives**……....83**

Bibliographie**…….85**

RESUME

La gravité est une stimulation permanente pour l'organisme sur terre. La microgravité induit des modifications de nombreuses fonctionnalités, y compris celles du système cardio-vasculaire. Le but de ce travail était d'étudier les mécanismes des modifications vasculaires induites par les modèles de microgravité.

Nous avons conduit nos études chez l'homme à l'aide de l'immersion sèche qui est considérée comme un des modèles reproduisant au mieux la microgravité. L'immersion sèche est le seul modèle de microgravité prolongée à imiter l'absence d'appui. Notre travail montre que l'immersion sèche induit une atteinte au niveau microcirculatoire avec une dysfonction endothéliale (étude des microparticules circulantes) et une atteinte de la fonction vasodilatatrice cutanée (étude par iontophorèse couplée au laser Doppler). Cette atteinte vasculaire s'intègre dans un syndrome de déconditionnement cardio-vasculaire plus global que nous avons également étudié. Il comprend un nouvel équilibre hydro-sodé qui survient très rapidement, dans les 24 premières heures. Pendant la période de récupération, nous avons observé une augmentation significative du NT-proBNP qui pourrait être un marqueur biologique du déconditionnement cardio-vasculaire.

Nos études sur l'animal avec les modèles d'hypokinésie et d'inclinaison tête en bas discontinues chroniques suggèrent qu'une translocation augmentée d'endotoxine et une réduction du métabolisme énergétique en microgravité pourraient participer aux atteintes endothéliales.

La dysfonction endothéliale deviendrait ainsi une cible thérapeutique pour des atteintes liées à l'inactivité physique et à la microgravité.

РЕЗЮМЕ

Гравитационный стимул является неотъемлемым атрибутом земных условий существования всех живых организмов. Модификация направленности действия гравитационного фактора и его интенсивности сопровождается изменением функционирования многих систем организма, в том числе и сердечно-сосудистой системы. Целью настоящей работы явилось изучение механизмов сосудистых изменений при моделировании микрогравитации.

Исследования на человеке проводили с использованием «сухой» иммерсии, предоставляющей уникальную возможность изучения физиологических эффектов безопорности, и являющейся одной из наиболее адекватных моделей, воспроизводящих эффекты микрогравитации. В работе установлено, что в условиях «сухой» иммерсии происходят изменения на уровне микроциркуляторного русла с развитием эндотелиальной дисфункции (исследования с количественным определением циркулирующих микрочастиц) и снижением вазодилатации сосудов кожи (исследования методом лазер-допплер флоуметрии в сочетании с ионофорезом вазоактивных субстанций). Выявленные сосудистые нарушения включаются в более общий синдром детренированности, анализ механизмов развития которого также являлся целью работы, в частности, в аспекте сдвигов водно-солевого баланса. Показано, что в течение первых суток иммерсии происходит стабилизация водно-солевого баланса на новом уровне. В период восстановления наблюдается достоверный рост плазматического NT-proBNP, что позволяет заключить о перспективности применения этого показателя в качестве маркера детренированности сердечно-сосудистой системы.

Проведенная работа на экспериментальных животных с использованием моделей горизонтальной и антиортостатической гипокинезии позволила сформировать гипотезу, что в условиях микрогравитации происходит увеличение транслокации эндотоксина из кишечника во внутреннюю среду организма и снижение интенсивности энергетического метаболизма. Эти факторы являются патогенетическими в развитии поражений эндотелия.

Таким образом, дисфункция эндотелия может рассматриваться как одна из мишеней в терапии патологий ассоциированных с физической инактивацией и микрогравитацией.

ABSTRACT

Gravitational stimulation is an inherent part of terrestrial environment. Modified gravity changes functioning of numerous body systems, including cardiovascular system. The aim of this work was to study the mechanisms of vascular modifications induced by modeled microgravity.

The human studies were performed using dry immersion. This model is considered as one of the most appropriate to reproduce the effects of prolonged microgravity and especially to mimic the lack of support. We found that dry immersion induces microcirculatory impairment with endothelial dysfunction (study of circulating microparticles) and impaired skin vasodilation (study by iontophoresis of vasoactive substances coupled with laser Doppler flowmetry). This vascular impairment is integrated to more general cardiovascular deconditioning syndrome. The global deconditioning was also considered in this work, including the rapid reset of water and sodium balance to a new steady state. During the recovery period, we observed a significant increase of plasmatic NT-proBNP which might reflect the degree of cardiovascular deconditioning.

Our studies with animal models of horizontal and antiorthostatic hypokinesia suggest that increased endotoxin translocation and reduced energy metabolism in microgravity could contribute to endothelial impairment.

Endothelial dysfunction may be regarded as one of the therapeutic targets for pathologies associated with physical inactivity and microgravity.

INTRODUCTION

La force de gravité, du fait de son caractère universel, a conditionné le vivant. Les travaux sur la gravité comme force physique sont très anciens et ont accompagné la naissance de la physique au temps de l'Antiquité. Plus tard, les découvertes de Galilée et d'Isaac Newton au XVII^{e} siècle ont constitué la base des sciences physiques contemporaines liées à la gravité.

Ce n'est que beaucoup plus tard que l'on s'est intéressé aux effets biologiques de la gravité, ce domaine prenant un essor particulier maintenant que l'homme cherche à se soustraire de la gravité. La première découverte, faite par Thomas Knight (1806), était le géotropisme des plantes. Les études des effets de la gravité sur les animaux ont débuté, indépendamment, en France et en Russie - par Salathé (1877) et Tsiolkovski (1879). A. Salathé a étudié l'anémie et la « congestion cérébrale » provoquée par la verticalisation et par un mouvement giratoire chez le chien et le lapin (Salathé 1877). C. Tsiolkovski étudiait la tolérance à l'accélération chez les insectes (pour les cafards il a trouvé une limite de tolérance de 300 G) et chez les poussins (une limite de tolérance de 10 G) (Kotovskaia & Will-Williams 2004). Dans les années 1920-1940, le développement de l'aviation a intensifié les études de physiologie et de pathophysiologie de l'hypergravité.

Mais jusque dans les années 1950, l'incapacité de supprimer le facteur gravité pour une période plus ou mois longue freinait le développement de la biologie gravitationnelle. Ce n'est qu'avec la conquête spatiale que l'étude des effets de l'hypogravité chez le vivant a commencée. La première expérience animale dans l'espace était faite en 1957, avec la chienne Laïka dans Spoutnik 2. En 1961, elle est suivie par le premier vol spatial humain. Au-delà de l'approche appliquée à la conquête spatiale, l'étude de la physiologie gravitationnelle répond à la démarche scientifique, selon le grand principe de Claude Bernard, qui consiste, pour mieux connaître un système, à étudier les conséquences de son exclusion. Pour les études en sciences physiques, les périodes courtes d'impesanteur pendant la chute libre permettaient déjà d'apporter des résultats. En revanche, pour le vivant,

les durées d'impesanteur nécessaires pour objectiver des modifications significatives sont beaucoup plus longues.

Les difficultés pour organiser des expériences en vol et le besoin de résoudre plusieurs questions fondamentales et appliquées rendent la simulation expérimentale de la microgravité sur Terre indispensable. Les moyens de simulation permettent de reproduire des effets de la microgravité au sol et de soustraire partiellement l'organisme de l'influence de la gravité. La méthode la plus répandue est l'alitement antiorthostatique (Pavy-Le Traon et al. 2007). Une autre alternative est l'immersion dans l'eau. Dans les œuvres brillantes de C. E. Tsiolkovski, le père de l'astronautique, on trouve déjà les allusions à la possibilité d'imiter les conditions de l'impesanteur sur Terre par l'immersion du corps dans un milieu liquide. Dans son livre « Au-delà de la Terre » (1920), Tsiolkovski décrit le passage à l'impesanteur après le décollage de la navette spatiale : « ... Quand l'explosion s'arrêtera et que la fusée cessera d'accélérer… la pesanteur relative devra disparaître… Les voyageurs… seront semblables aux poissons dans l'eau, ne ressentant seulement à chacun de leur mouvement… la résistance de l'eau ». Dans l'autre ouvrage remarquable « L'exploration de l'espace cosmique par des engins à réaction » (1903), en discutant la question d'action de la gravité sur l'organisme humain, Tsiolkovski a écrit : « Pendant une baignade sur Terre, le poids de notre corps est presque tout autant neutralisé par l'effet contraire de l'eau. Cette absence de poids peut être illimité, pourvu que l'eau soit suffisamment chaude ». Tsiolkovski a estimé que dans les conditions terrestres « …les expériences spécifiques pour prouver que le milieu privé de la pesanteur est inoffensif étaient à peine nécessaires ». Cependant, le développement des études cosmiques et le passage aux missions spatiales ont montré qu'en l'absence de gravité, un « déconditionnement » se développe, se manifestant par la perte de capacité à réagir de manière adéquate à la charge de gravité.

Pour système cardio-vasculaire, le syndrome de déconditionnement comporte une intolérance à l'orthostatisme, une tachycardie et une baisse de la capacité à l'exercice. Plusieurs hypothèses ont été étudiées jusqu'à présent pour expliquer ce déconditionnement cardio-vasculaire : diminution du volume plasmatique (Buckey et al. 1996, Convertino 1995) ;

altération de la sensibilité du baroréflexe (Convertino 2002, Engelke et al. 1996) ; atteinte des fonctions végétatives (Convertino 2002) ; majoration de la compliance veineuse (Convertino 1995), cette dernière pouvant être renforcée encore par la diminution du tonus musculaire (Grigor'ev et al. 2004) ; atteintes vasculaires (Zhang 2001), y compris celle des fonctions microcirculatoires (Coupé et al. 2009), favorisée par l'inactivité physique et la diminution prolongée de forces de cisaillement au niveau vasculaire (De Groot et al. 2006).

L'atteinte vasculaire apparaît actuellement comme un facteur primordial du déconditionnement cardio-vasculaire. Mais les mécanismes de cette atteinte et ses liens avec les symptômes du déconditionnement cardio-vasculaire restent à étudier.

OBJECTIFS DE CE TRAVAIL

Les changements les plus importants en microgravité sont observés dans les systèmes gravité-dépendants, y compris dans le système cardio-vasculaire. Une des raisons de ce déconditionnement cardio-vasculaire est l'atteinte vasculaire. Le cadre général de ce travail concerne l'étude des modifications vasculaires induites par les modèles de microgravité. Quels sont les mécanismes de la dysfonction vasculaire dans les conditions de changement d'action du vecteur gravité ? Pour y répondre, nous avons mené des études sur les modifications cardio-vasculaires chez l'homme induites par le modèle d'immersion sèche. Nous avons aussi proposé de nouvelles hypothèses de la dysfonction vasculaire en utilisant les modèles animaux.

Il semble que ce soient les atteintes endothéliales, surtout microcirculatoires, qui apparaissent les premières avec le changement en microgravité des forces physiques qui s'exercent sur l'organisme. De nombreuses questions demeurent autour de ces fonctions microcirculatoires : par quels mécanismes sont-elles affectées par la microgravité et comment peuvent-elles s'intégrer dans le syndrome de déconditionnement cardio-vasculaire ? Dans ce travail nous voulons étudier spécifiquement l'état de l'endothélium et l'état fonctionnel de la microcirculation en microgravité simulée et leurs liens avec les changements globaux cardio-vasculaires.

Parmi les mécanismes évoqués, nous nous sommes intéressés principalement aux effets spécifiques de l'inactivité physique. Nous avons aussi voulu explorer de nouvelles hypothèses. Chez l'animal la microgravité influe-t-elle la translocation des endotoxines ? L'augmentation des taux plasmatiques d'endotoxines pourrait endommager l'endothélium directement et indirectement. Nous avons aussi étudié les effets de microgravité simulée sur l'intensité métabolique.

LE CONTEXTE GENERAL

A. La microgravité

1. Les paramètres environnementaux à prendre en compte dans l'espace

2. Les modèles

B. Régulation de la pression artérielle et de la perfusion régionale

C. Comment les paramètres environnementaux liés à l'espace peuvent modifier le fonctionnement vasculaire

A1. LA MICROGRAVITE :

LES PARAMETRES ENVIRONNEMENTAUX A PRENDRE EN COMPTE DANS L'ESPACE

La compréhension des paramètres environnementaux spécifiques à l'espace est indispensable pour créer l'habitat, envisager les systèmes de protection et réaliser toutes les conditions de vie nécessaires pour permettre les performances physique et mentale des spationautes. Prenant en compte les projets de conquête de Mars au XXI^{e} siècle, la médecine spatiale doit se préparer pour des missions plus prolongées, plus compliquées et plus ambitieuses.

Les facteurs agissants dans le véhicule spatial

Pour l'instant et dans un proche avenir, l'environnement pendant le vol peut être qualifié de restrictif (Nicogossian et al. 1993). Confinement, atmosphère et rythme nycthéméral artificiels, isolements physique et social, monotonie des stimuli visuels, auditifs, olfactifs, tactiles, gustatifs et thermiques sont inhérents à la vie dans l'espace, ainsi que l'hypodynamie (diminution de la charge s'exerçant sur les muscles posturaux) et l'hypokinésie (réduction de l'activité motrice) (Kovalenko & Gurovsky 1980). Tous ces facteurs peuvent provoquer un stress, d'autant plus que le vol spatial entraîne une charge émotionnelle non négligeable pour le spationaute après sa longue période d'entraînement et une sélection très compétitive (Karemaker & Berecki-Gisolf 2009). Au-delà de la protection par la magnétosphère terrestre, le facteur radiation représente également un danger non négligeable (Nicogossian et al. 1993) et requiert des systèmes de protection (Petrov et al. 2001).

Mais de tous les facteurs agissants dans l'espace, la microgravité est le seul spécifique du vol spatial et inimitable dans les conditions terrestres, hormis sur une courte période. Pour étudier la microgravité au sol nous avons donc recours à des modèles qui reproduisent non

pas la microgravité elle-même, mais ses effets. Les effets primaires de la microgravité (Grigor'ev & Egorov 1988) sont :

- L'absence de poids supprimant les tensions s'exerçant sur les différentes structures du corps et les déformations qui en résultent habituellement,
- La diminution significative des efforts physiques, grâce à la mise en décharge des structures d'appui et des muscles posturaux, ainsi que l'absence quasi complète d'efforts à fournir pour se mouvoir,
- La disparition des gradients hydrostatiques, induisant la redistribution liquidienne vers le pôle thoraco-céphalique,
- La modification des influx sensoriels, diminués pour la proprioception, altérés pour le système vestibulaire et supprimés pour la perception d'appui.

La possibilité de reproduire l'absence d'appui fait toute l'originalité et tout l'intérêt du modèle d'immersion sèche en comparaison aux autres modèles de simulation de microgravité. La suppression du gradient de pression mécanique en immersion crée des conditions similaires à l'absence totale de soutien structurel, contrairement à la position debout ou couchée habituelle avec appui constant sur des zones limitées des pieds ou du dos (Kovalenko & Gurovsky 1980).

A2. LA MICROGRAVITE :

LES MODELES

Sur Terre, nous pouvons expérimenter la microgravité pendant une fraction de seconde lors d'un saut par exemple. Les vols paraboliques permettent une durée de microgravité réelle mais en général trop courte pour l'étude du vivant (25 s maximum). Pour simuler les effets physiologiques de la microgravité prolongée, plusieurs modèles humains et animaux sont utilisés. Nous proposons de classer ces différentes méthodes de simulation en fonction du degré d'inactivité physique induite.

Chez l'homme

• L'immobilisation partielle par décharge d'un membre (Kovalenko 1977, Bleeker et al. 2005a), induit une inactivité physique segmentaire uniquement (Fig. 1).

Figure 1. Modèle de décharge unilatérale (ULLS)

(d'après Berg et al. 1991)

• L'infrastructure de suspension de corps avec harnais spécial pour diminution d'appui chez l'homme (Spady 1969) est utilisée pour imiter la gravité diminuée, par exemple lunaire (0.17 G) (Fig. 2).

Figure 2. Simulateur de gravité lunaire, Centre de Recherche Langley de NASA, Virginia, Etats-Unis. Le sujet habillé en costume spécial est supporté par des fils de façon qu'un-sixième de son poids soit appliqué sur un mur incliné dans le but de simuler la marche sur la surface lunaire

http://www.nasa.gov/centers/langley/news/factsheets/Landmarks.html

• Le maintien prolongé de la position assise (Lamb et al. 1964, 1965, Panferova 1976) a été proposé dans les années 1960 - 1970. Il induit une inactivité plus importante mais sa mise en œuvre est difficile. Le modèle le plus couramment utilisé reste l'alitement prolongé, horizontal ou antiorthostatique avec différents angles d'inclinaison (Fortney et al. 1996, Pavy-Le Traon et al. 2007) (Fig. 3).

Figure 3. Alitement antiorthostatique

• En immersion sèche (Shulzhenko & Vil-Vilyams 1975) l'inactivité physique est encore plus importante que lors de l'alitement prolongé, car la force de flottabilité compense les efforts induits par les mouvements et l'absence d'influx des zones d'appui annule le tonus des muscles posturaux (Fig. 4).

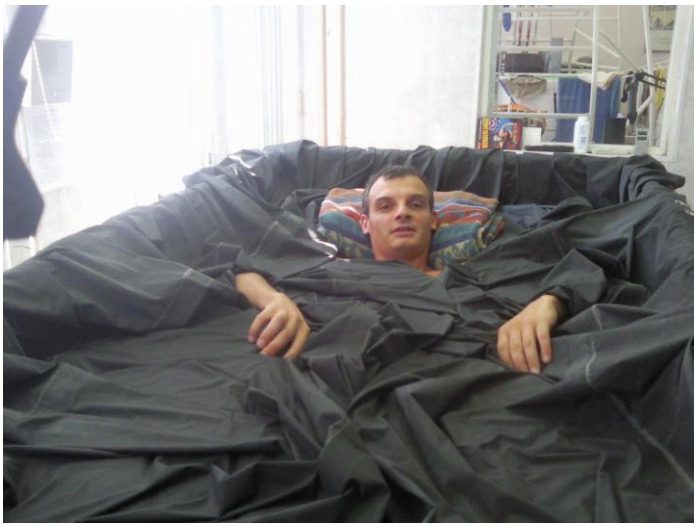


Figure 4. Immersion sèche

• La microgravité réelle (Fig. 5), avec sa baisse significative d'efforts physiques, induit l'inactivité physique la plus poussée. Cependant, l'interprétation des données des missions spatiales se complique par le manque de contrôle, vu que tous les membres d'équipage sont obligés de s'entraîner si la mission excède 10 jours (Adams et al. 2003).

Figure 5. Microgravité réelle

Chez l'animal (rongeur)

• Chez les rongeurs, les modèles d'immobilisation partielle sont la décharge du train postérieur par la queue proposé par Morey-Holton (Morey-Holton & Globus 2002) (Fig. 6) et en modification « anneau-queue » (deux boucles passés via les espaces intervertébraux de la queue pour fixer le câble de suspension) (Brown et al. 2005) (Fig. 7). Il y a également la suspension du rat avec un harnais : modèle de Mussachia et coll. (Mussachia et al. 1980), et sa modification par Overton-Tipton (Stump et al. 1990) (Fig. 8).

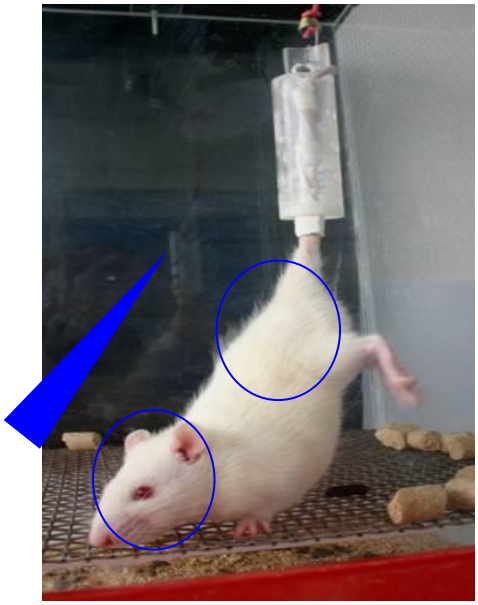


Figure 6. Décharge du train postérieur par la queue

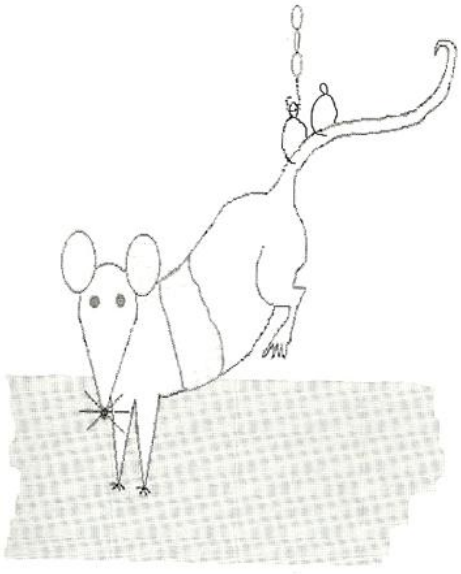


Figure 7. Modification de décharge du train postérieur « anneau-queue » (d'après Brown et al. 2005)

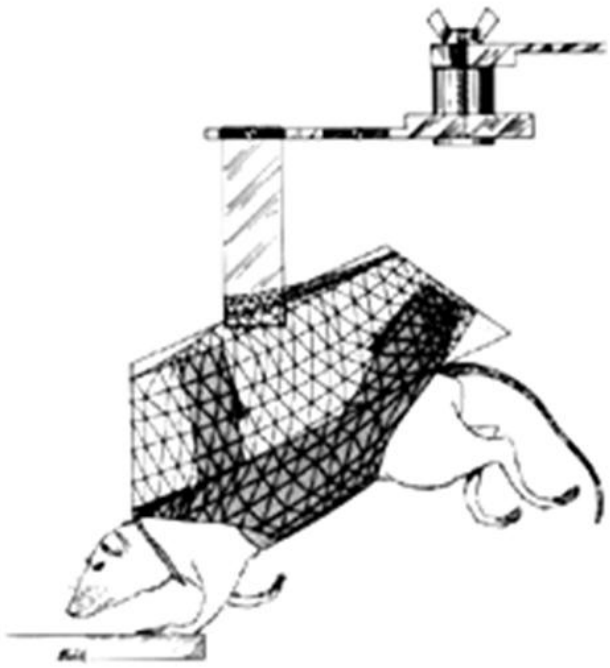


Figure 8. Harnais de décharge du train postérieur (d'après Mussachia et al. 1980)

• L'hypokinésie antiorthostatique et horizontale chez le rongeur est effectuée à l'aide de cages de contention. L'hypokinésie antiorthostatique continue chez le rat est largement utilisée par l'équipe de Monos (Raffai et al. 2009, Monos et al. 2007) (Fig 9).

Figure 9. Hypokinésie antiorthostatique continue (d'après Monos et al. 2007)

• Une stimulation discontinue (head-down tilt) chez rat est utilisée par notre équipe en Biélorussie, et aussi par Suzuki et al. (2008) (Fig. 10).

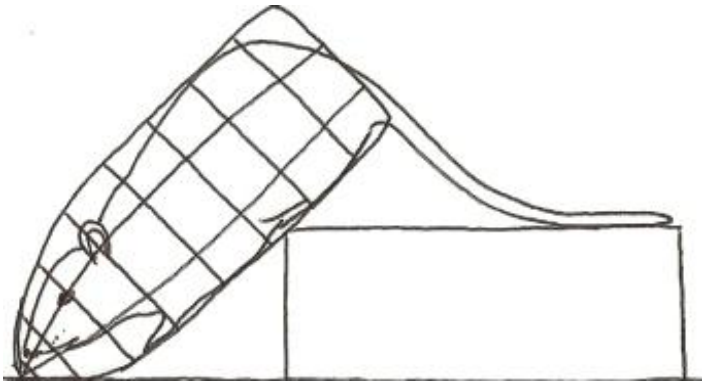


Figure 10. Hypokinésie antiorthostatique discontinu (utilisée par Kulchitsky et collègues)

Si les modèles humains sont bien admis pour étudier les effets de la microgravité sur le système cardio-vasculaire, les modèles chez les rongeurs demeurent utiles pour étudier le mécanisme des atteintes cardio-vasculaires liées à l'environnement. Il faut cependant interpréter les résultats obtenus grâce à ces modèles animaux avec prudence pour le domaine cardio-vasculaire : les rongeurs étant des quadrupèdes, la tolérance à l'orthostatisme est d'évaluation difficile chez eux.

B. REGULATION DE LA PRESSION ARTERIELLE ET DE LA PERFUSION REGIONALE

Les artères de résistance déterminent la pression artérielle systémique et le flux sanguin local

Régulation de la pression artérielle

La régulation de la pression artérielle implique plusieurs mécanismes qui contrôlent la fréquence cardiaque, la contractilité, les résistances périphériques et le volume sanguin. Ces mécanismes sont à réponse rapide ou à long terme. La régulation immédiate s'effectue principalement avec la boucle du baroréflexe. La régulation à long terme implique toujours le cœur et les vaisseaux, mais aussi le volume sanguin. La mise en jeu et l'importance des différents facteurs du contrôle de la pression artérielle sont schématisées dans la Fig. 11.

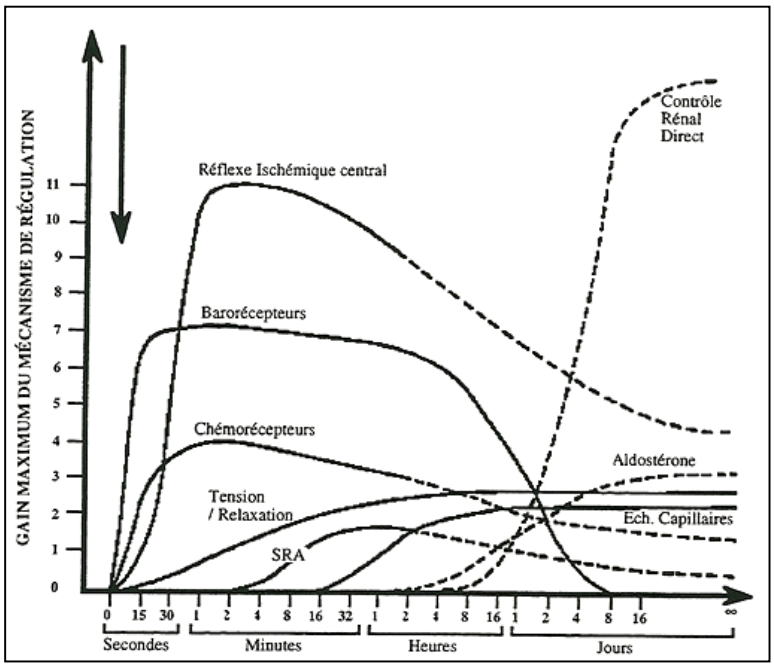


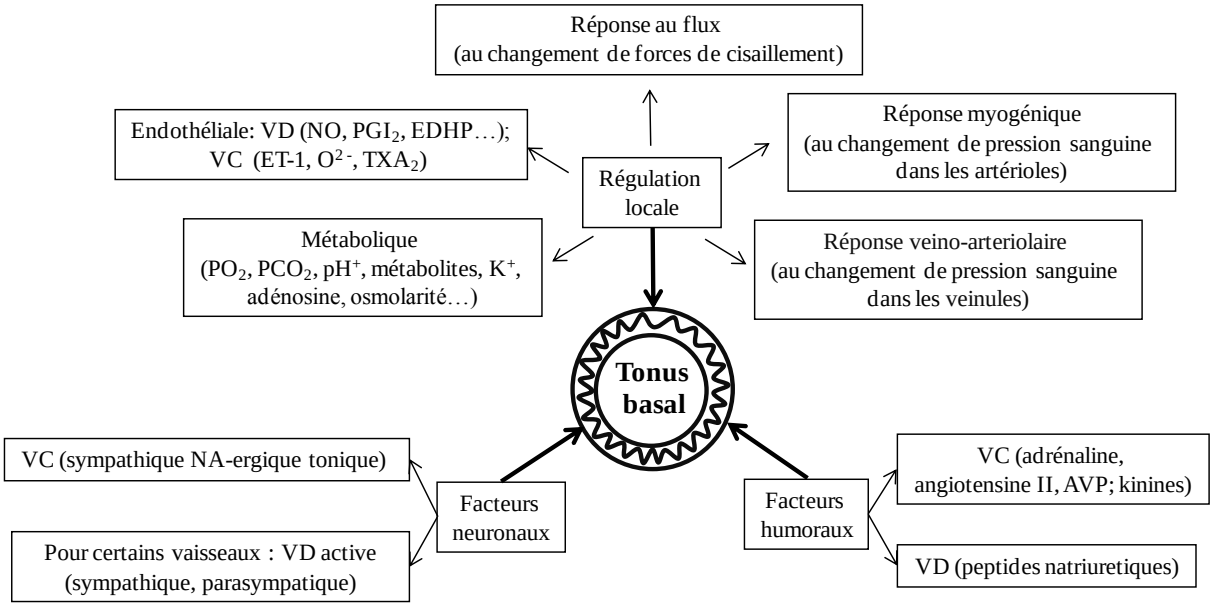
Figure 11. Délai de mise en jeu et importance relative des mécanismes intervenant dans la régulation de la pression artérielle. SRA, système rénine-angiotensine

(d'après Guyton 1991)

Régulation de la perfusion régionale

La perfusion des tissus est la fonction principale de la circulation sanguine. En situation physiologique et dans des conditions de pression artérielle stable, le flux régional est déterminé par le tonus artériolaire. Les mécanismes de régulation du tonus artériolaire sont récapitulés dans la Fig. 12.

Figure 12. Les mécanismes de la régulation du tonus artériolaire. AVP, arginine-vasopressine ; EDHP, facteur hyperpolarisant dérivé de l'endothélium ; ET-1, endothéline 1 ; NA, noradrénaline ; NO, monoxyde d'azote; O^{2-}, anion superoxyde ; PCO_2, pression partielle en dioxyde de carbone; PGI_2, prostacycline ; PO_2, pression partielle en oxygène ; TXA_2, thromboxane A_2 ; VC, vasoconstriction ; VD, vasodilatation



La régulation de la perfusion régionale est spécifique pour un territoire donné. On évoque ainsi le contrôle des circulations coronaire, cérébrale, musculaire, cutanée, pulmonaire ou rénale. Ceci rend toute étude globale des fonctions vasculaires relativement difficile.

C. COMMENT LES PARAMETRES ENVIRONNEMENTAUX LIES A L'ESPACE PEUVENT MODIFIER LE FONCTIONNEMENT VASCULAIRE

L'environnement spatial, comme nous l'avons dit, est complexe et associe l'isolement, le confinement, le stress, l'inactivité physique poussée, la microgravité avec l'annulation de la composante hydrostatique de pression et des transferts liquidiens thoraco-céphaliques. L'absence d'appui est caractéristique de l'environnement spatial. Tous ces facteurs peuvent affecter le système cardio-vasculaire.

Les facteurs psychosociaux causent une altération hémodynamique importante (Plante 2002). **L'isolement, le confinement et le stress chronique** augmentent l'activité neuroendocrinienne (Hornstein 2004) et modifient le tonus vasculaire chez l'homme. Ainsi, les jeunes adultes isolés socialement montrent une résistance vasculaire augmentée. Ce mécanisme de contrôle de la pression artérielle est associé à la réponse passive aux facteurs de stress (Cacioppo & Hawkley 2003). L'isolement social pendant l'enfance est associé à l'hypertension et à un risque vasculaire plus élevé 20 ans plus tard (Caspi et al. 2006). Chez les adolescents et les adultes, le stress chronique, l'isolement social et la solitude entraînent une augmentation plus importante de la pression artérielle en réponse aux facteurs de stress (Low et al. 2009, Steptoe et al. 2004). Un stress chronique augmente rapidement la rigidité des artères majeures (Plante 2002).

L'isolement social et le stress augmentent directement le taux de fibrinogène (Steptoe et al. 2004) et le taux de plaquettes dans le sang (Hornstein 2004). Par ailleurs, le stress altère la fonction endothéliale (Hornstein 2004) et la vasodilatation induite par le flux (FMD) dans l'artère humérale (Cooper et al. 2010). *In vitro*, les hormones de stress aigu (adrénaline et noradrénaline) et chronique (beta-endorphine, corticotropin releasing hormone) induisent la libération d'ET-1 par les cellules endothéliales (Nickel et al. 2009).

L’inactivité physique a une influence spécifique sur le risque cardio-vasculaire global, indépendamment de son action sur les autres facteurs de risque liés à la pression artérielle comme l’indice de masse corporelle ou les taux plasmatiques de cholestérol, de triglycérides et de glucose. Soixante pour cent de la réduction du risque cardio-vasculaire relatif associé à l'exercice physique peuvent être expliqués par une action sur les autres facteurs de risque (Fig. 13), suggérant qu'au moins 40% de cette réduction reposent sur les effets directs de l'activité physique.

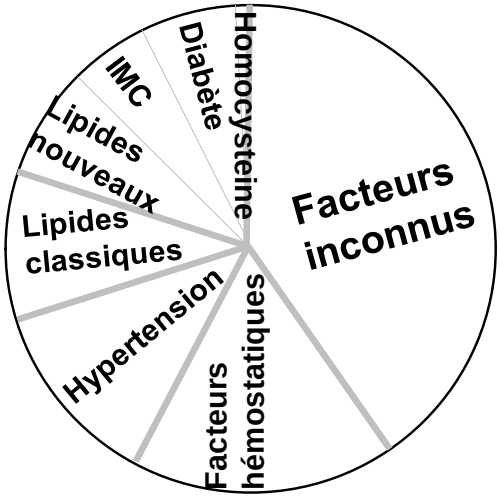


Figure 13. (Thijssen et al. 2010). Pourcentages de réduction des événements cardio-vasculaires associés à l'activité physique expliqués par les facteurs de risque traditionnels ou nouveaux.

La gravité et le niveau d’activité physique agissent directement sur les forces physiques qui s’appliquent sur les vaisseaux. L’effet cardio-vasculaire primaire de la microgravité est la redistribution de la pression vasculaire transmurale lorsque les gradients hydrostatiques disparaissent. L’inactivité diminue le flux sanguin, ce qui modifie les forces de cisaillement et la pulsatilité du flux (Thijssen et al. 2010).

On comprend bien l’importance de l'exercice aérobie comme le vélo pour préserver un bon fonctionnement cardio-vasculaire. En outre il serait intéressant de regarder les effets des exercices physiques qui induisent des déplacements inertiels des liquides corporels le long de l’axe tête-pieds. Les « chocs » hydrodynamiques longitudinaux pendant la marche, la course ou des sauts pourraient être importants pour préserver la régulation adéquate du tonus vasculaire (Stepantsov et al. 1974).

Les forces physiques modifient le fonctionnement et la structure des vaisseaux. Ainsi les variations de flux sanguin et de pression transmurale induisent à court terme une adaptation du tonus vasomoteur et à long terme des modifications structurales des vaisseaux appelées remodelage vasculaire. Ce remodelage implique les différentes couches des vaisseaux, en particulier la couche des cellules endothéliales et celle des cellules musculaires lisses (De Groot et al. 2006).

Mis à part la modification des forces physiques au niveau vasculaire, certains facteurs circulants devraient aussi être pris en compte. En particulier, l'activation de facteurs proinflammatoires pourrait participer à l’atteinte endothéliale associée au déconditionnement cardio-vasculaire.

TRAVAIL EXPERIMENTAL CHEZ L'HOMME :

L'IMMERSION SECHE

A. L'immersion sèche: revue et perspectives

B. Etude des modifications cardio-vasculaires induites par l'immersion sèche

1. Présentation de notre protocole
2. État de l'endothélium et fonctions microcirculatoires
3. Réponse cardio-vasculaire à l'orthostatisme
4. Métabolisme hydro-sodé et sa régulation hormonale

A. L'IMMERSION SECHE : REVUE ET PERSPECTIVES

L'immersion sèche reproduit de près les effets physiologiques principaux de la microgravité

"LONG TERM DRY IMMERSION: REVIEW AND PROSPECTS"

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En immersion sèche, un film élastique imperméable isole le sujet immergé jusqu'aux aisselles ou jusqu'au cou, ce qui évite tout contact direct avec l'eau. Les résultats de nombreuses expériences ont montré que l'immersion sèche peut être considérée comme l'un des modèles les plus adéquats pour étudier les effets physiologiques de la microgravité. Outre l'environnement monotone, les changements hémodynamiques et les effets hypokinétiques (déjà modélisés par l'alitement prolongé), l'immersion sèche reproduit en particulier l'absence d'appui et la diminution des influx proprioceptifs. Les changements majeurs induits par une immersion sèche prolongée sont observés particulièrement dans les systèmes dépendants de la gravité : le système neuromusculaire, l'équilibre liquidien et le système cardio-vasculaire. La vitesse de développement et le degré des désordres neuromusculaires fonctionnels et structurels en immersion sèche sont très semblables à ceux du vol spatial, voire plus intenses. Une atonie immédiate des muscles posturaux, une diminution de la force et de la vitesse des contractions musculaires, une détérioration de la coordination et un retard des réactions sensitivo-motrices sont observées. Les modifications adaptives de la balance hydro-sodée incluent la redistribution liquidienne rapide entre les secteurs intravasculaire et extravasculaire et une augmentation de l'élimination d'eau. Le système cardio-vasculaire subit le déconditionnement. En Russie, l'immersion sèche à court terme a été pratiquée dans un but thérapeutique. Elle peut être proposée en pédiatrie pour la réhabilitation des enfants prématurés, en cardiologie et néphrologie pour le traitement de l'hypertension et des œdèmes, en neurologie pour affiner le diagnostic de certains troubles dissimulés et pour réduire une hypertonie musculaire. Elle peut également être proposée comme méthode de relaxation.

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REVIEW ARTICLE

Long-term dry immersion: review and prospects

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Abstract Dry immersion, which is a ground-based model of prolonged conditions of microgravity, is widely used in Russia but is less well known elsewhere. Dry immersion involves immersing the subject in thermoneutral water covered with an elastic waterproof fabric. As a result, the immersed subject, who is freely suspended in the water mass, remains dry. For a relatively short duration, the model can faithfully reproduce most physiological effects of actual microgravity, including centralization of body fluids, support unloading, and hypokinesia. Unlike bed rest, dry immersion provides a unique opportunity to study the physiological effects of the lack of a supporting structure for the body (a phenomenon we call 'supportlessness'). In this review, we attempt to provide a detailed description of dry immersion. The main sections of the paper discuss the changes induced by long-term dry immersion in the neuromuscular and sensorimotor systems, fluid–electrolyte regulation, the cardiovascular system, metabolism, blood and immunity, respiration, and thermoregulation. The long-term effects of dry immersion are compared with those of bed rest and actual space flight. The actual and potential uses of dry immersion are discussed in the context of fundamental studies and applications for medical support during space flight and terrestrial health care.

Keywords Microgravity simulation · Muscle deconditioning · Cardiovascular deconditioning · Water-electrolyte balance · Countermeasures

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Introduction

Immersion

Immersion in fluid is the natural state for our cells, for our aquatic ancestors, and even for ourselves during the 9-month prenatal period. The curative properties of immersion in water have been known and exploited for centuries (Epstein 1992; Somody et al. 1999). Somewhat ironically, however, the current growing scientific interest in water immersion is due not to prior experience, but rather to the development of space programs. Weightlessness causes numerous physiological changes, which affect the musculoskeletal, cardiovascular, sensory, nervous, and other systems. Experimental opportunities during actual space flight are limited, so ground-based models are necessary. These models allow the assessment of microgravity-induced deconditioning effects, and reveal gravitational mechanisms in the body's physiological systems, as well as mechanisms involved in adaptation of the body to microgravity. In particular, they allow researchers to develop and test measures to counter the deleterious effects of weightlessness. Immersion is one such model, because it creates conditions that closely resemble the gravity-free environment.

Classification of immersion models

A number of different approaches are covered by the term "immersion". The immersion model of weightlessness can be classified as follows. With regard to the immersion medium, virtually all studies use tap water, but sodium chloride and silicon solutions have also been tested. According to the position of the body axis, immersion can be upright (standing, sitting, and even inverted) or horizontal. Concerning depth, a distinction is made between whole-body immersion, immersion up to the neck (also called head-out or deep immersion), and gradual immersion (up to the umbilicus, up to the xiphisternum, etc.). Regarding the temperature of the medium, immersion can be thermoneutral (around 34.5°C or 94°F), hot, cool or cold. With regard to insulation of the subject from the medium, immersion can be without insulation ("wet" immersion, the most widespread model) or with insulation; by means of a waterproof fabric ("dry" immersion, Figs. 1, 2), or a diving suit ("suit" immersion). With respect to exposure time, immersion can be short or prolonged; with the "wet" model, immersion lasts no more than several hours, whereas for the "dry" model it can last for several days or even weeks. In this review, "immersion", unless otherwise specified, refers to head-out, thermoneutral immersion in water.

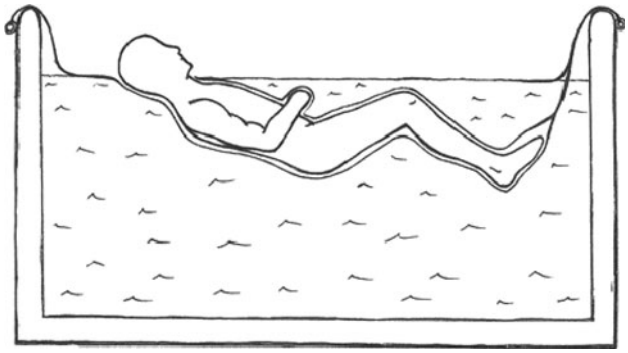

Fig. 1 Dry immersion method: the subject is immersed up to the neck and separated from the water with waterproof fabric

Fig. 2 Dry immersion experiment

The main effects of immersion

Several factors act simultaneously on the human body during immersion.

Supportlessness

During horizontal immersion, pressure forces are distributed nearly equally around the entire surface of the body (only the head and neck are not entirely supported by water). This is in contrast to the usual standing and supine positions, in which limited areas of the body carry the weight (feet or back). The absence of mechanical support of specific zones during immersion creates a state akin to weightlessness that we call supportlessness. This supportlessness itself induces physiological changes in the body's motor and other systems. The removal of afferent signals from the support zones of the feet has been shown to trigger a dramatic fall in activity of the postural muscle system (Gevlich et al. 1983; Grigor'eva and Kozlovskaia 1983; Kozlovskaia 2008; Kozlovskaya and Kirenskaya 1987; Miller et al. 2004) as we shall describe later.

Extensive physical inactivity

Hypokinesia and hypodynamia are the principal characteristics of physical inactivity induced by dry immersion. Hypodynamia involves a decrease in postural muscle load, whereas hypokinesia is a reduction in motor activity. In addition to the acute limitation of normal muscular activity and the reduced stress on muscles and bones, thermoneutral immersion swiftly causes a significant decrease in muscle tone and muscle tension (Grigor'ev et al. 2004; Kozlovskaia et al. 1984) that cannot be achieved even with the prolonged bed rest model (Kovalenko and Gurovsky 1980).

Hydrostatic compression

When a subject is immersed, hydrostatic compression of superficial tissues and vessels lowers the peripheral vascular capacity, which causes a pronounced reduction in the vascular volume of the skin, and a sustained increase in transcapillary reabsorption (movement of fluid from the interstitial to the intravascular space). The main reason for this transcapillary shift of fluid is assumed to be the pressure gradient between the interstitial and intravascular compartments (Miki et al. 1989). The shift results in the prompt centralization of fluid and an increase in total blood volume. Hydrostatic pressure increases with the depth of immersion and thus acts more on the lower surfaces of the body. The internal hydrostatic pressures are counterbalanced exactly by those of the surrounding water regardless of posture in the water. The hydrostatic compression induces a prompt redistribution of body fluids towards the thoracocephalic region (Epstein 1996; Somody et al. 1999).

Many studies have been published on "wet" immersion; however, they have mostly focused on short-term exposure (up to 6–9 h). Dry immersion was first developed and has been used mainly by Russian scientists (Grigor'ev et al. 2004; Kozlovskaia 2008; Shulzhenko and Vil-Vilyams 1975, 1976, and many other authors whose results are discussed below). Only a few non-Russian scientists have mentioned the specific model of dry immersion (Clement and Pavy-Le Traon 2004; Iwase et al. 2000; Nicogossian et al. 1993; Somody et al. 1999; Watenpaugh and Hargens 1996). To our knowledge, apart from those performed in Russian facilities, dry immersion studies have only been conducted in India (Modak and Banerjee 2004) and Austria (Berger et al. 2001; Saling et al. 2002; Struhal et al. 2002). Given the efficiency of the long-term dry immersion method on the one hand, and the relative lack of information on dry immersion for the English-speaking reader on the other, we provide here a detailed review of the experiments that have been performed with the dry immersion model.

Dry immersion: history and description

Creation of the method: a short history

The main limitation of the usual method of "wet" immersion in water is its duration, which cannot exceed 6–12 h. Early attempts to extend the immersion time by using isotonic or hypertonic sodium chloride or silicon solutions were abandoned because of maceration of the skin by the salt solutions and the expense and technical difficulty of the silicon technique. In contrast, dry immersion is simple, easy to replicate, and allows the experimental period to be lengthened (Leach Huntoon et al. 1998). The dry immersion method was first proposed during the early 1970s as a means of simulating prolonged microgravity during an era of active development of space programs. It was first described by E.B. Shulzhenko and I.S. Vil-Vilyams (Shulzhenko 1975; Shulzhenko and Vil-Vilyams 1975) who were Soviet specialists in space biology and medicine and worked at the Institute of Aviation and Space Medicine (now called the Institute of Biomedical Problems, Moscow). The dry immersion model makes it possible, for a relatively short period, to reproduce closely the physiological effects of microgravity.

The first experiments lasted 10 h, and later 3-, 7-, 13- and 28-day dry immersions were tested, with the longest duration being 56 days (Shulzhenko 1975; Shulzhenko and Vil-Vilyams 1976). By comparison, the longest head-down bed rest study to date lasted 370 days and was conducted in the former Soviet Union in 1986–1987 (Leach Huntoon et al. 1998).

Detailed description of the dry immersion method (Figs. 1, 2).

The experiments are performed in a specially designed bath filled with tap water. The bath used by Shulzhenko (Shulzhenko and Vil-Vilyams 1976) for one subject was 2 m × 1 m × 1 m; for simultaneous immersion of two subjects, a water pool with an area of 7.2 m^2 and a depth of 2.1 m was used. Nowadays in Russia, a bath that is 2.2 m long, 1.1 m wide, and 0.85 m deep is used for one subject (the length was increased to avoid accidental or intentional touching of the ends of the bath with the feet, which could provide stimulation for the foot support zones and thus compromise the results). Baths for two (Grigor'ev et al. 2004) or three volunteers are also available in Russia. Austrian researchers use a round swimming pool for the simultaneous immersion of several subjects. A special highly elastic waterproof fabric is attached to a metal rim around the external margin of the bath. The area of the fabric considerably exceeds the area of the water surface (if the bath were empty, the fabric would reach the bottom).

The bath has a built-in lift for lowering and raising the subject. The subject, dressed in a comfortable sport suit, is placed on the waterproof fabric after the fabric has first been covered with a cotton sheet for hygiene reasons. The subject is lowered slowly into the water on the lift and his body is gradually covered with the folds of fabric together with the water they contain**.** The fabric is thin and of sufficient area to allow the subject to appear to be "freely suspended" in the water mass, under conditions that are similar to a complete lack of structural support. Depending on the protocol, the depth of immersion used to be either up to the neck, or up to the imaginary line that connects the armpits (head and upper chest out of the water). Now, the accepted depth is up to the neck ("clavicles hidden"), in order to maximize the effects of dry immersion. The subjects are permitted to put their hands out to work with a computer, eat, read, and perform experimental tasks. The water temperature is regulated automatically and checked by a thermometer floating in the middle of the water mass. It is set to 32–34.5°C (thermoneutral), and is adjusted for comfort within these limits at the subject's request (Kozlovskaia 2008; Shulzhenko and Vil-Vilyams 1976). The air temperature is approximately 24°C, in order to maintain the heat balance when the subject is raised from the bath. The subject remains under constant medical observation 24 h a day. For physical examinations and visual inspections of the skin, the folds of fabric can be moved apart without changing the experimental conditions substantially. Recordings from any site on the body can be taken without risk of wetting the probes or electrodes. Urine is collected into clinical urine pouches. Once a day, the subject is allowed to get out for 15 min for hygiene procedures and weighing.

E.B. Shulzhenko and I.S. Vil-Vilyams defined their method as "water immersion hypokinesia". They provided evidence that the use of dry immersion allowed prolonged experimentation without concomitant complications (Shulzhenko et al. 1977), and recommended it for large-scale experimental use. To date, dry immersion has been performed at the Institute of Biomedical Problems for more than 30 years (since 1975), usually with 1–2 sessions per year and 8–12 subjects per session. The most common durations of immersion are 3 and 7 days, depending on the experimental task. Up to now, only male volunteers have been studied, primarily because mainly men have participated in actual Russian space missions, but also because urination in the dry immersion bath is more complicated for women.

Tolerance and subjective sensations

In general, the subjects find dry immersion to be comfortable during the initial period (Atkov and Bednenko 1992; Iwase et al. 2000; Nicogossian et al. 1993; Shulzhenko and Vil-Vilyams 1976). Volunteers' comments have included: "As if you were lying in thick jelly and didn't sink…"; "It is like a soft feather bed or a slightly inflated rubber mattress…". The sensation of headward blood transfer (heaviness in the head), slight obstruction of the nose, and some difficulty in inhaling occur later. Nearly all subjects complain of backache after the first 6–8 h of dry immersion (possibly due to loss of tone in the back muscles), but this disappears by day 2–3. This backache is especially severe in persons whose muscles have undergone intensive training. Sleep disturbance, loss of appetite, and diarrhea can also occur (Fomin et al. 1985; Shulzhenko 1975; Shul'zhenko et al. 1984). When several subjects are immersed in one bath, some of them report symptoms of motion sickness during water movement (Struhal et al. 2002). Similar complaints have been described during the first few days of space flight (Egorov 1996; Kornilova 1997; Williams et al. 2009) or bed rest (Baum and Essfeld 1999; Greenleaf 1984).

Dry immersion: initial responses

The initial responses to dry immersion are induced by immediate changes in the distribution of body fluids and the removal of support stimulation. This section discusses the acute effects that become apparent within the first 12 h.

Fluid–electrolyte and cardiovascular changes

Initial renal and cardiovascular responses are common for both dry immersion and "wet" immersion, with an acute increase in water and electrolyte excretion and changes in hemodynamic parameters. Most "wet" immersion studies use the upright, sitting or standing, posture. Some evidence indicates that posture does not significantly influence the response. Larina et al. (1999) compared data from experiments that involved dry immersion or horizontal and vertical suit immersion, and found that the quantitative effects of dry immersion and vertical suit immersion on water and electrolyte excretion were nearly identical for the first 3–5 h of exposure. Genin et al. (1988) found that hemodynamic parameters did not change significantly as the subject transferred between horizontal and vertical immersion, which suggests that these positions in water are hemodynamically similar. With respect to acute cardiovascular responses to dry immersion, Modak and Banerjee (2004) showed that, during 4 h of dry immersion ($n = 11$), there was a significant decrease in heart rate of about 5 bpm and in diastolic blood pressure of about 5 mmHg, as well as a 9% increase in stroke volume and a 7% decrease in total peripheral resistance during the first 2 h (Fig. 3).

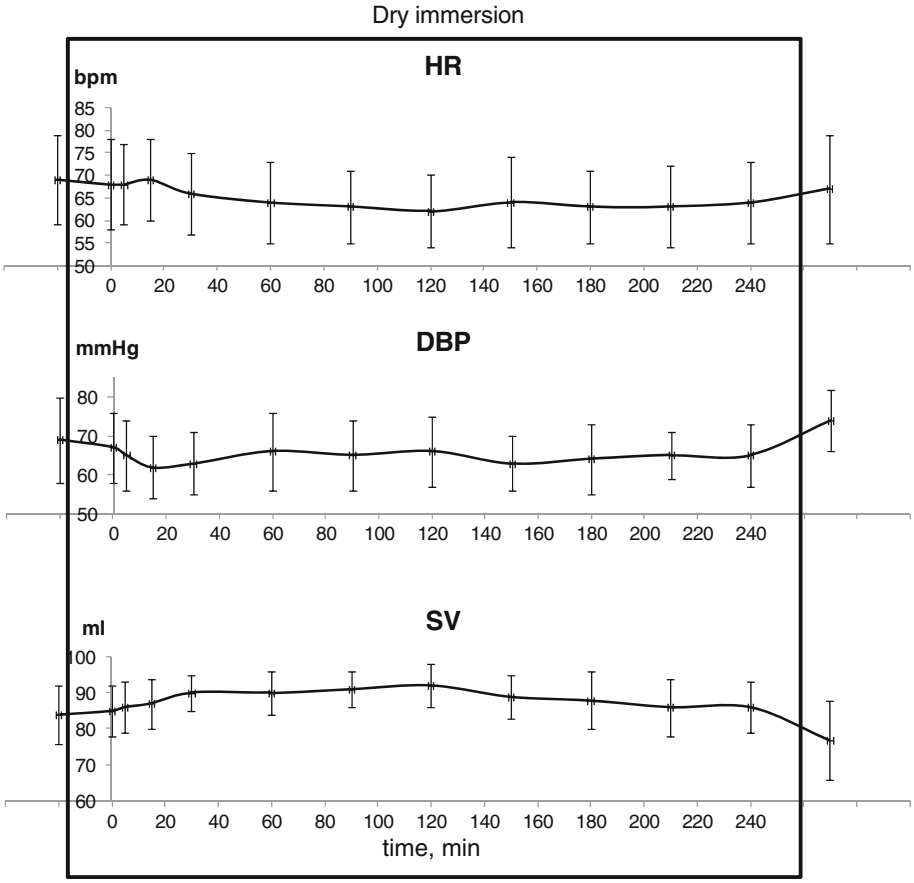

Fig. 3 Heart rate (*HR*), diastolic blood pressure (*DBP*), and stroke volume (*SV*) before, during and after 4 h of dry immersion, mean ± SD (adapted from Modak and Banerjee 2004)

The main stimulus that triggers water–electrolyte changes during immersion is assumed to be congestion of the central vascular regions (Epstein 1996; Somody et al. 1999). Under immersion, the hydrostatic pressure lowers the peripheral vascular capacity and increases vascular perfusion. This leads to the prompt redistribution of fluid with relative central hypervolemia. The resulting changes are an increase in cardiac output (Arborelius et al. 1972), a decrease in sympathetic nervous system activity, suppression of the renin–angiotensin–aldosterone system (Epstein 1996), an increase in atrial natriuretic peptide (Epstein et al. 1989), a decrease in vasomotor tone, and high natriuresis and diuresis (Nicogossian et al. 1993). There are no available data about changes in central venous pressure during the acute phase of dry immersion. For head-out vertical water immersion with a duration of 10 min, Arborelius et al. (1972) reported an acute increase in central venous pressure from 3 to 15 mmHg. During chronic dry immersion, Fomin et al. (1985) measured central venous pressure in two healthy young men and four men with borderline arterial hypertension using the Valdman apparatus (catheter). The maximal increase (+40% for the healthy volunteers and more than +50% for the subjects with borderline arterial hypertension) was observed on day 3 of dry immersion, with a tendency to return to normal by day 7.

However, in space, an unexpected decrease in central venous pressure has been observed (Buckey et al. 1996a; Foldager et al. 1996; Kirsch et al. 1984). Surprisingly, cardiac output did not decrease. Several hypotheses have been suggested, which include thoracic (Foldager et al. 1996) and cardiac (Koenig et al. 1998) biomechanical changes that are specific to real conditions of weightlessness. During weightlessness, the chest relaxes and thus the volume of the closed chest cavity increases. Intrathoracic pressure is decreased more than central venous pressure, so the real transmural filling pressure increases, with subsequent increases in heart size and stroke volume (White and Blomqvist 1998). Thus, it should be taken into account that actual weightlessness and terrestrial models for weightlessness, namely dry immersion and head-down bed rest, induce quite different changes in central venous pressure.

Muscle tone and afferent input

Whereas fluid centralization is inherent to both dry immersion and head-down bed rest, the swift drop in muscle tone and the acute alteration in afferent input is specific to dry immersion. Under terrestrial conditions, muscle tone—the continuous partial muscle contractions that help to maintain posture without causing fatigue—is

always maintained. Supportlessness immediately induces a marked decrease in muscle tone. The dramatic loss of postural muscle tone is one of the early manifestations of exposure to dry immersion (Gevlich et al. 1983; Kozlovskaia 2008; Kozlovskaya et al. 1988, 2007a). For the postural muscles, the 40–50% drop in tone occurs within the first few hours of dry immersion (Gevlich et al. 1983; Kozlovskaya 2002; Kozlovskaia et al. 1984). Kozlovskaya's team revealed that these changes are due to the lack of support stimuli, which, under terrestrial conditions, seem to be a natural trigger for activity of the postural muscles (Grigor'ev et al. 2004; Kozlovskaia 2008; Kozlovskaya et al. 2007a, b; Vinogradova et al. 2002a).

Dry immersion: principal changes induced by long-term exposure

Neuromuscular and skeletal system

Muscles and muscular deconditioning

The alteration of skeletal muscle structure and function is one of the characteristic features of the body's adaptation to microgravity. Studies of the neuromotor effects of weightlessness have shown that microgravity motor syndrome is characterized by a decrease in muscle tone, changes in muscle structure (atrophy, myofibrillar destruction, alterations in muscle fiber composition), and a corresponding reduction in the force of muscle contraction. These changes are most profound in antigravity tonic leg and body muscles (Adams et al. 2003; Berry 1973; Il'yina-Kakueva et al. 1979; Kakurin et al. 1971; Williams et al. 2009). The physiological mechanisms involved in the adaptation of tonic muscles to microgravity are summarized in Fig. 4. Neuromotor changes also involve disturbances in coordination, which result in decreased accuracy in muscle effort, decreased performance with respect to

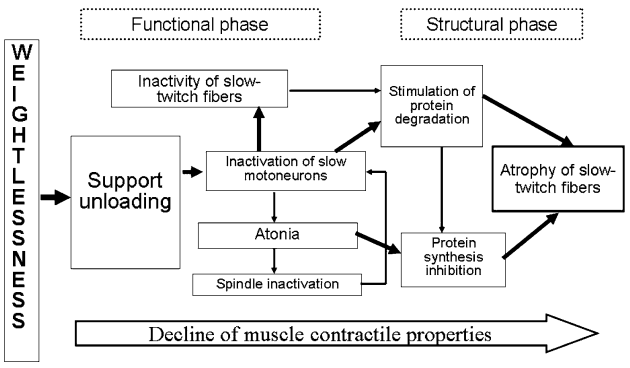

Fig. 4 Schematic diagram of the physiological mechanisms for the adaptation of tonic muscles to microgravity (adapted from Kozlovskaya 2007)

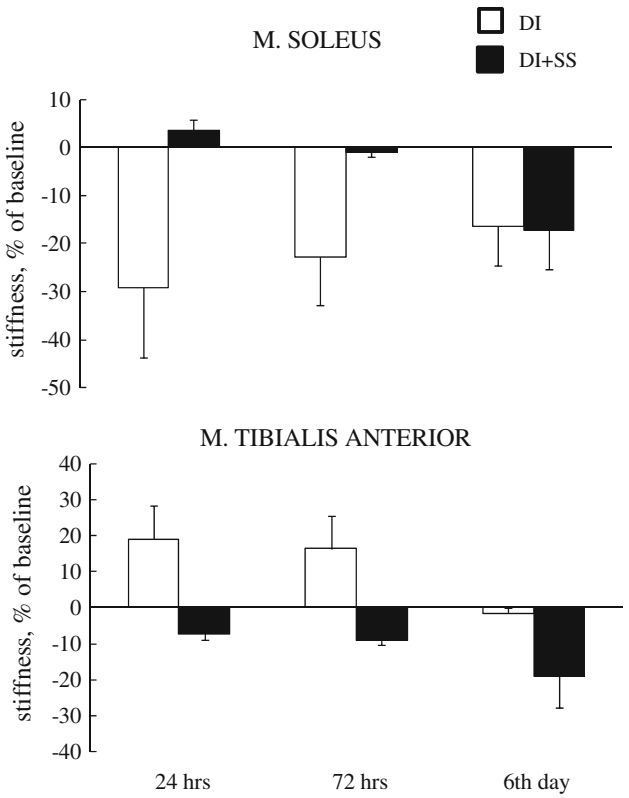

Fig. 5 Changes in transverse stiffness in the *m. soleus* and *m. tibialis anterior* after 6 days of dry immersion (*DI*) with and without support zone stimulation (*SS*) as a percentage of the baseline value, mean ± SD. Adapted from Kozlovskaya et al. (2007b)

motor tasks, decreased postural stability, and locomotion disturbances (Chkhaidze 1968; Gazenko et al. 1987; Homick et al. 1977; Kozlovskaya et al. 1981).

Changes in muscle functions

Dry immersion induces an obvious decline in postural muscle tone (as evidenced by decreasing stiffness) and a decrease in the electromyographic activity of extensor muscles with a simultaneous increase in flexor activity within the first few hours (Grigor'ev et al. 2004; Grigor'eva and Kozlovskaia 1983; Kozlovskaya 2002; Kozlovskaia et al. 1984). After 24 h of dry immersion, a sharp drop of approximately 30% in the stiffness of the leg antigravity muscles (*m. soleus)* has been reported (Fig. 5) (Grigor'ev et al. 2004; Grigor'eva and Kozlovskaia 1983; Kozlovskaya 2002; Miller et al. 2004; Vinogradova et al. 2002a). A decrease in the maximal voluntary force of leg antigravity muscles (*m. triceps surae*) was recorded by isokinetic dynamometry on days 4–5 of dry immersion (Grigor'eva and Kozlovskaia 1983; Khusnutdinova et al. 2004; Netreba et al. 2004, 2005). In experiments reported by Koryak (2002a), the muscle force of *m. triceps surae* was reduced by 19% on day 7 of dry immersion. This reduction is

thought to be a consequence of reduced motor drive and muscle atonia, because the electrically evoked tetanic forces were not reduced (Koryak 2002a, b). Supporting this idea, Khristova et al. (1986, 1988), during electromyographic studies (*m. biceps brachii*), observed a significant decline (up to 30%) in the propagation velocity of muscle potentials for low threshold motor units on day 3 of dry immersion, which indicated neuromotor impairment of slow muscle fibers. After 7 days of dry immersion, the force–velocity properties of leg antigravity muscles had declined by as much as 30–40% (Grigor'ev et al. 2004; Kozlovskaya 2002), and the endurance of these muscles also decreased (Kozlovskaya 2002).

Changes to muscle morphology and functional changes at the micro-level

After 3–7 days of dry immersion, deep structural changes in leg antigravity muscles have been recorded. Atrophy of muscle fibers during dry immersion has been reported by several authors (Litvinova et al. 2004; Moukhina et al. 2004; Nemirovskaya and Shenkman 2002; Shenkman et al. 1997), predominantly for slow fibers. The atrophy that occurs during dry immersion is associated with a slight, but significant, shift of myosin to the fast type (6%) (Grigor'ev et al. 2004; Shenkman et al. 1997) and a dramatic decrease in key sarcomeric proteins (a 30% decrease in titin and nebulin after 7 days of dry immersion (Grigor'ev et al. 2004; Shenkman et al. 2004a, b). More precisely, for *m. vastus lateralis*, the decrease in the cross-sectional area of both slow and fast fibers is 5–9% after 3 days of dry immersion and 15–18% after 7 days of dry immersion (Shenkman et al. 2004a). However, most of this reduction is due to dehydration of the muscle fibers, rather than to atrophy of the contractile apparatus, which has not developed to a significant extent after 3–7 days of exposure, as shown by the simultaneous decrease in the cross-sectional area and increase in the total protein concentration of the fibers (Shenkman et al. 2004a). For the most common durations of dry immersion (3–7 days), muscle atrophy is barely visible at a clinical level. For the entire lower leg, Vinogradova et al. (2002b) found only a 1.5–2.5% decrease in cross-sectional area after 3 days of dry immersion, and less than 5% after 7 days of dry immersion.

In addition, the sensitivity of muscle fibers to Ca^{2+} decreases at the level of single skinned muscle fibers, which might influence muscle contractile characteristics (Grigor'ev et al. 2004; Shenkman et al. 2004a).

Comparison with head-down bed rest and space flight

The intensity and rate of neuromuscular changes that occur during dry immersion are much higher than those

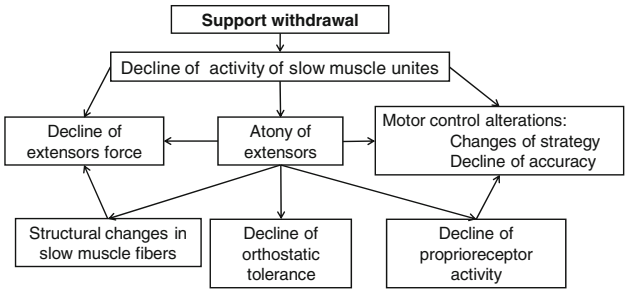

Fig. 6 Schema of motor effects of support withdrawal (adapted from Kozlovskaya 2007)

that occur during head-down bed rest. A decrease in muscle stiffness is seen immediately with dry immersion, with a maximal decrease of 40–50% after 6 h, whereas with head-down bed rest, decreased muscle stiffness can only be detected after 3–4 days, and reaches a maximal decrease of 30–40% after 14–30 days (Kozlovskaya 2002; Kozlovskaya and Kirenskaya 1987). The loss in contractile properties during dry immersion, both at the system and cellular level, is extremely rapid, reaching high values after just 5–7 days. For head-down bed rest, 60–90 days are needed to reach a similar level of impairment (Koryak 2006; Shenkman et al. 2004a). The main difference between the head-down bed rest and dry immersion models appears to be the level of removal of support; therefore deprivation of support might play a leading role in the genesis of the microgravity-induced syndromes of muscular deconditioning and hypogravitational ataxia (Gazenko et al. 1987; Grigor'ev et al. 2004; Kozlovskaia 2008; Kozlovskaya et al. 1988). The motor effects of withdrawal of support are summarized in Fig. 6.

The rate, degree, and maximal extent of structural and functional muscular disorders during dry immersion are very close to those that occur during space flight (Kozlovskaya 2002; Kozlovskaia 2008). In fact, they might be even more intensive during dry immersion, as shown by measurements of stiffness and force for *m. triceps surae* after 7 days of dry immersion and 7 days of space flight (stiffness and maximal isometric force had decreased by 25–30% after dry immersion, whereas after space flight they fell by 15–20%) (Kozlovskaia et al. 1984). The interpretation of space flight data is complicated by heterogeneity in the data collection methodology, lack of controls (for missions lasting more than 10 days all crew members must exercise), and interference from any unrecorded physical activity. After short-term missions (8–17 days), decreases in the cross-sectional area of the muscle of 4–10% have been reported for *m. gastrocnemius* and of 5–15% for the quadriceps group (Adams et al. 2003).

Sensorimotor changes: posture and locomotion

Motor control involves the integration of sensation and movement in order to achieve a certain goal (Bernstein 1947). In microgravity, motor control is complicated by the deficit and distortion of specific information (for example, vestibular inputs and support inputs) that is needed to build motor programs (feed-forward) and control their execution (feedback). Moreover, it is complicated by a deficit of non-specific information that is necessary to activate mechanisms of motor control.

Changes in motor control that are induced by dry immersion include spinal hyperreflexia (Gazenko et al. 1987; Sayenko 2007), suppression of tonic motoneuron activities and alteration of the pattern of motor neuron recruitment (Kirenskaia et al. 1985; Kozlovskaya 2002; Kozlovskaya et al. 1988), sensory and motor ataxia, and alterations in strategies for movement control (Gazenko et al. 1987; Grigor'eva and Kozlovskaia 1985; Kirenskaia et al. 1985). All these changes are reproduced with 3–7 days of dry immersion (Grigor'ev et al. 2004; Kozlovskaia 2008). Disorders of postural and voluntary motor control are unvarying consequences of exposure to dry immersion, and are due to the resetting of neuromuscular control (Kozlovskaya and Kirenskaya 1987; Sayenko et al. 2005). The state of the nervous system during dry immersion is considered by Grigor'ev et al. (2004) and Kornilova et al. (2004) to be the result of partial deafferentation linked to the initial withdrawal of support stimulation, which is complemented by the decrease in muscle proprioceptive input because of atony and hence unloading of muscle proprioceptors (Kozlovskaya 2002; Kozlovskaia 2008). It is assumed that denervation decreases the thresholds for specific and non-specific stimuli because of a lack of neural inhibition, which results from a lack of afferentation (Grigor'ev et al. 2004). Such facilitation (resulting from functional denervation), which occurs within the first few hours and becomes more pronounced after 3–7 days of dry immersion, is seen in the significant hypersensitivity of the proprioceptive pathways of the spinal cord, as evaluated by T- and H-reflex parameters (Kozlovskaya 2002; Kozlovskaya et al. 2007a, b; Sayenko 2007).

Sensorimotor changes that occur after dry immersion include disturbed maintenance of vertical posture and a decline in the ability to withstand postural perturbations (Sayenko et al. 2005), disturbances in locomotor activity (Mel'nik et al. 2006) and in the coordination and precision of movement (Grigor'eva and Kozlovskaia 1985; Kirenskaia et al. 1985), disturbances in the control of eye movement, including pursuit function (Kornilova et al. 2004, 2008) and gaze fixation (Kirenskaia et al. 2006; Kreidich et al. 1982; Tomilovskaya 2007; Tomilovskaya et al. 2004, 2008).

The proprioceptive stimulation countermeasure

Functional deafferentation with respect to support stimuli and the effects of compensation for this deafferentation have been extensively studied in dry immersion experiments. The "compensator of support unloading" was developed by the Institute of Biomedical Problems and Zvezda Co., Moscow, Russia, and consists of pneumatic insoles with inflatable bladders that impart a pressure of 40 kPa alternately to the heel and metatarsus. During dry immersion, the daily stimulation protocol is 20 min per hour for 6 h, i.e. 2 h/day. Two stimulation regimes are applied, which simulate slow walking (75 steps/min) and fast walking (120 steps/min), respectively. Several dry immersion experiments over 3 and 7 days have shown that such periodic short-term mechanical stimulation at a natural rhythm fully eliminates or considerably suppresses all the negative effects of microgravity on the motor system (Grigor'ev et al. 2004; Khusnutdinova et al. 2004; Kozlovskaya 2002; Kozlovskaya et al. 2007a, b; Layne and Forth 2008; Mel'nik et al. 2006; Moukhina et al. 2004; Netreba et al. 2004, 2005, 2006; Popov et al. 2003; Shenkman et al. 2004a, b; Vinogradova et al. 2002a).

The support stimulation countermeasure seems to be much more effective than electromyostimulation. Analysis of the state of single fibers of postural muscles after dry immersion with either support stimulation or electromyostimulation has revealed that electrostimulation does not prevent muscle fiber degradation, in contrast to support stimulation (Ogneva et al. 2009).

Bones

Kotov et al. (2003) reported that, after 7 days of dry immersion, the bone mineral density of the lower part of the skeleton (proximal epiphysis of the femur) tends to be approximately 2% lower and that of the upper part (skull, hand, costal bones) tends to be approximately 2% higher than the values at baseline. Moreover, 3 weeks of recovery after dry immersion are sufficient to reverse these changes in bone density. These alterations are assumed to be a secondary effect of a cephalad transfer of fluid to the upper part of the body, upon which the increased hydrostatic pressure promotes the shift of ions and proteins into bone. Thus, dry immersion seems to have a similar effect as bed rest on bone resorption in certain areas of the body. During bed rest, it has been demonstrated that markers of bone resorption increase as early as day 2 (Baecker et al. 2003). The data of Kotov et al. (2003) support a rapid onset of bone degradation during conditions of immobilization, such as dry immersion or bed rest.

In general, the state of bone tissue during dry immersion for 7 days is rather stable (Markin et al. 2008), as detailed below.

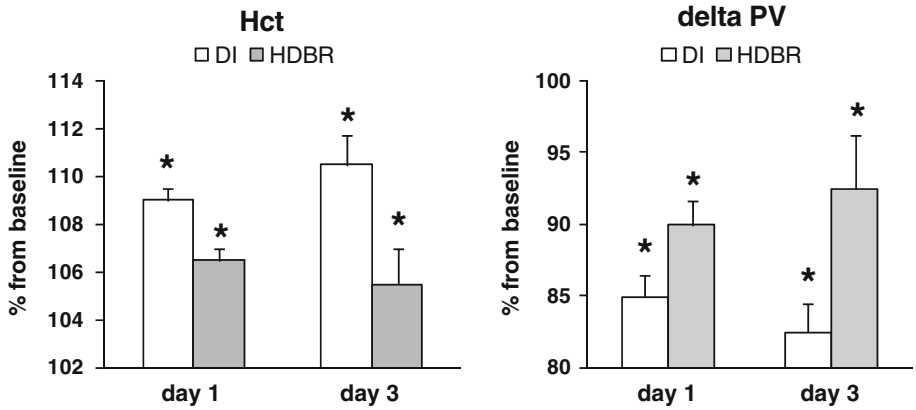

Fig. 7 Relative changes in hematocrit (*Hct*) and calculated plasma volume (*PV*) during dry immersion (*DI*) and −6° head-down bed rest (*HDBR*) after 1 and 3 days of exposure, mean ± SEM; $*p < 0.05$ versus respective baseline (adapted from Gogolev et al. 1980)

Blood calcium

With 7 days of dry immersion, concentrations of total and ionized calcium in the blood do not change (Kopp 2008; Markin et al. 2008). With 56 days of dry immersion, blood Ca^{2+} increases slightly during the second week. This increase persists until the end of the experimental period, which might indicate calcium loss from the bones (Leach Huntoon et al. 1998).

Urinary calcium

Urinary excretion of calcium increases significantly on the first day of a 7-day period of dry immersion, which might be explained by acutely increased diuresis, and returns to the basal level on day 2, after which there is only a tendency for it to increase until the end of the experimental period (Kopp 2008; personal data).

Bone formation markers

Processes involved in bone formation are not affected by dry immersion for 7 days, as shown by the lack of changes in alkaline phosphatase (Markin et al. 2008), PINP (serum procollagen aminoterminal propeptide), and BAP (serum bone-specific alkaline phosphatase) (Kopp 2008).

Bone resorption markers

Markers of bone tissue resorption, such as TRAP (serum tartrate-resistant acid phosphatase) and CTX (urinary carboxyterminal cross-linked telopeptide of type I collagen) increased slightly during dry immersion for 7 days (Kopp 2008). This might indicate that mechanical unloading of bones leads to the activation of osteoclastic processes. However, Markin et al. (2008) did not find any changes in the activity of total acid phosphatase during the 7 days of dry immersion.

Water–electrolyte balance

Plasma volume and fluid compartments

Following an initial decrease (approximately −15% within the first day of dry immersion) (Leach Huntoon et al. 1998), plasma volume remains stable thereafter (Gogolev et al. 1980; Larina et al. 2008; Navasiolava et al. 2010b; Nesterovskaia et al. 2008; Pakharukova et al. 2009a). During head-down bed rest, plasma volume decreases by approximately 10%, and this also occurs mainly within the first 24 h (Greenleaf et al. 1977; Pavy-Le Traon et al. 2007) (Fig. 7). Surprisingly, protein concentrations in the plasma do not increase during dry immersion despite the reduction in plasma volume (Chaika and Balakhovskii 1982; Navasiolava et al., submitted EJAP), and they might even decrease (Gogolev et al. 1980). This might be explained by the partial transfer of plasma proteins to the interstitial fluid due to an increased transcapillary escape rate and decreased lymphatic return. During immersion, when there is hydrostatic counterpressure of superficial tissues and continuous transcapillary "autotransfusion" (Krasney 1996), there is a greater loss of interstitial fluid. The redistribution of proteins from the plasma to the interstitial fluid increases the oncotic pressure of the interstitial fluid and prevents further tissue dehydration (Gogolev et al. 1980). Similar findings have been observed in head-down bed rest studies (Greenleaf et al. 1977).

The maximal renal losses of fluid and sodium occur on the first day of dry immersion (Leach Huntoon et al. 1998; Iunusov et al. 1985; Shulzhenko et al. 1980). Then a new steady state for water–sodium homeostasis is established that involves increased excretion of water and a stable decreased partial water balance, while sodium excretion returns to the pre-immersion level (Leach Huntoon et al. 1998; Navasiolava et al., submitted EJAP) (Fig. 8). During head-down bed rest, in contrast to dry immersion, no pronounced natriuresis occurs at the onset (Millet et al. 2001) (Fig. 8). It is important to mention that extrarenal

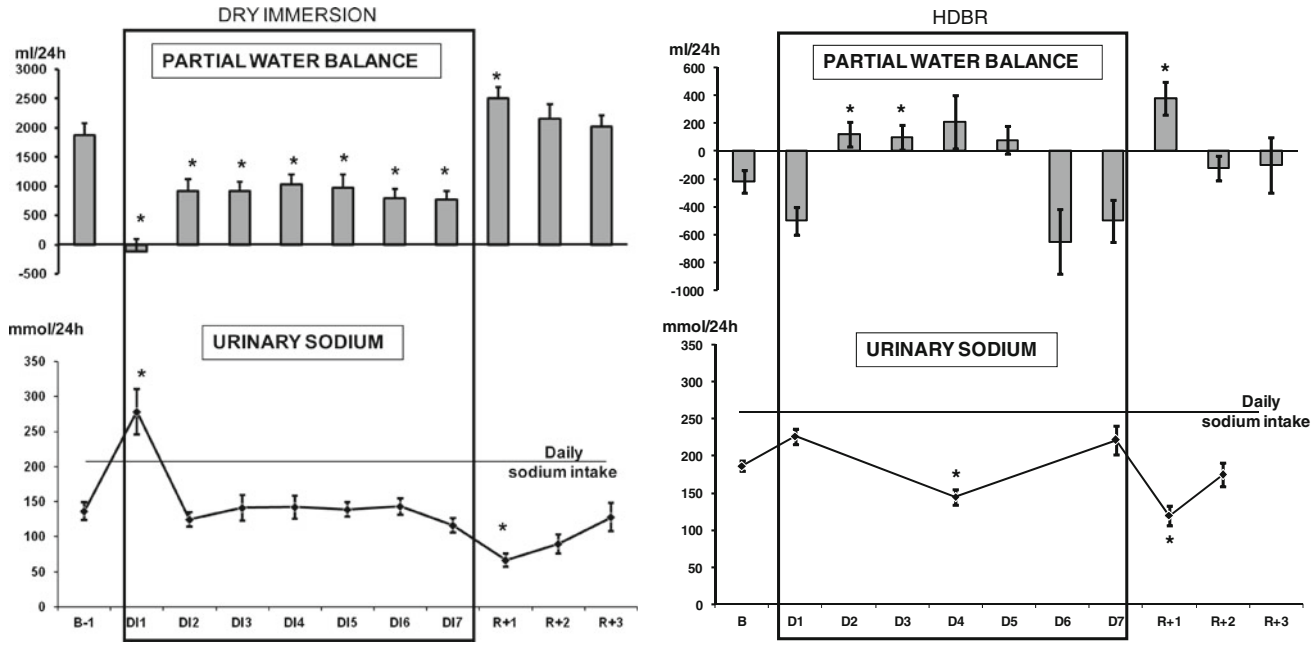

Fig. 8 Partial water balance and urinary sodium before, during, and after 7 days of dry immersion (Navasiolava et al., submitted EJAP) and 7 days of head-down bed rest (HDBR), mean ± SEM (adapted from Millet et al. 2001); $*p < 0.05$ versus baseline

water loss has a significant impact on water balance during immersion. It has never been measured during dry immersion, but in the case of suit immersion for 3 days, it has been shown that this extrarenal loss can account for 25–75% of total water loss (Lakota and Larina 2002; Larina and Lakota 2000).

Volume-regulating hormones

Vasopressin The immediate suppression of vasopressin in normally hydrated subjects with the onset of immersion has been reported by many authors (Somody et al. 1999; Epstein 1996; Larina et al. 1999). During dry immersion, the vasopressin level drops in the first 1.5 h (0.8 ± 0.2 pg/ml vs. 2.3 ± 0.6 at baseline), then recovers to the baseline level by 5 h, and remains close to the baseline after 24 h (Sukhanov 1985). At days 6 and 7 the vasopressin level remains unchanged, and at R + 1 (first recovery day) it is increased significantly (8.6 ± 1.2 pg/ml at R + 1 vs. 2.4 ± 0.7 at day 7) (Sukhanov 1985).

Renin–angiotensin–aldosterone system The immediate response to immersion is a suppression of plasma renin activity and plasma aldosterone (Epstein 1996; Iunusov et al. 1985; Sukhanov 1985). With respect to the plasma renin chronic response, neither we (Navasiolava et al., submitted EJAP) nor Sukhanov (1985) found any significant change on day 3, 6, or 7 of the 7 days of dry immersion, but Shulzhenko et al. (1980) found a small but significant increase on day 7 and R + 2 (second recovery day). Plasma aldosterone is unchanged during 7 days of dry immersion (Iunusov et al. 1985; Shulzhenko et al. 1980; Sukhanov 1985; Navasiolava et al., submitted EJAP) but is significantly increased at R + 1 (Sukhanov 1985).

Natriuretic peptides Upon immersion, an immediate increase in atrial natriuretic peptide has been identified (Epstein et al. 1989). The only study to date of natriuretic peptides during chronic dry immersion analyzed N-Terminal proB-type natriuretic peptide (NT-proBNP), whose levels tended to decrease. During recovery, NT-proBNP increased significantly, which might be related to the increase in cardiac work during normal daily activity after immersion, induced by increased cardiac wall stress and increased heart rate (Navasiolava et al., submitted EJAP).

Cortisol During dry immersion for 7 days, urinary free cortisol increased significantly only on the first day and not subsequently, which probably reflects the emotional stress experienced at the beginning of immersion (Navasiolava et al., submitted EJAP).

Cardiovascular system

Cardiovascular deconditioning syndrome

Cardiovascular deconditioning is a state in which the cardiovascular system does not respond efficiently to challenge. It is characterized by a predisposition to orthostatic intolerance, reduced capability for exercise, and

Table 1 Summary of published data on tilt/stand tolerance after dry immersion

Reference	Duration of dry immersion	Number of subjects (men)	Orthostatic test type	Number of non-finishers on R0/R + 1	Reason for intolerance	Heart rate in standing position, after dry immersion versus before	Blood pressure in standing position, after dry immersion versus before
Vinogradova et al. (2002a)	7 h	8	Tilt for 10 min	1 (5th min)	Drop in blood pressure	95 vs. 85 bpm	Pulse pressure decreased in 4 men
Eshmanova (2009)	24 h	10	Stand for 10 min	2	Heart rate increased more than 30 bpm and systolic pressure decreased more than 20 mmHg	+55 vs. +35% at 3rd min	Systolic pressure decreased more than 20 mmHg in 4 men
Shul'zhenko et al. (1984)	36 h	6	Tilt 70° for 20 min	1	NA	+90 vs. +76%	NA
Iwase et al. (2000)	3 days	6	Tilt 60° for 5 min	1 (2nd min)	Drop in blood pressure	97 vs. 78 bpm	Diastolic pressure decreased from 75 to 65 mmHg at the 5th min
Iarullin et al. (1987)	7 days	6	Tilt (no details)	2	NA	From 70 to 113 vs. from 69 to 80 bpm	NA
Eshmanova (2009)	7 days + daily myostimulation	6	Stand for 10 min	2 (7th–8th min)	Drop in blood pressure	+36 vs. +36% (equal)	NA
Eshmanova (2009)	7 days	8	Stand for 10 min	1 (4th min)	Vertigo, acute weakness	86 vs. 128 bpm (5th min of stand)	Systolic pressure 123 vs. 111, Diastolic pressure 92 vs. 91 mmHg (5th min of standing)
Evdokimova et al. (1989)	7 days	10 (with neuro-circulatory dystonia)	Tilt 75° for 20 min	6	Collapse on 5th–15th min	+20 and more vs. +10 bpm	Delta of systolic pressure increase −22 vs. −7 mmHg
Panferova (1976)	10 days	9	Tilt 85° for 5 min	NA		+39 vs. +11 bpm (from 63 to 77 bpm vs. from 74 to 116 bpm)	Diastolic pressure increased [from 63 (rest) to 72 (tilt) vs. from 71 to 83 mmHg]

NA data not available

Table 2 Summary of published data on tolerance to acceleration after dry immersion

Reference	Duration of dry immersion (days)	Number of subjects (men)	Test	Result
Vil-Viliams et al. (2001)	3	NA	+3Gz acceleration	Time of tolerance reduced by 21%
Vil'-Viliams and Shul'zhenko (1978), Shulzhenko and Vil-Vilyams (1975)	3	10	+3Gz acceleration for 300 s maximum, rate of increase 0.2 G/s	Time of tolerance reduced to 249 s; test stopped because of altered vision (grey veil) in 50% of subjects
Kokova (1984)	7	6	Centrifugation with a slow onset, rate of increase 0.003 G/s	Acceleration tolerance was decreased from 4.8 to 3.6 G, or by 24%
Vil-Viliams et al. (2001)	7	4	+3Gz acceleration	Time of tolerance reduced by 56%
Vil'-Vil'iams and Shul'zhenko (1980)	7	4	+3Gz acceleration for 300 s maximum	Time of tolerance reduced by 65% (test stopped because of altered vision or decrease of blood pressure in the ear lobe vessels <40 mmHg)
Vil-Viliams et al. (1996)	7	4	+4Gz for 10 s	In 50% of cases, marked vegetative vascular reactions seen as skin pallor and hyperhidrosis (did not occur in preimmersional test)
Vil'-Viliams and Shul'zhenko (1978), Shulzhenko and Vil-Vilyams (1975)	13	6	+3Gz acceleration for 300 s maximum, rate of increase 0.2 G/s	Time of tolerance reduced to 226 s; test stopped because of altered vision (grey veil) in 50% of subjects

NA data not available

tachycardia (Coupe et al. 2009). The negative effects of cardiovascular deconditioning mainly become evident upon the "return" to conditions of gravity during active or passive standing, centrifugation or application of lower body negative pressure (LBNP), or physical exercise.

Orthostatic tolerance

Even short-term dry immersion (for 7 h) decreases orthostatic tolerance, and as the period of immersion is prolonged, the number of intolerant subjects increases. Thus, after dry immersion for 24 h, approximately one-eighth of healthy subjects are intolerant on R + 1 (Eshmanova 2009; Vinogradova et al. 2002a). After 1.5–3 days of dry immersion, the figure is approximately one-sixth (Iwase et al. 2000; Shul'zhenko et al. 1984), and after 7 days of dry immersion, approximately one-third of the subjects are intolerant (Eshmanova 2009; Iarullin et al. 1987). An increase in heart rate on orthostasis after dry immersion was shown by all the studies cited above. Table 1 gives details of data in the literature on the orthostatic response after dry immersion for different durations, taking into account the type of antiorthostatic test used. To imitate the return to Earth after a space mission, +Gz acceleration has been widely used. The published data on tolerance to centrifugation after dry immersion for different durations are summarized in Table 2. In general, in the absence of countermeasures, after 3 days of dry immersion, tolerance to +3G acceleration is reduced by approximately 20%, and after 7 days by 50–60% (Vil-Viliams et al. 2001). Iarullin et al. (1987), who studied the same six subjects during 7 days of dry immersion and 7 days of head-down bed rest, found that cardiovascular deconditioning (as estimated by orthostatic tolerance to the tilt test) occurred to the same extent under both conditions, despite more profound hemodynamic changes after dry immersion.

Exercise capacity (and maximal oxygen consumption)

Maximal oxygen consumption ($\dot{V}O_2$ max), which is the maximal capacity to transport and use oxygen during incremental exercise, is an indicator of physical fitness. To estimate $\dot{V}O_2$ max, graded exercise tests (treadmill or cycle ergometer) with maximal or submaximal loading are used. Other parameters that characterize exercise capability, and which are analyzed during dry immersion, are cardiac load index [(working heart rate − resting heart rate)/working heart rate], oxygen pulse (oxygen consumption per beat, ml/beat), $\dot{V}O_2$ peak, oxygen ventilatory equivalent ($\dot{V}_E/\dot{V}O_2$), stroke volume, delta heart rate, and total workload. The published data on *exercise capacity* after dry immersion for different durations are summarized in Table 3. A decline in the adaptive ability to exercise has been observed after dry immersion for just 1 day (Beliaev

Table 3 Summary of published data on exercise capacity after dry immersion

Reference	Duration of dry immersion	Number of subjects (men)	Test type	Results
Beliaev (1981)	24 h	8	Cycle ergometer, submaximal load	Increased heart rate (11.4%) and cardiac load index (14%), and decreased oxygen pulse (16.3%) and systolic volume (5.8%), suggesting a decline in the adaptive ability to exercise after just 1 day of dry immersion
Shul'zhenko et al. (1983a)	3 days	7	Cycle ergometer, submaximal load 15 min	13–18% decrease in $\dot{V}O_2$
Stoida and Vinogradova (1996), Vinogradova et al. (2002b)	3 days	6	Test-108 (graded walking and jogging)	Increased heart rate (178 bpm during exercise before dry immersion vs. 191 bpm after dry immersion) and increased oxygen ventilatory equivalent (29.3 before vs. 33.5 after); $\dot{V}O_2$ did not change; i.e. the heart and the respiratory system worked more intensively
Sonkin et al. (1996)	4 and 7 days	5	Two-load cycle ergometer test up to exhaustion	Tendency for heart rate to increase and $\dot{V}O_2$ to decrease, and significant decrease in lung ventilation, suggesting increased physiological cost of exercise
Anashkin and Beliaev (1982)	7 days	6	Cycle ergometer, maximal load	8.7% decrease in total volume of work (from 13,450 to 12,283 kg m) and 11.4% decrease in $\dot{V}O_2$max

1981). With 3–7 days of dry immersion, the metabolic cost of exercise increases, and $\dot{V}O_2$ max decreases by 10–18% (Anashkin and Beliaev 1982; Shul'zhenko et al. 1983a; Sonkin et al. 1996; Stoida and Vinogradova 1996; Vinogradova et al. 2002b). A comparable decrease in exercise capacity is observed after head-down bed rest and space flight. During bed rest, the value of $\dot{V}O_2$ peak decreases rapidly the first 3–6 days, and then declines more gradually, being reduced by approximately 22% after 30 days of bed rest (Fortney et al. 1996). After 2 weeks of space flight, the intensity of submaximal upright exercise at a given heart rate decreases by approximately 20% (Watenpaugh and Hargens 1996).

Resting heart rate and blood pressure

After the onset of dry immersion, acute changes in heart rate and blood pressure are not always significant when compared with the supine baseline values (Fig. 3). Furthermore, most authors have not found significant changes in resting heart rate (Fig. 9) or resting blood pressure at the end of 3–7 days of dry immersion (Bravyi et al. 2008; Eshmanova 2009; Iwase et al. 2000; Mikhailov et al. 1995; Navasiolava et al. 2010a, b; Vil'-Viliams and Shul'zhenko 1978). Some authors found a 15–20% increase in diastolic pressure after 7 days (Vil'-Vil'iams and Shul'zhenko 1980) and 10 days (Panferova 1976) of immersion. Similarly, during head-down bed rest, resting blood pressure does not vary significantly. An increase, decrease or no change have all been reported (Fortney et al. 1996; Pavy-Le Traon et al. 2007). Resting heart rate does not change or increases slightly, depending on the duration of the bed rest (Pavy-Le Traon et al. 2007; Traon et al. 1995). During space flight, systematic 24-h recording of heart rate and blood pressure for 5–10 days has shown a 7% decrease in resting heart rate, a significant decrease in diastolic pressure, and a tendency for systolic pressure to diminish (Fritsch-Yelle et al. 1996).

Cardiac morphology and functions

To our knowledge, the cardiology studies performed so far during dry immersion have focused on the regulation of cardiac function and on electrophysiological parameters (Eshmanova 2009; Eshmanova et al. 2009; Kabulova and Eshmanova 2008). The amount of directly acquired data on cardiac characteristics, such as size, morphology, mass, and contractility, during dry immersion remains insufficient. We only know of one such study, with dry immersion for 3 days, which revealed a slight diminution in the diameter of the left atrium, and an increase in the diameter

of the left ventricle of 18% on day 1, and of 11% on days 2 and 3 (Atkov and Bednenko 1992). As a consequence, we could postulate that dry immersion induces changes similar to those that occur during head-down bed rest or space flight. During head-down bed rest and space flight, the size of the heart is known to decrease, due to hypovolemia and ventricular remodeling/cardiac atrophy (Leach Huntoon et al. 1998; Pavy-Le Traon et al. 2007). During dry immersion, there is an initial large increase in stroke volume and cardiac output, which lasts for the first few minutes and is followed by a significant 16–30% decline by days 2–3 (Atkov and Bednenko 1992; Fomin et al. 1985; Iarullin et al. 1987; Shulzhenko et al. 1980). There is a tendency for stroke volume to recover by day 7 (Fomin et al. 1985; Shulzhenko et al. 1980), although it can still remain decreased by 12–14% (Iarullin et al. 1987; Vil'-Vil'iams and Shul'zhenko 1980) (Fig. 9). Similarly, during space flight and head-down bed rest, stroke volume initially increases and then decreases as the circulating blood volume is reduced (Prisk 2000). Eshmanova et al. (2009) applied dispersion electrocardiogram (ECG) mapping (the method described for example by Sula et al. 2007 and Cruz-Gonzalez et al. 2009), which is based on the analysis of natural small oscillations in the amplitude of the PQRST complex with calculation of the "myocardial" index (integral index of all microalternations). These authors showed that dry immersion causes a sequence of electrophysiological shifts, first in the ventricles and then in the left atrium. Some deterioration of myocardial electrophysiological properties occurs from the first day of dry immersion.

Cardiac problems

No systematic disturbances of cardiac rhythm have been observed during dry immersion. However, some individual cases have been mentioned. For example, one subject developed paroxysmal tachycardia after 23 h of dry immersion (Vil'-Viliams and Shul'zhenko 1978). As for the response to gravitational load, Vil'-Viliams and Shul'zhenko (1978) reported that the number of extrasystolic arrhythmias provoked by +3Gz acceleration after 3 or 13 days of dry immersion was 4–6 times greater, and that more severe arrhythmias—repetitive premature ventricular beats—developed, than in controls. Similarly, during space flight and bed rest no systematic increase in the risk of arrhythmias was observed. No clinically relevant ECG changes have been reported (Pavy-Le Traon et al. 2007; Watenpaugh and Hargens 1996). Nevertheless, during extra-vehicular activity in open space, on the surface of the moon and during functional testing, the number of arrhythmias increases (Egorov 1996). With long-term space flight, changes in the conduction system of the heart are suspected that might possibly provoke arrhythmias (Mikhailov et al. 2001). The question of a possible cardiac risk in very long-term space flight remains unanswered (Navasiolava and Custaud 2009).

Vascular morphology and function

Arterial level

With 7 days of dry immersion, Iarullin et al. (1987) suspected, after indirect measurement, that the vascular muscle tone of large and medium diameter arteries had decreased. However, direct analysis of the vascular wall with ultrasound has not yet been performed for dry immersion. Linear blood velocity in the arteries of the head and lower extremities was slowed down during dry immersion for 7 days (days 2 and 5 of dry immersion) with recovery on R + 2 (Moreva 2008). This decrease in blood velocity is probably the result of the overall lowering of cardiovascular stimulation due to physical inactivity and muscle atonia.

Microcirculatory level

Total peripheral resistance is an integrative characteristic of the overall resistance of the peripheral vasculature in the systemic circulation. During most head-down bed rest studies, total peripheral resistance is unchanged (Arbeille et al. 1999; Shoemaker et al. 1998) or increased (Pavy-Le Traon et al. 2007). After 9–14 days of space flight, total peripheral resistance did not change relative to that of a supine control on the ground (Buckey et al. 1996b). However, most studies have reported an increase in total peripheral resistance during dry immersion (Fomin et al. 1985; Shulzhenko et al. 1980; Vil'-Vil'iams and Shul'zhenko 1980). With respect to regional circulation, increased resistance with decreased blood flow at the level of the calf was observed at the end of 7 days of dry immersion (Iarullin et al. 1987). Stoida and Vinogradova (1996), Stoida et al. (1998), and Vinogradova et al. (2002b) observed a diminution in the blood supply to working muscles after 3 or 7 days of dry immersion. After 7 days of dry immersion, the maximal blood flow at the level of the calf (as registered with plethysmography) was diminished slightly (by 7%). The authors surmised that the probable reasons are structural changes in the vascular bed that lead to a decrease in perfusion capacity. Endothelial functions are impaired by dry immersion and might contribute to changes in perfusion. Seven days of dry immersion induced endothelial dysfunction with reduced endothelium-dependent vasodilation at the skin level and an increase in circulating endothelial microparticles. Furthermore, plasmatic vascular endothelial growth factor decreased significantly during dry

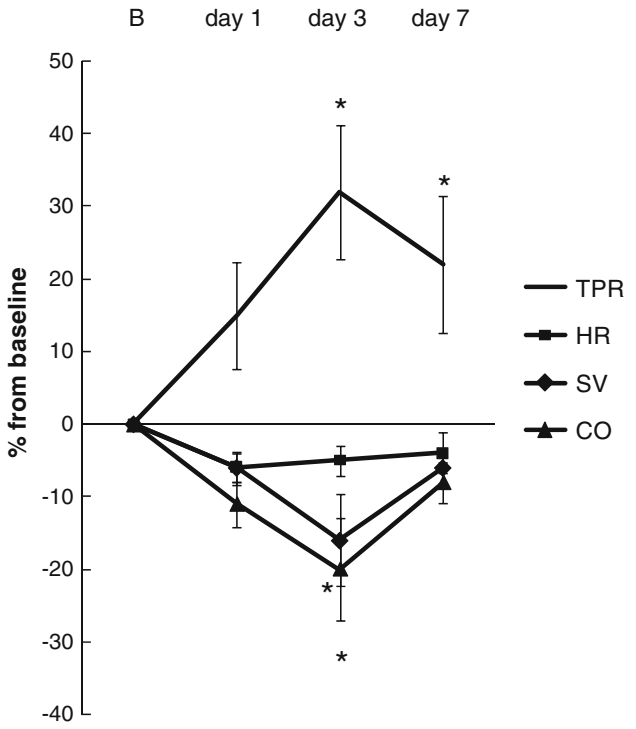

Fig. 9 Variations in cardiovascular parameters during 7 days of dry immersion: heart rate (*HR*), stroke volume (*SV*), cardiac output (*CO*), total peripheral resistance (*TPR*), mean ± SEM; $*p < 0.05$ (adapted from Shulzhenko et al. 1980)

immersion whereas soluble E-selectin was unchanged, which indicated a decrease in antiapoptotic tone rather than endothelial activation (Navasiolava et al. 2010a).

Venous level

Venous properties during space flight have been studied since the beginning of the space era. Increased leg venous compliance with increased pooling of blood in the lower body during standing is thought to be one of the reasons for orthostatic intolerance. Acute immersion immediately reduces venous tone; this reduction is maintained for at least 1 h after 3 h of immersion (Greenleaf 1984). Increased calf venous compliance after dry immersion is well documented (Gogolev et al. 1980; Grigor'ev et al. 2004; Popov et al. 2003; Vinogradova et al. 2002a). A 7% increase in calf venous compliance was observed by Vinogradova et al. (2002a) as early as after the first 7 h of dry immersion. Gogolev et al. (1980) observed increased compliance of leg tissues in response to venous occlusion with the thigh cuff up to 50 mmHg (pressure normally observed in the calf muscle when standing quietly) on day 1 and, to a lesser extent, on day 3 of dry immersion, whereas head-down bed rest did not cause any change in these parameters. A similar increase in leg volume in response to occlusion was observed by Thornton during space flight and was explained by hypohydration in the lower body (Gogolev et al. 1980; Nicogossian et al. 1993).

Autonomic regulation of cardiovascular functions

The effects of actual and simulated microgravity on the autonomic neural system have been studied extensively, because sympathetic neural control is extremely important for the maintenance of blood pressure homeostasis against gravity (Mano 2005). The first 12 h of dry immersion induces a decrease in sympathetic and an increase in parasympathetic modulation (Eshmanova 2009). With prolonged dry immersion the response is reversed.

At rest

After 3 days of dry immersion, an alteration of the autonomic balance towards the sympathetic dominant state was reported by Miwa et al. (1997) from a study of variability of heart rate and blood pressure, and by Iwase et al. (2000) from a study of muscle sympathetic nerve activity. Similar findings were also reported with 7 days of dry immersion by Eshmanova et al. (2008) from a study of heart rate variability. In the studies of Miwa et al. and Iwase et al., the testing was performed after the end of dry immersion, with the subject out of the water tank in a supine position on the tilt bed. Eshmanova recorded the data both during dry immersion at days 2, 4 and 6, and after dry immersion at R + 2, during supine stand-test measurements. During dry immersion, there was a tendency for the stress index [which characterizes the activity of sympathetic regulation and load of regulatory systems (Baevsky 1979)] to increase and for pNN50 (percentage of adjacent cardiac cycles differing by more than 50 ms, which characterizes the vagal modulation of heart rate) to decrease at days 4 and 6. The concentration of epinephrine and its metabolites in the blood and urine increases during 7 days of dry immersion, whereas the levels of norepinephrine and its metabolites diminish, which suggests that hormonal activity increases and the transmitter activity of the sympathetic system decreases during dry immersion (Davydova et al. 1981). During the post-dry immersion recovery period, the level of catecholamines and their metabolites in the blood (Davydova et al. 1981) and urine (Davydova et al. 1981; Navasiolava et al. 2010b) increases, and is normalized by R + 5 (Davydova et al. 1981).

In response to tilt/stand

Muscle sympathetic nerve activity in response to tilt is unchanged after 3 days of dry immersion, although

increased tachycardia is observed in response to tilt (Iwase et al. 2000). Blood pressure variability in response to tilt is unchanged after 3 days of dry immersion. However, for heart rate variability, the decrease in HF (high frequency band of the spectrum; an indicator of cardiac vagal modulation) and the increase in LF/HF (the ratio of low-to-high frequency spectral power; an indicator of sympatho-vagal balance) are more pronounced than at baseline (Miwa et al. 1997). Similarly, after 7 days of dry immersion, increased activation of the sympathetic nervous system in response to standing is observed, as shown by an increase in the stress index (Eshmanova et al. 2008).

Altogether, most of these data suggest that dry immersion induces increased activation of the sympathetic nervous system.

For head-down bed rest, an increase in resting sympathetic modulation was also observed during (day 6) and after 7 days of bed rest in a study of heart rate variability (Baevsky et al. 2004). In addition, an increase in resting muscle sympathetic nerve activity was observed after 6 (Kamiya et al. 2000a), 14 (Kamiya et al. 2000b) and 120 days of head-down bed rest (Kamiya et al. 2000c). However, for blood and urinary catecholamines, the data are contradictory. During 42 days of head-down bed rest, urinary catecholamines decreased (Sigaudo et al. 1998). Nevertheless, it was recently reported that plasma catecholamines increased during 20 days of head-down bed rest (Kanikowska et al. 2008).

With respect to space flight, during the first 5–10 days, sympathetic modulation is lowered as suggested by reductions in in-flight awake heart rate and diastolic pressure (Fritsch-Yelle et al. 1996), but with prolonged exposure to microgravity, sympathetic neural traffic seems to increase. This increase is implied by an increase in noradrenalin spillover and clearance (Ertl et al. 2002), and unchanged or even increased muscle sympathetic nerve activity during and after flight (Cox et al. 2002; Ertl et al. 2002; Fu et al. 2002; Levine et al. 2002). The results of assessment of heart rate variability (Cooke et al. 2000) suggest that cardiac vagal modulation is reduced by prolonged space flight. Interestingly, spectral analysis of heart rate variability after a long-term flight suggested higher parasympathetic modulation of the RR interval in the supine position in non-finishers, who failed to remain in the standing position during the orthostatic test, and the predominance of sympathetic modulation in finishers (Sigaudo-Roussel et al. 2002).

Proteins, lipids and carbohydrate metabolism

Models of microgravity are very useful for understanding the effects of physical inactivity on metabolism. Compared to bed rest, very few studies devoted to metabolic changes have been undertaken during dry immersion.

Weight changes

After 3 days of dry immersion, weight loss of approximately 1 kg (1.3%) has been reported (Iwase et al. 2000). Larina et al. (2008) have reported a weight decrease of appproximately 2 kg, or 3%, by the end of a 7-day period of dry immersion. Dry immersion always induces weight loss, mainly due to increased fluid loss, and also through decreased fluid intake due to diminished thirst (Grigor'ev and Shul'zhenko 1979). With 56 days of dry immersion, a decrease in caloric intake was observed (the subjects had an ad libitum diet) (Kovalenko and Gurovsky 1980).

Energy expenditure

In addition to total energy expenditure, even the basal metabolic rate decreases during prolonged immersion (as shown for 56 days of dry immersion), as well as during prolonged bed rest (Kovalenko and Gurovsky 1980).

Lipid profile

During 7 days of dry immersion (18 men with borderline arterial hypertension), Kirichenko et al. (1985) reported a significant increase in general blood lipids and nonesterified fatty acids on day 3, followed by recovery on R + 2. More recently, Markin et al. (2008) also found a tendency for the concentrations of total plasma cholesterol and low density lipoprotein to increase, and an increase in triglycerides, although within normal limits. Pakharukova et al. (2009a), in a serum proteome study, reported an increase in apolipoproteins C1 and C3 on day 7 of dry immersion, which suggested complex changes in lipid metabolism.

Glucose metabolism

Blood glucose did not change significantly during 7 days of dry immersion (Markin et al. 2008). However, blood insulin was significantly increased on R + 2 after dry immersion for 7 days (Afonin and Sedova 2009). This favors the hypothesis that insulin resistance develops during dry immersion, similar to that observed during head-down bed rest (Blanc et al. 2000).

Blood and immune systems

In general, real and simulated microgravity causes a decrease in the mass of red blood cells, as well as changes in the physical properties of red blood cells.

Red blood cells

Hemoconcentration during dry immersion is reflected by an increase in the levels of erythrocytes (Kozinets et al. 1983; Navasiolava et al. 2010a), hemoglobin (Kozinets et al. 1983; Larina et al. 2008; Navasiolava et al. 2010a) and hematocrit (Fomin 1981; Gogolev et al. 1980; Iarullin et al. 1987; Larina et al. 2008; Navasiolava et al. 2010a; Pakharukova et al. 2009a) (Fig. 7), although all these values remain within physiological limits. Dry immersion induces only slight changes in the form and function of red blood cells. Over the course of time, a small change in the morphology of erythrocytes occurs (Ivanova et al. 2009; Kozinets et al. 1983). With dry immersion for 7 days, Ivanova et al. observed a trend towards changes in red blood cell morphology (microcytosis), and a decrease in erythropoiesis (as evidenced by an increase in serum iron and a tendency for erythropoietin to decrease on day 7). On day 7, oxygen binding in venous blood tended to decrease, oxygen release declined significantly, and the HbO_2/Hb ratio increased significantly. These changes might promote hypoxia at the cellular level (Ivanova et al. 2009).

Coagulation system and viscosity

No cases of thrombosis have been reported during dry immersion experiments, although hemoconcentration, the redistribution of body fluids, hypodynamia, and possible endothelial impairment might increase the risk of thrombosis. Similarly, no evidence of thrombosis was observed in subjects who underwent bed rest, although it can clinically be expected in bedridden patients (Adams et al. 2003; Fortney et al. 1996). An increase in the viscosity of the blood was observed after just 24 h of dry immersion, with a further increase by day 7 (Ivanov et al. 1983). Seven days of dry immersion induced a significant increase in the number of platelets and hypercoagulopathic changes to platelet hemostasis (Kirichenko et al. 1985, 1988), a change in hemostatic factors (Kirichenko et al. 1988), and moderate fibrinogenemia (Fomin 1981).

Immune system

Both short-term and long-term space flights modify innate immunity, which presumably reflects an acute response to the stress of landing. Prolonged flights induce a remarkable depression in immunological function, affecting both innate and adaptive immunity (Rykova et al. 2008). For dry immersion protocols, on the whole, the relatively short duration of dry immersion (mostly 7 days) seems insufficient to cause significant alterations in the immune system.

Surface barriers

Exposure to dry immersion influences the commensal microflora. Significant dysbacteriosis and changes in mouth–throat and intestinal flora were observed after 7 days of dry immersion, which is natural for long-term isolation in a confined habitat (Il'in et al. 2008a, Ilyin et al. 2008b). A decrease in gut lactoflora after dry immersion for 7 days was observed in 9 out of 10 subjects (Il'in et al. 2008a). A study of cutaneous and nasal microflora revealed an increase in staphylococci, gram-negative bacilli, and yeasts, which confirms the increased risk of inflammatory diseases (Il'in et al. 2008a).

Peripheral leukocytes in general: distribution and function

Seven days of dry immersion either do not change the number of leukocytes (Kozinets et al. 1983; Navasiolava et al. 2010a), or increase it (Berendeeva et al. (2009) found a 40% increase). The leukocyte differential count remains unchanged (Berendeeva et al. 2009; Kozinets et al. 1983). In general, leukocyte functions appear unaltered by 7 days of dry immersion (Kozinets et al. 1983).

Innate immunity

Complement system Proteome studies have revealed that the complement fragment C4a increases during dry immersion for 7 days (Pakharukova et al. 2009b; Pastushkova et al. 2010).

Cytokines Dry immersion might induce changes in the functioning of the cytokine network, as shown by an increase in the production of proinflammatory cytokines by mononuclear cells in response to in vitro lipopolysaccharide stimulation after dry immersion for 7 days (Berendeeva et al. 2009).

Natural killer cells and phagocytes No change in the number of natural killer cells (Berendeeva et al. 2009) or in the cytochemistry of granulocytes was found during 7 days of dry immersion (Kozinets et al. 1983).

Adaptive immunity

Lymphocytes Berendeeva et al. 2009 found that the populations of T and B cells increased at day 7 of dry immersion, with recovery at R + 7. However, decreased expression of the early activation marker was reported in response to pokeweed mitogen, which might indicate the functional impairment of the cell-mediated immune response after 7 days of dry immersion (Berendeeva et al. 2009).

Immunoglobulins No significant changes in serum IgA, IgM, and IgG were found (Berendeeva et al. 2009).

Other functions

Respiration

Some studies have focused on the respiratory system during dry immersion (Genin and Galichii 1995; Golovkina 1982; Kamenskii et al. 1976; Tikhonov et al. 1983). In these studies, only minor changes were found. No increase in the number of non-ventilated alveoli nor any development of atelectasis or edema of the lungs was detected during dry immersion (Atkov and Bednenko 1992). The pressure of the water on the abdominal and thoracic areas creates additional, though not significant, respiratory load. The intrapulmonary pressure remains equal to atmospheric pressure, whereas the external pressure on the thorax increases in proportion to the depth of immersion, which practically replicates respiration under negative pressure [which also favors a headward fluid shift (Tikhonov et al. 1983; Park et al. 1999)]. The moderate changes observed are concerned with decreases in respiratory volume, vital lung capacity, maximal pulmonary ventilation, and breathing retention time during inhalation and expiration, which occur during the acute period (the first 1–3 days, and especially the first few hours) of adaptation to dry immersion (Golovkina 1982; Kamenskii et al. 1976). It is worth reiterating here that during the initial period of dry immersion the subjects experience muscle discomfort (backache, lumbar and chest pain), which is aggravated by deep respiration, and especially by attempts at maximal ventilation. As a consequence, the subjects naturally limit the depth and intensity of their respiration, which could affect the measured indices (Kamenskii et al. 1976). By day 5 of dry immersion, most of the respiratory indices are restored; however, the functional capabilities of external respiration (response to functional tests) remain significantly diminished, as shown with dry immersion for 13 days (Kamenskii et al. 1976).

Thermoregulation

The question of thermoregulation is important for space flight. With respect to thermoregulation, heat is lost through convection (which under normal conditions accounts for 15–30% of total heat loss), conduction (2–5%), radiation (45–55%), and evaporation (20–25%). Convective heat transfer is possible due to gravity (as warmed air becomes lighter and rises, it makes way for cooler air, which can be warmed in turn); therefore, it is impaired under microgravity (Gunga et al. 2009). Evaporation by perspiration has not been studied sufficiently during space flight; nevertheless, it is assumed that the formation of a film of sweat that covers the entire body might inhibit further perspiration (Larina and Lakota 2000). Actual and simulated microgravity seem to decrease heat conduction from the core to the periphery by inhibiting peripheral vasodilation. This has been shown by the acceleration of the rate of increase and the final core temperature during exercise after 6 h of thermoneutral "wet" immersion, as well as after prolonged head-down bed rest and space flight (Greenleaf 1997). Theoretically, dry immersion conditions could diminish convection, conductive heat loss would depend on water temperature, radiation would be unchanged, and evaporation would be disrupted by the use of plastic film. Surprisingly, very few dry immersion studies on thermoregulation have been performed. However, one study on suit immersion has shown that mean skin temperature and foot temperature falls, whereas rectal temperature does not change; thus, "core-to-shell" and "chest-to-foot" temperature gradients increase (Lakota and Larina 2002). Moreover, under suit immersion, the heat is redistributed headwards (similar to body fluids) and this transfer occurs ten times faster than under head-down bed rest (Lakota and Larina 2002).

Dry immersion: applications

Fundamental context

Gravity is a universal and omnipresent force; it has fashioned organisms throughout millions of years of evolution. The experience of space flight has revealed the crucial role of gravity with respect to health. Daily gravitational stimulation appears to be necessary to maintain good health and fitness, as well as the body's adaptation to gravity. Dry immersion, by diminishing the effects of gravity, provides an approach for studying the influence of gravity under normal conditions (how we adapt to gravity), and in pathology.

Space context: test of countermeasures

The dry immersion model improves our understanding of the mechanisms of microgravity-induced changes and allows countermeasures and combinations of such measures to be developed and tested in order to prevent deconditioning in astronauts after their return to Earth. The following have all been tested using dry immersion as a model of microgravity: periodic acceleration (Clement and Pavy-Le Traon 2004; Grigor'ev and Shul'zhenko 1979; Shulzhenko et al. 1977; Vil-Viliams 1994; Vil'-Vil'iams and Shul'zhenko 1980), the antigravity suit (Shul'zhenko et al. 1983b; Vil-Viliams et al. 1996), cycle ergometer

training (Vil-Viliams 1994; Vil'-Vil'iams and Shul'zhenko 1980), electromyostimulation (Eshmanova 2009; Eshmanova et al. 2008, 2009; Koryak et al. 2008; Kozlovskaia 2008; Ogneva et al. 2009), water–salt supplements (Kokova 1984; Shulzhenko et al. 1977), the use of thigh cuffs and the simultaneous use of thigh and forearm cuffs (Atkov and Bednenko 1992), the compensator of support unloading by mechanic stimulation of foot support zones (Grigor'ev et al. 2004; Khusnutdinova et al. 2004; Kozlovskaya 2002; Kozlovskaya et al. 2007a, b; Kornilova et al. 2004; Layne and Forth 2008; Mel'nik et al. 2006; Moukhina et al. 2004; Netreba et al. 2005, 2006; Popov et al. 2003; Shenkman et al. 2004a, b; Vinogradova et al. 2002a), and pharmacological remedies [the stimulant sydnocarb, *N*-phenylcarbamoyl-3-(beta-phenylisopropyl)sydnonimine (Anashkin and Beliaev 1982), amlodipine (sarcolemmal L-Ca^{2+} channel blocker; Eshmanova et al. 2008), peroral dry lactobacterin, topical collagen-immobilized lactobacterin (Kriukov et al. 2007; Il'in et al. 2008a), and probiotics based on lactobacilli auto-strains for dysbacteriosis prophylaxis (Ilyin et al. 2008b)].

Dry immersion principles have been applied effectively to the design and construction of a new anti-G protective suit for pilots proposed by Barer et al. (Zvezda Aerospace medicine Department; Barer 2007). In addition, dry immersion was one of the models used to develop objective formalizable physiological indices of working capacity that were suitable for reliable algorithmization of the control of the physical state, in order to create a future on-board autonomous computer expert system (Sonkin et al. 1998).

Healthcare context

Cardiology, nephrology

Dry immersion has been used successfully to treat edema, especially refractory edema, of different etiologies (cardiovascular diseases, burns, edemas caused by liver cirrhosis and by renal pathologies). In patients with edema, the 1.5- to 3-fold increase in diuresis observed after a short-term dry immersion procedure (4–6 h) remained at that level for 2 days (Iunusov et al. 1985; Ivanov and Bogomazov 1988). Dry immersion has been proposed as therapy for treatment-resistant hypertensive crisis (Ivanov and Markova 1990) and shows a rapid, prolonged hypotensive and sedative effect after 1.5 h (or less) of dry immersion.

Pediatrics

Dry immersion could be especially valuable when included in the complex of rehabilitative measures for premature infants who arrive before term and are exposed early to gravity from the intra-uterine environment. Improvements in neurological status and stabilization of hemodynamics have been observed. Dry immersion is used in the recovery treatment of infants and young children with malfunctions of the central nervous system (perinatal encephalopathy; Kazanskaya 2008). Exposure to dry immersion might help to prevent the development of cerebral palsy, diminish muscle hypertonia and hyperactivity, and facilitate the disappearance of primitive reflexes (labyrinthine, cervical; Yatsyk 2002).

Neurology

Dry immersion is proposed for rehabilitation for diseases associated with spastic symptoms (cerebral palsy, multiple sclerosis or other neuromuscular diseases) because of the rapid fall in muscle tone during dry immersion: after 2 h, the body becomes "plasticine (play-dough)-like", ready for massage and other physiotherapeutic procedures. Dry immersion might also be useful in revealing hidden compensated neurological disturbances. In young healthy subjects who presented with no neurological symptoms before experimentation, different neurological signs (such as symptoms of disintegrated cerebellar function and signs of peripheral neural function deterioration, as well as signs of posterior tract lesions and pyramidal, extrapyramidal, and frontal cortex signs) appeared after 24, 48, and 72 h of dry immersion (Berger et al. 2001; Gerstenbrand and Kozlovskaya 1990). The hypothetical explanation for this phenomenon is that almost all individuals suffer some functional brain disbalance, but due to brain plasticity its consequences are usually fully compensated, and it is hidden during normal life. Altered support and proprioceptive afferentation during dry immersion deeply alters the body scheme, a conscious representation of sensory–motor aspects of the body, which disrupts the compensation and thus reveals hidden neurological disturbances.

The mechanical support stimulator ("medical boots") developed and tested at the Institute of Biomedical Problems using the dry immersion method was one of the first pieces of "space" equipment adopted by neurological clinics to apply mechanical stimulation to the foot support zones in the same way as during slow or fast walking. It is useful in cases of cerebral palsy in children, ischemic stroke, and pathologies associated with long-term immobilization (Miller et al. 2005; Yatsyk 2002).

It should be noted that there are quite a few contraindications to the clinical application of dry immersion, which include myocardial infarction, severe arrhythmias, chronic respiratory diseases with decompensated cor pulmonale, thrombophlebitis, significant resting dyspnea (Ivanov and Markova 1990), acute inflammation, and generalized skin diseases (Yatsyk 2002).

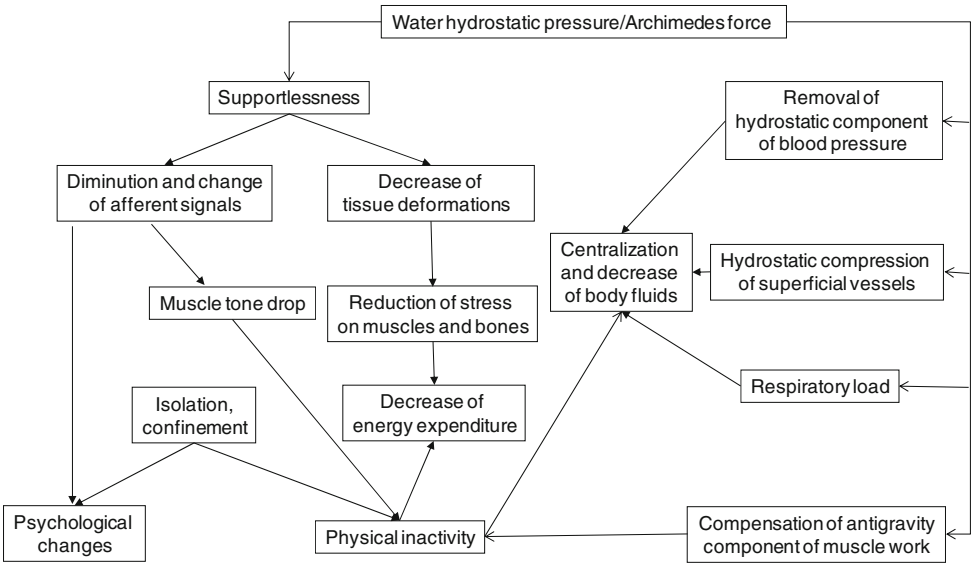

Fig. 10 Schematic diagram of changes induced by dry immersion

Examples of benefits for healthy people

Short-term dry immersion could be valuable for endurance athletes after heavy training (Radzijewska and Radzijewski 2007). Short-term dry immersion is both easy to apply and therapeutically promising. It might also be beneficial as a comfortable, quick, and profound relaxation method. In spa therapy, it is known as dry floatation.

Conclusion

The results of extended investigations have shown that long-term dry immersion can be regarded as one of the most satisfactory models of the physiological effects of microgravity. In addition to the monotonous environment, posture–motion limitations, hemodynamic changes, and hypokinetic effects (which are all modeled already by head-down bed rest), dry immersion also mimics other aspects of space flight: support unloading and decreased proprioceptive input (Fig. 10). This support unloading itself induces very significant physiological changes. Under conditions where there is a lack of support, muscle tone diminishes dramatically, especially the tone of antigravity muscles. Dry immersion enables subjects to be exposed to the most enhanced level of physical inactivity, and induces rapid gravitational deconditioning with quick re-establishment during the recovery period. Simulation by dry immersion can be used to design and validate countermeasures against muscle impairments that occur in microgravity and hypokinesia, and to conduct pharmacological studies under simulated microgravity.

Currently, the medical world is cautiously testing the possibility of using changes in gravitation as a therapeutic measure. Broad applications of short-term dry immersion can be proposed in pediatrics for the rehabilitation of premature infants, in cardiology and nephrology for the treatment of hypertension and edemas, in neurology to detect hidden disturbances and relieve muscle spasms, and also for relaxation.

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Conflict of interest The authors declare that they have no conflict of interest.

Appendix: Variations of English transcription of the names of Russian authors cited in this paper

Baevsky, Baevskiĭ RM
Chernicova, Chernikova AG
Grigor'ev, Grigoriev, Grigoryev AI
Grigor'eva, Grigorieva LS
Il'in, Ilyin VK
Kirenskaia, Kirenskaya AV
Koriak, Koryak, Korijak Iu(Yu, Y)A
Kozlovskaya, Kozlovskaia, Kozlovskaja IB
Kreidich IuV, Kreydich YV
Mel'nik, Melnik KA
Shul'zhenko, Shulzhenko EB
Tomilovskaia, Tomilovskaya ES

Vil-Vilyams, Vil-Viliams, Vil'-Viliams, Vil'-Vil'iams, Will-Williams IF

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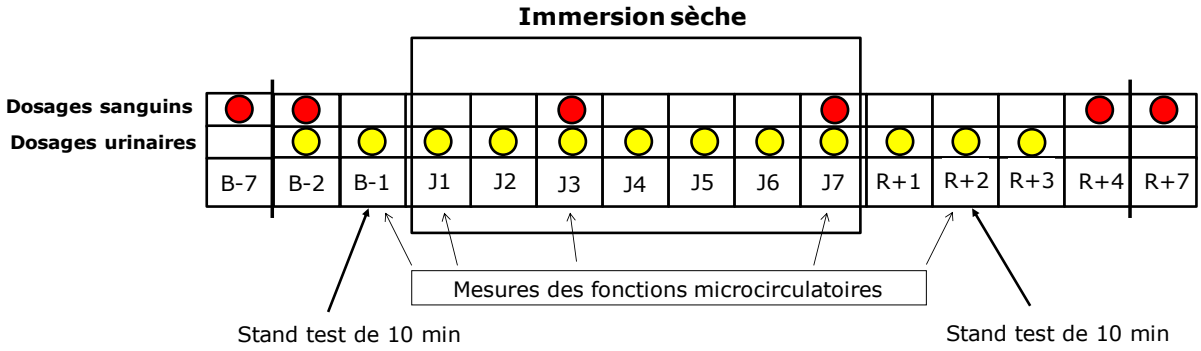
B1. ETUDE DES MODIFICATIONS CARDIO-VASCULAIRES INDUITES PAR L'IMMERSION SECHE :

PRESENTATION DE NOTRE PROTOCOLE

L'étude comportait un échantillon de 8 hommes volontaires sains, dont l'âge s'échelonnait entre 20 et 25 ans, la taille entre 1m 65 et 1m 87, et le poids entre 56 et 94 kg. Tous les sujets restaient au sein de l'Institut pendant 11 jours : 2 jours de contrôle avant l'immersion sèche (B-2 et B-1), suivis de 7 jours d'immersion (J1 – J7) et de 2 jours de récupération (R+1 et R+2). De plus, un prélèvement sanguin chez chaque sujet était pratiqué 7 jours avant le début de l'immersion (B-7) et 4 jours après la fin de l'immersion (R+4). Les sujets ont été immergés l'un après l'autre.

Le chronogramme de nos études est présenté sur la Fig.14**.**

Figure 14. Chronogramme des études



Les axes principaux de notre recherche étaient :

- L'état de l'endothélium et des fonctions microcirculatoires,
- La réponse cardio-vasculaire à l'orthostatisme,
- Le métabolisme hydro-sodé et sa régulation hormonale.

B2. ETUDE DES MODIFICATIONS CARDIO-VASCULAIRES INDUITES PAR L'IMMERSION SECHE :

ETAT DE L'ENDOTHELIUM ET DES FONCTIONS MICROCIRCULATOIRES

Les fonctions endothéliales sont rapidement altérées par l'immersion sèche

"ENFORCED PHYSICAL INACTIVITY INCREASES ENDOTHELIAL MICROPARTICLE LEVELS IN HEALTHY VOLUNTEERS"

Navasiolava NM, Dignat-George F, Sabatier F, Larina IM, Demiot C, Fortrat JO, Gauquelin-Koch G, Kozlovskaya IB, Custaud MA. - American Journal of Physiology 299:H248-256, 2010

Commenté : Widlansky ME. The danger of sedenterism: endothelium at risk. Am J Physiol 299:H243-244, 2010

L'immersion sèche, modèle efficace de microgravité, induit rapidement un déconditionnement cardio-vasculaire. Cependant les fonctions endothéliales demeurent assez peu étudiées dans ces conditions. L'étude que nous avons menée avait pour but de rechercher les effets de 7 jours d'immersion sèche chez les volontaires sains sur les fonctions endothéliales.

Les études fonctionnelles étaient effectuées au niveau de la microcirculation cutanée. Nous avons observé une atteinte de la vasodilatation endothélium dépendante en immersion. La vasodilatation endothélium-indépendante tendait à diminuer mais sans significativité statistique. Nous avons observé une augmentation spécifique des microparticules endothéliales, tandis que les taux relatifs des microparticules circulantes d'autres sources cellulaires étaient stables. Le dosage de marqueurs solubles témoignant de l'état de l'endothélium a montré une diminution significative du sVEGF au cours de l'immersion, alors que E-sélectine restait inchangée, indiquant que le mécanisme potentiellement responsable de l'augmentation de microparticules est une répression de la voie anti-apoptotique plutôt que l'activation endothéliale. La dysfonction endothéliale pourrait être une cible thérapeutique pour des atteintes liées à l'inactivité physique et à la microgravité.

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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 ± 6% vs. 12 ± 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.

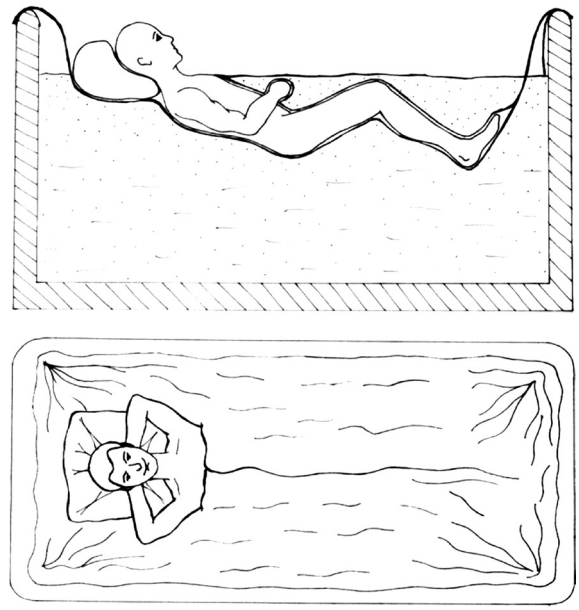

Fig. 1. Dry immersion (DI) method. The subject is immersed up to the armpits and separated from the water by waterproof fabric. *Top*: side view; *bottom*: view from above.

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·kg^{-1}·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·kg^{-1}·day^{-1}, daily calcium intake was adjusted to 1,000–1,150 mg, and protein intake was 1.15 g·kg^{-1}·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.

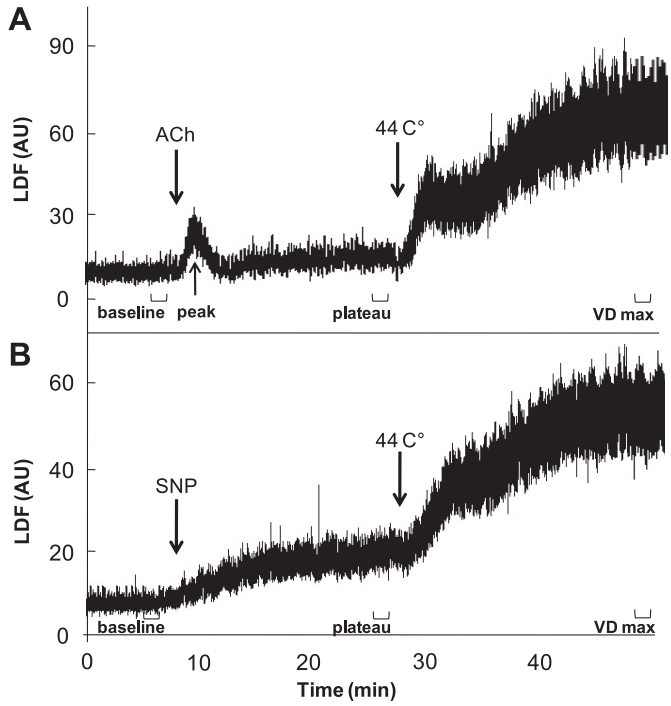

Fig. 2. Examples of laser-Doppler flow [LDF; in arbitrary units (AU)] recordings with the ACh probe (*A*) and sodium nitroprusside (SNP) probe (*B*) at baseline, responses to iontophoresis of ACh (peak and plateau phases) and SNP (plateau), and responses to local heating [maximal vasodilation (VD max)]. 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: $\Delta PV\ (in\ \%) = 100 \times [Hb_{B\text{-}2}(1 - 0.01Hct_i)]/[Hb_i(1 - 0.01Hct_{B\text{-}2})] - 100$, 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 ± 0.11 × 10^{12} erythrocytes/l at baseline vs. 5.5 ± 0.08 × 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 ± 0.6 × 10^9 vs. 6.3 ± 0.5 × 10^9 white blood cells/l and 252 ± 19 × 10^9 vs. 232 ± 21 × 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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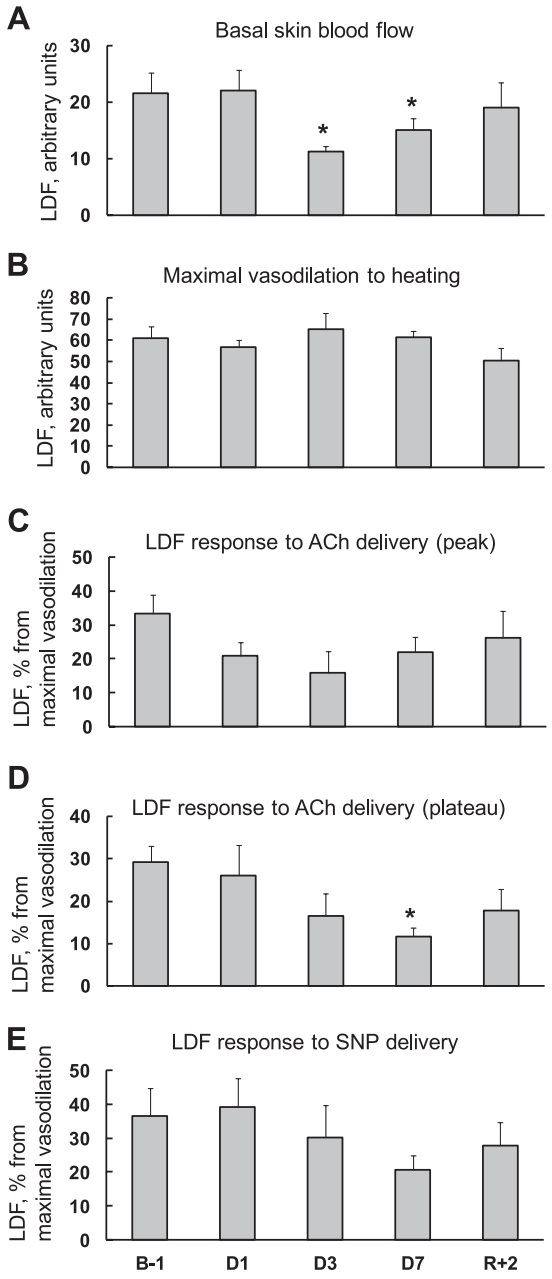

Fig. 3. Effect of physical inactivity induced by 7 days of DI on calf skin blood flow defined by the laser-Doppler technique. *A*: mean basal flow. *B*: flow stimulated by local heating up to 44°C. *C* and *D*: flow stimulated by iontophoresis of ACh on peak (*C*) and plateau (*D*) phases. *E*: flow 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

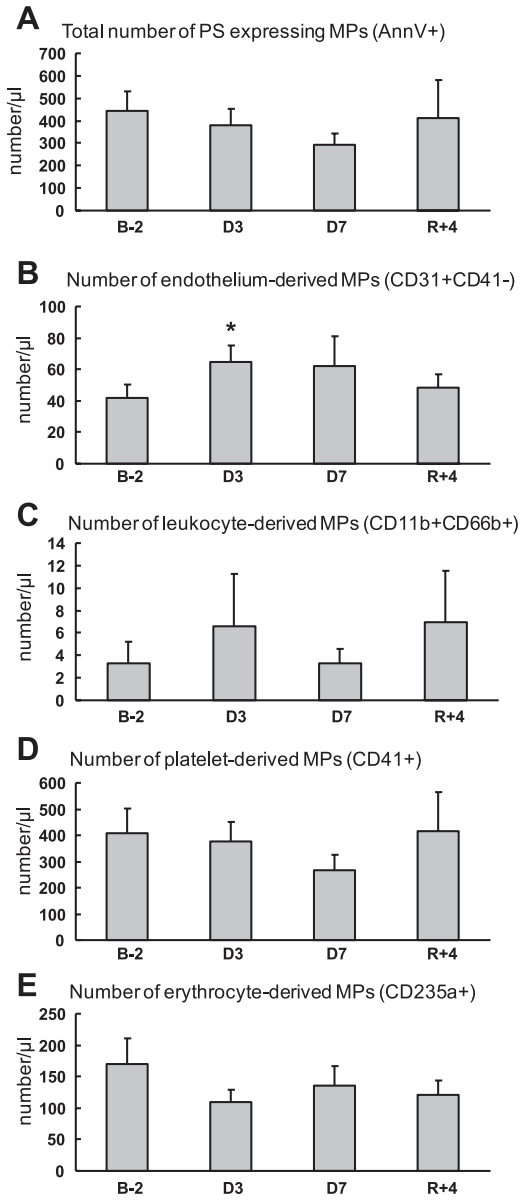

Fig. 4. Numbers of microparticles (MPs) as assessed by flow cytometry before (*day B-2*), during (*days D3* and *D7*), and after (*day R+4*) DI. Values are means ± SE. *$P < 0.05$ vs. *day B-2*.

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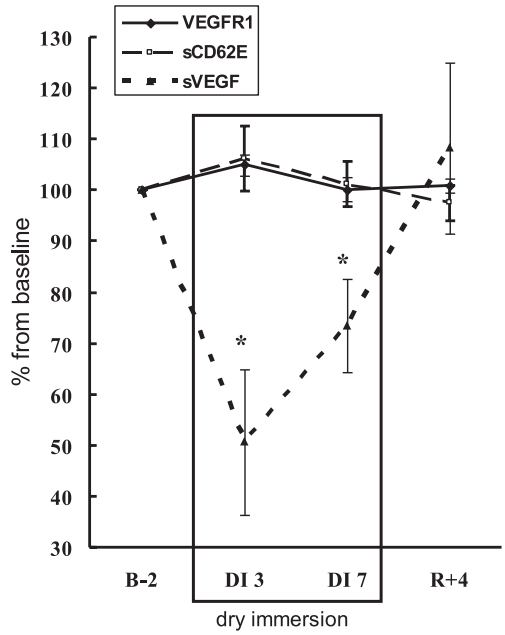

Fig. 5. Variations in soluble endothelial factors in plasma. VEGFR1, VEGF receptor 1; sCD62E, soluble CD62E; sVEGF, soluble VEGF. Values are means ± SE. *Significant difference vs. baseline ($P < 0.05$).

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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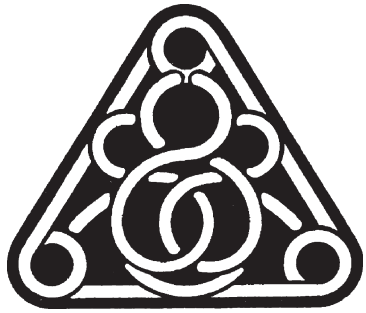

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The danger of sedenterism: endothelium at risk

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HABITUAL PHYSICAL inactivity, otherwise known as sedenterism, is a leading cause of excessive and preventable cardiovascular risk (4). Less than half of U.S. adults met recommended physical activity standards in 2007, and nearly one-quarter of the U.S. population reported no leisure time physical activity in 2008 with rates of sedenterism above 30% for those over the age of 65 (3). While the exact magnitude of the societal cost of sedenterism is difficult to quantify, estimates suggest that the direct medical costs of sedentary individuals are nearly one-third higher than those of their physically active counterparts and likely amounts to at least 100 billion U.S. dollars of excess healthcare costs today (10).

Sedentarism's deleterious mechanisms of action remain unclear. Much of the risk of physical inactivity has been attributed to the onset of obesity secondary to excessive caloric intake relative to energy requirements. Obesity is a well-recognized risk factor for the development of dyslipidemia, systemic inflammation, hypertension, and insulin resistance. In this setting, the vascular endothelium becomes dysfunctional. Endothelial dysfunction is characterized by a proinflammatory, prothrombotic, and vasoconstrictive phenotype and is a known harbinger of adverse cardiovascular events (13). While obesity has been linked to endothelial dysfunction and insulin resistance and physical activity has been shown to improve both measures in at-risk populations, the strong colinearity of sedenterism and obesity significantly complicates attempts to determine the contribution of physical inactivity alone to both endothelial dysfunction and insulin resistance.

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To isolate the potential adverse effects of sedenterism alone, multiple research groups have employed model protocols that enforce strict, acute bed rest on otherwise healthy persons to determine the vascular, hemodynamic, and metabolic consequences of sedenterism (1, 5, 7, 8, 11). The results of these studies are delineated in Table 1. While the conclusions resulting from the comparisons of these studies need to be tempered by their heterogeneity of methods and outcome measurements, these data suggest that strict bed rest as a model of acute physical inactivity results in significant alterations to vascular homeostasis. These alterations appear to be most prominent in vessels feeding the most affected muscle beds and include rapid impairment of microvascular endothelial function, reduction of resting blood flow, and reduced conduit vessel luminal diameter suggesting an increase in resting arterial tone. Furthermore, studies with comparison groups that performed recumbent exercise during the bed rest period consistently report a blunting of adverse effects of strict bed rest on microvascular function and conduit vessel homeostasis (1, 5, 8). Only one of these studies concomitantly evaluated the inflammatory and metabolic impact of severely restricted physical activity (7). In that study, an acute insulin-resistant state developed in parallel with endothelial dysfunction in these otherwise healthy subjects. These data are in agreement with prior work demonstrating insulin resistance due to short-term physical inactivity (12). When examined together, these data also suggest that physical inactivity can acutely impart a metabolic syndrome-like state, even in the absence of obesity.

In this issue of the *American Journal of Physiology-Heart and Circulatory Physiology*, Navasiolava and colleagues (9) employed a novel, "dry immersion" protocol to simulate weightlessness for 7 days in a group of eight healthy volunteers

Table 1. *Strict physical inactivity studies measuring endothelial function*

Study	*N*	Duration of Bed Rest, days	Basal Conduit Vessel	Conduit Vessel Function	Microvascular Function	Blood Pressure	Lipid Profile	Insulin Sensitivity
Shoemaker et al., 1998 (11)	20	14	N/A	N/A	Impaired postischemic hyperemia (10-min occlusion period, arm)	No change in MAP	N/A	N/A
Bleeker et al., 2005 (1)	16	25 and 52	Reduced femoral artery diameter	Increased FMD	N/A	No changes	N/A	N/A
Hesse et al., 2005 (8)	10	13	N/A	N/A	Impaired (forearm)	No change in MAP	N/A	N/A
Hamburg et al., 2007 (7)	20	5	Reduced brachial luminal diameter	No effect on brachial FMD	Impaired by *day 3* and on *day 5* in both forearm and calf	↑ SBP	↑ Triglycerides and total cholesterol	Impaired by *day 5*
Demiot et al., 2007 (5)	16	56	N/A	N/A	Impaired post-bed rest in calf	No change in MAP	N/A	N/A
Navasiolava et al., 2010 (9)	8	7	N/A	N/A	Impaired by *day 7*	↑ DBP on *day 1*	No significant changes (trend increase in triglycerides)	N/A

N, number of subjects; FMD, flow-mediated dilation; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/A, not applicable (data not reported).

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to investigate the effects of extreme physical inactivity on endothelial homeostasis. This protocol involves suspending volunteers in an elastic waterproof film that suspends them in a pool of water, more completely reducing muscle tonic activity relative to bed rest protocols by eliminating support stimuli. Using laser Doppler and iontophoresis, the authors found that basal skin blood flow and microvascular function began to be impaired within 3 days of dry immersion. These data largely confirm prior data from bed rest studies, consistently showing an impairment of microvascular function with strict physical inactivity.

The experimental results involving the measurement of circulating microparticles and soluble biomarkers are novel and provide interesting insight into potential mechanisms behind physical inactivity-induced endothelial dysfunction. While the mechanisms of microparticle formation remain incompletely understood, they appear to originate from blebs in the plasma membrane of endothelial cells, platelets, and leukocytes. Elevations in circulating levels of microparticles represent increased cellular inflammatory activation and/or apoptotic signaling and are associated with increased cardiovascular risk (6). In the case of endothelial cells, both apoptosis and factors also associated with the activation of endothelial cell proinflammatory and prothrombotic phenotypes, including low shear stress, inflammatory cytokines exposure, and excessive oxidative stress, have been shown to lead to an increased endothelial microparticle production (2). Endothelial microparticles also appear to be functional and can reduce local endothelial nitric oxide bioavailability, thus impairing endothelial function both locally and systemically.

Interestingly, Navasiolava and colleagues (9) showed that circulating endothelial cell microparticles increased within 3 days of dry immersion, concomitant with the development of microvascular dysfunction and impaired basal blood flow. Furthermore, the adverse changes in endothelial cell homeostasis occurred in the absence of evidence of endothelial inflammation as measured by circulating E-selectin and leukocyte and platelet activation as measured by microparticle levels. While more definitive measurements of microparticle content, mononuclear cell activation, and direct measurements of endothelial cell inflammatory markers would have more clearly defined the overall vascular homeostasis and inflammatory state in the study participants before and during dry immersion, their findings do suggest that physical inactivity-induced endothelial dysfunction may at least in part originate from an apoptotic-centered process rather than an inflammatory one.

Unfortunately, measurements of insulin sensitivity in this study were not robust. The authors did measure fasting glucose levels (which were unchanged throughout the study) and did note a trend toward an increase in circulating triglyceride levels during dry immersion. However, the study lacked a direct assessment of insulin sensitivity. Simple measurements of insulin sensitivity such as the insulin sensitivity index or homeostatic model assessment tracked throughout the study would have given a stronger sense of the timing of the onset of insulin resistance relative the endothelial dysfunction. The lack of evidence of inflammatory activation suggests that the onset of insulin resistance either occurs in parallel or following the development of endothelial dysfunction, but further evidence is needed to corroborate this hypothesis.

The investigative groups that have gone through the effort and expense to perform physical inactivity study protocols should be commended for their efforts. This investigative model has the potential to reveal the origins of vascular and metabolic dysregulation in inactive humans given its ability to rapidly induce both endothelial dysfunction and insulin resistance in previously healthy individuals. Future work in this area should concentrate on a broad measurement approach that includes measurements of vascular function, insulin sensitivity, and local and systemic inflammation to better elucidate the origins of and relationships between these physical inactivity-induced derangements.

DISCLOSURES

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

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B3. ETUDE DES MODIFICATIONS CARDIO-VASCULAIRES INDUITES PAR L'IMMERSION SECHE :

REPONSE CARDIO-VASCULAIRE A L'ORTHOSTATISME

L'immersion sèche augmente la vasoconstriction cutanée à l'orthostatisme

"SKIN VASCULAR RESISTANCE IN STANDING POSITION INCREASES SIGNIFICANTLY AFTER 7 DAYS OF DRY IMMERSION"

Navasiolava NM, de Germain V, Levrard T, Larina IM, Kozlovskaya IB, Diquet B, Le Bouil A, Custaud MA, Fortrat JO. - Autonomic Neuroscience: Basic and Clinical 2010 Nov 9 [Epub ahead of print]

La peau peut être considérée comme un lit vasculaire facile d'accès pour examiner les effets cardio-vasculaires négatifs de la microgravité réelle et simulée.

L'objectif était d'étudier les effets de la position débout sur la microcirculation cutanée, ses relations avec l'hémodynamique générale et sa régulation autonome avant et après simulation de microgravité par immersion sèche de 7 jours.

Le déconditionnement cardio-vasculaire était observé après immersion sèche avec une tachycardie plus importante en position debout. Cependant, la préservation inattendue de la tolérance orthostatique dans cette étude par rapport aux études avec alitement prolongé pourrait s'expliquer par l'effet d'une brève stimulation orthostatique quotidienne durant la période d'immersion.

Nous avons montré une élévation de la résistance vasculaire cutanée en position debout après immersion sèche. Nous supposons que ce phénomène résulte de l'augmentation du réflexe veino-artériolaire induite par la compression chronique des tissus en immersion sèche.

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Skin vascular resistance in the standing position increases significantly after 7 days of dry immersion

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ABSTRACT

Actual and simulated microgravity induces hypovolemia and cardiovascular deconditioning, associated with vascular dysfunction. We hypothesized that vasoconstriction of skin microcirculatory bed should be altered following 7 days of simulated microgravity in order to maintain cardiovascular homeostasis during active standing. Eight healthy men were studied before and after 7 days of simulated microgravity modeled by dry immersion (DI). Changes of plasma volume and orthostatic tolerance were evaluated. Calf skin blood flow (laser-Doppler flowmetry), ECG and blood pressure signal during a 10-min stand test were recorded, and skin vascular resistance, central hemodynamics, baroreflex sensitivity and heart rate variability were estimated. After DI we observed increased calf skin vascular resistance in the standing position (12.0 ± 1.0 AU—after- vs. 6.8 ± 1.4 AU—before), while supine it was unchanged. Cardiovascular deconditioning was confirmed by greater tachycardia on standing and by hypovolemia ($-16 \pm 3\%$ at day 7 of DI). Total peripheral resistance and indices of cardiovascular autonomic control were not modified. In conclusion, unchanged autonomic control and total peripheral resistance suggest that increased skin vasoconstriction to standing involves rather local mechanisms–as venoarteriolar reflex–and might compensate insufficient vasoconstriction of other vascular beds.

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1. Introduction

The need to counteract gravity has supplied the cardiovascular system with regulatory mechanisms. They maintain adequate perfusion of each organ despite postural changes. Reduced action of gravity (during space flight or simulated microgravity) rapidly induces hypovolemia and cardiovascular deconditioning (orthostatic intolerance, reduced exercise capacity, and increased heart rate). A number of mechanisms may contribute to cardiovascular deconditioning (for a review see Convertino, 2002; Zhang, 2001). However, vascular functions (Zhang, 2001) including microcirculation (Coupe et al., 2009) are worthy of special interest. Vasoconstriction is needed for cardiovascular homeostasis in standing position, while cardiovascular deconditioning is associated with microcirculatory dysfunction, promoted by physical inactivity and prolonged decrease in shear stress forces (De Groot et al., 2006). Physical inactivity and modified thermoregulation inherent to microgravity might alter directly skin vascular functions. In this regard, skin microcirculation is of particular interest not only because of easy access, but also because of systemic consequences of vasomotricity of a vast vascular bed. In fact, overall hemodynamics is dependent on how much blood is distributed to skin. Under normothermic conditions, skin blood flow is about 5% of cardiac output; however it can vary from nearly zero to as much as 60% of cardiac output (Rowell, 1974). However, most studies deal with skin vasodilation. Indeed, actual and simulated microgravity is known to attenuate skin vasodilation with respect to heating (Crandall et al., 1994, 2003; Michikami et al., 2004), exercise (Greenleaf, 1997) and pharmacological stimuli (Demiot et al., 2007). Skin vasoconstriction in microgravity is less studied and data are contradictory. Increased and unchanged skin vasoconstriction was observed in response to various gravitational challenges (leg lowering, lower body negative pressure and head-up tilt test) after actual and simulated microgravity (Aratow et al., 1991; Gabrielsen et al., 1995, 1999; Gabrielsen and Norsk, 2007).

We hypothesized that vasoconstriction of skin microcirculation should be altered following 7 days of simulated microgravity in order

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to maintain cardiovascular homeostasis during active standing, the physiological gravitational challenge.

2. Materials and methods

2.1. Subjects

Eight healthy Russian men participated in the study. Their age, body height and weight were 22.9 ± 0.5 years old, 175 ± 3 cm and 76 ± 4 kg (mean ± SEM). Based on their interview, they were non-athletic, but they were also not sedentary: they had regular physical activity resulting from their daily life, working occupation and leisure sport. 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 written consent to participate. The experimental protocol was approved by the Russian National Committee on Bioethics of the Russian Academy of Sciences and conformed to the standards set by the *Declaration of Helsinki*.

2.2. Simulated microgravity

Dry immersion (DI) is a simulation of microgravity largely used in Russia. DI involves immersing the subject into thermoneutral water (between 32 and 33 °C) covered with a special elastic waterproof fabric film (Shulzhenko and Vil-Vilyams, 1976) (Fig. 1). The subject is kept dry, since the film separates him from 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 hydrostatic pressure is equally distributed over the body surface, providing conditions similar to complete lack of structural support (Grigor'ev et al., 2004). The present study was undertaken at the Dry Immersion Facility affiliated to the Institute of Biomedical Problems, State Scientific Centre of Russia, Moscow, Russia.

All subjects remained at the Dry Immersion Facility for 11 days, including two ambulatory control days before immersion (B − 2 and B − 1), 7 days of immersion (DI 1–DI 7) and two recovery days (R + 1 and R + 2). Subjects were immersed one after another. During DI, the subjects remained in a supine position continuously for all activities except for two 15 min long extractions at 9 a.m. and 9 p.m. for weighing (in a standing position) and shower (in a sitting position), which is common for DI protocols. The subjects were continuously observed by video monitoring.

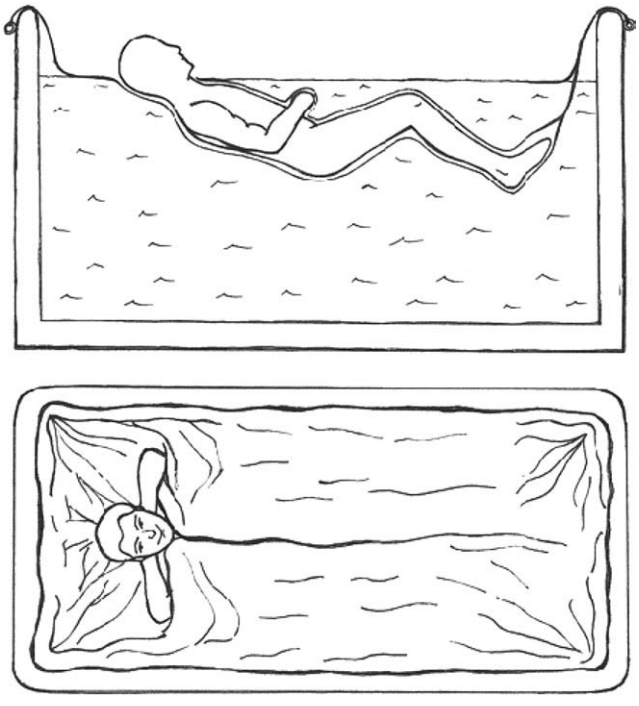

Fig. 1. Dry immersion method. The subject is separated from the water by waterproof fabric.

2.3. Hypovolemia and cardiovascular deconditioning

Change in plasma volume was determined by the Dill and Costill method (Dill and Costill, 1974) based on hemoglobin and hematocrit measurements (MEK 6318 hematological auto analyzer, Japan). Antecubital venous blood was taken in the morning, before breakfast, in the supine position on B − 2, DI 3, DI 7.

Cardiovascular deconditioning induced by DI was evaluated by estimation of orthostatic tolerance and heart rate (HR). Orthostatic tolerance was evaluated by a specific 10-min active standing test at B − 2 and R + 1 (first rising after the end of DI) by another team (Eshmanova, 2009). HR was determined on the data obtained during the active standing test on B − 2, B − 1, R + 1 (first rising) and R + 2.

2.4. Standing skin vascular resistance and central hemodynamics

Cardiovascular responses to active standing were studied before DI (day B − 1) and in the recovery period (day R + 2). Assessments were performed in the morning in a quiet room at ambient air temperature of 24–26 ° C. The subject remained supine for 30 min, following which data were recorded for 10 min. After this, the subject was asked to stand up and to keep this position for 10 min. Active standing was performed with continuous supervision and monitoring in order to stop the test upon any signs of pre syncope (a feeling of faintness, rapid drop in systolic blood pressure (SBP) more than 25 mm Hg, HR more than 160 bpm). Should they feel any discomfort, the subjects were advised to stop the test and lie down.

Skin blood flow was measured with laser-Doppler flowmetry. The measurements were performed on the left leg at the calf level, with a laser-Doppler multifiber probe (wavelength 780 nm) connected to a laser-Doppler flowmeter (Periflux PF4001, Perimed, Sweden). Skin temperature was controlled by monitoring with thermocouples 5 cm apart connected to an electronic thermometer (BAT-12, Physitemp Instruments, Clifton, New Jersey). In the supine position skin temperature was maintained at the thermoneutral level between 33.5 and 34.5 °C by means of a blanket. Mean values for 10 min of supine position and 10 min of standing for laser-Doppler flow (LDF) were obtained. Skin vascular resistance (SVR) was estimated by dividing mean blood pressure (MBP) by LDF, and expressed in arbitrary units (AU), which is common for laser-Doppler method (Okazaki et al., 2005).

Finger blood pressure wave (Finapres, Ohmeda, Englewood, Col.) and standard ECG (Biopac, ECG 100 C, USA) were recorded continuously and registered with the data acquisition system (Biopac MP 150, USA). The finger cuff was maintained at the heart level. SBP and diastolic blood pressure (DBP), and the R–R interval (RRi) at each beat were extracted from the signal using a software package (Acknowledge® 3.9.0). MBP was calculated as DBP + (1/3 * (SBP − DBP). Stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were estimated from the blood pressure signal using the modelflow® method (Beatscope® software, TNO, the Netherlands). We performed power spectrum analysis of heart rate variability (HRV) by Fast Fourier Transform using online software and service (www.televasc.fr). Low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz) power were determined and normalized by the total power. Spontaneous baroreflex sensitivity (SBRs) (Hughson et al., 1993) was calculated using online software and service (www.televasc.fr). A spontaneous baroreflex sequence was

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defined as same-direction changes in RRi and SBP for at least three beats. A linear regression was applied to each sequence and the mean slope was taken as the spontaneous baroreflex sensitivity.

2.5. Urine sampling and analysis

Urine pools were collected over 24 h each day of the study, urine volume was measured, and samples were stored at −20 °C pending analysis. Normetanephrine concentration was determined by high-pressure liquid chromatography with amperometric detection. The limit of quantification of the method was 0.05 μmol L^{-1} and the coefficient of variation was 5%.

2.6. Data analysis

All values are presented as means ± SEM. The data collected during DI and in the recovery period were compared with data obtained before DI. For statistical analysis, a Friedman test was used, followed by post-hoc Wilcoxon signed-rank test if the global significant differences were obtained. These non-parametric tests are appropriate for matched pairs of data where the samples are related and a normal distribution is not assumed. *P* values of less than 0.05 were considered statistically significant. Analyses were performed with SPSS 15.0 for Windows.

3. Results

3.1. Hypovolemia and cardiovascular deconditioning

A significant decrease in plasma volume was observed on the third and seventh day of DI (−15 ± 4% and −16 ± 3%, respectively, $P<0.05$). One intolerant subject out of eight was found on R + 1 while all the subjects completed a 10-min active standing test before DI. Heart rate in the standing position was significantly greater after DI (96 ± 6 bpm at B − 1 vs. 108 ± 6 bpm at R + 2, $P<0.05$). At B − 2 and R + 1 heart rate in the standing position was 86 ± 4 bpm and 128 ± 3 bpm ($P<0.05$), respectively (Eshmanova, 2009). Resting heart rate did not change significantly (64 ± 3 bpm at B − 2 and 59 ± 3 bpm at B − 1 vs. 72 ± 3 bpm at R + 1 and 63 ± 4 bpm at R + 2) (Fig. 2).

3.2. Standing skin vascular resistance and central hemodynamics

Skin vascular resistance increased much more on R + 2 in the standing position (6.8 ± 1.4 AU before vs. 12.0 ± 1.0 AU after, $P<0.05$), whereas it was unaltered in the supine position (3.4 ± 0.7 AU before vs. 3.5 ± 0.6 AU after). Standing calf skin temperature was significantly lower for both days of measurements in comparison with supine. However, there was no significant difference in skin temperature before and after immersion, neither in supine position (33.5 ± 0.2 °C on B − 1 vs. 33.6 ± 0.2 °C on R + 2) nor in standing position (31.7 ± 0.9 °C on B − 1 vs. 31.9 ± 0.4 °C on R + 2).

Standing position led to the expected changes in central hemodynamics variables (increased HR, DBP and TPR, decreased SV, and unchanged SBP and CO), as well as HRV spectral power and SBRs (decreased HF, increased LF, and decreased SBRs) with no significant difference between before DI (B − 1) and after DI (R + 2) (Table 1, Fig. 2).

3.3. Urinary normetanephrine

Urinary normetanephrine was significantly increased on the first and second days after DI (0.82 ± 0.08 μmol 24 h^{-1} baseline vs. 1.03 ± 0.16 μmol 24 h^{-1} on R + 1 and 0.99 ± 0.12 μmol 24 h^{-1} on R + 2, $P<0.05$) (Fig. 3).

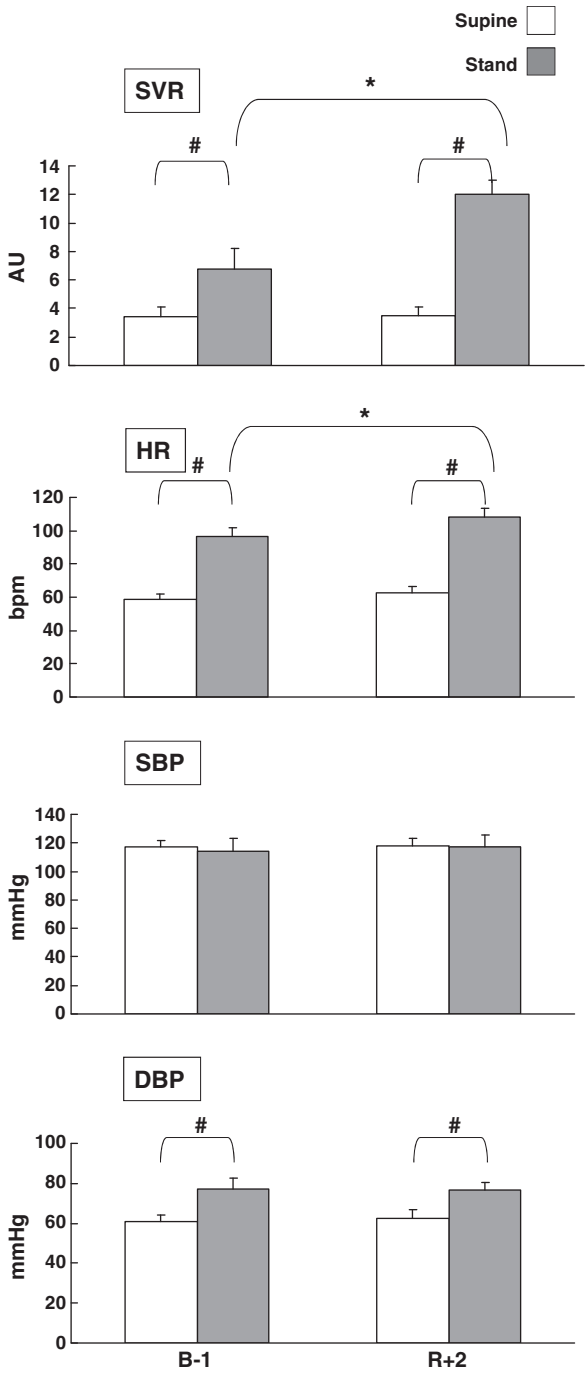

Fig. 2. Results of stand test before (B − 1) and after (R + 2) 7 days of dry immersion: skin vascular resistance (SVR), heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure in supine and standing position. AU, arbitrary units; bpm, beats per min. * Significant difference R + 2 vs. B − 1, $P<0.05$; # significant difference standing vs. supine, $P<0.05$.

We did not notice any specific differences in the measured parameters between the subject with orthostatic intolerance, and seven other subjects.

4. Discussion

The major new finding of the present study was a more pronounced increase in skin vascular resistance in standing position after 7 days of dry immersion, while total peripheral resistance and overall central hemodynamics were not significantly impaired.

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Table 1
Cardiovascular results of stand test.

	Supine B−1	Standing B−1	Supine R+2	Standing R+2
Stroke volume (ml)	92.7±4.0	50.5±4.9*	95.0±6.1	52.5±6.5*
Cardiac output (L min^{-1})	5.56±0.43	4.70±0.32	5.91±0.52	5.48±0.57
TPR (dynes sec cm^{-5})	850±80	1240±120*	860±120	1100±150*
SBRs (msec mm Hg^{-1})	37±8	9±1*	26±3	7±1*
HF RRi normalized	0.5±0.03	0.16±0.02*	0.49±0.04	0.18±0.04*
LF RRi normalized	0.5±0.03	0.84±0.02*	0.5±0.04	0.82±0.04*
LF/HF RRi	1.11±0.20	6.22±1.08*	1.15±0.24	6.34±1.47*

TPR, total peripheral resistance; SBRs, spontaneous baroreflex–response slope; HF, high frequency band; LF, low frequency band; RRi, R-R interval; * significant difference supine vs. standing, $P<0.05$.

4.1. Dry immersion

Dry immersion (DI) as a long-term microgravity model is widely used in Russia. This model is less known in western countries where prolonged head-down bed rest (HDBR) is preferred as simulated weightlessness. The special features of DI are the absence of support gradient and the constant water compression. Physical inactivity in DI is very enforced due to acute limitation of the voluntary muscular activity, reduction of gravitational stress on muscles, and also deep atonia. It has been shown that in supportless conditions muscle tone diminishes dramatically, especially the postural muscle tone (Grigor'ev et al., 2004). DI induces centralization of body fluids mainly as a result of hydrostatic compression. Also it was demonstrated that after initial decrease of sympathetic activity in the first 12 h, DI is associated with increased sympathetic activation (Eshmanova et al., 2008), which might promote vasoconstriction.

4.2. Altered skin vasoconstriction: potential role of VAR

Orthostatic vasoconstriction is mediated by central (mainly sympathetic) and local mechanisms. When standing up, lower body venous pooling induces baroreflex-mediated sympathetic activation and liberation of vasoconstrictive hormones, both leading to general vasoconstriction. In addition to baroreflex control, vasomotor tone is also increased by local mechanisms such as myogenic autoregulation and venoarteriolar reflexes, which increase vascular resistance in response to elevated local arteriolar and venular pressures, respectively. We observed increase in urinary normetanephrine, which suggests sympathetic activation after DI. However, it has been shown that orthostatic stimulus does not increase sympathetic discharge to skin as demonstrated by means of skin sympathetic nerve activity by microneurography during lower body negative pressure (LBNP) and head-up tilt test (Vissing et al., 1997; Cui et al., 2004; Wilson et al., 2005). Moreover, unchanged spontaneous baroreflex sensitivity and heart rate variability suggest absence of significant alteration in cardiovascular autonomic control during our protocol. It is thus more likely that changes observed in skin microcirculation are due to local mechanisms such as venoarteriolar reflex (VAR). Vissing et al. (1997) have demonstrated the primary role of VAR in triggering vasoconstriction in cutaneous circulation during upright posture. Gabrielsen and Norsk (2007) observed an unchanged VAR after actual microgravity while HDBR led to increased (Gabrielsen et al., 1999), or attenuated (Wilson et al., 2003) VAR. These controversial conclusions may not only result from the differences in studied models (actual microgravity and HDBR) but also from the different procedures and methods used to assess VAR. DI might provide interesting model in order to clarify the role of VAR in the cardiovascular deconditioning. Chronic hydrostatic compression inherent to DI might explain the exaggerated VAR. Indeed, long-term elastic compression therapy is known to improve impaired VAR (Mancini et al., 2003; Belcaro et al., 1988, 1993).

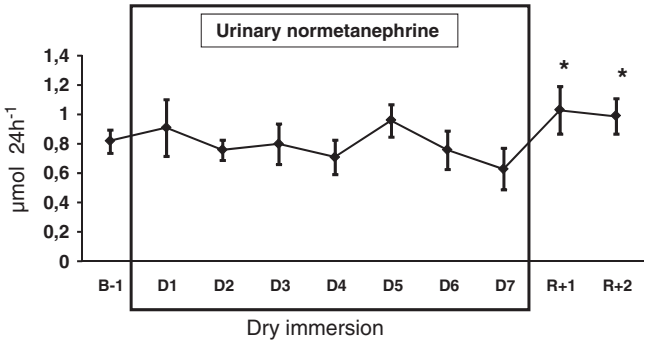

Fig. 3. Variation in urinary normetanephrine before, during and after dry immersion; * significant difference vs. B−1, $P<0.05$.

4.3. Altered skin vasoconstriction: potential role of endothelial dysfunction

Another local factor which might act on vasoconstriction is endothelial control. Endothelium maintains fine balance between vasoconstriction and vasodilation. Simulated microgravity, being associated with physical inactivity, is known to induce endothelial dysfunction (Hesse et al., 2005; Demiot et al., 2007; Hamburg et al., 2007). DI was shown to impair endothelial vasodilative properties at the skin level (Navasiolava et al., 2010). The reduction of endothelial "vasodilative tone" might shift the balance towards vasoconstriction. Consistently, a decrease in plasma NO release was shown after 14-day HDBR, whereas MSNA was increased (Kamiya et al., 2000), which supports the idea of vasoconstrictor–vasodilator imbalance following simulated microgravity.

4.4. Integrative physiology: total peripheral resistance, overall central hemodynamics

We observed that exaggerated skin vasoconstriction to standing was after DI, while TPR was maintained at the initial level. This combination of increased skin vasoconstriction and unchanged TPR suggests that other territories (probably, splanchnic and skeletal muscles vascular beds) fail to provide efficient vasoconstriction after DI. In simulated microgravity, vascular adaptation in the different vascular areas might differ depending on hemodynamic changes induced by the fluid shift (Pavy-Le Traon et al., 2007).

In conclusion, 7 days of DI increased calf skin vascular resistance in the standing position. Changes observed for skin microcirculation are most likely due to local mechanisms such as venoarteriolar reflex and/or endothelial impairment. Unaltered total peripheral resistance might indicate that increased skin vasoconstriction compensates insufficient vasoconstriction in other vascular beds.

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B4. ETUDE DES MODIFICATIONS CARDIO-VASCULAIRES INDUITES PAR L'IMMERSION SECHE :

METABOLISME HYDRO-SODE ET SA REGULATION HORMONALE

L'immersion sèche engendre rapidement un nouvel état d'équilibre hydro-sodé

"NT-proBNP LEVELS, WATER AND SODIUM HOMEOSTASIS IN HEALTHY MEN: EFFECTS OF 7 DAYS OF DRY IMMERSION"

Navasiolava N, Pajot A, Gallois Y, Pastushkova L, Kulchitsky V, Gauquelin-Koch G, Kozlovskaya I, Heer M, Hand O, Larina I, Custaud MA. - European Journal of Applied Physiology (soumis)

Les effets initiaux de l'immersion sur les liquides corporels sont assez bien connus. En revanche, les effets de l'immersion sur une plus longue durée restent moins étudiés. Nous avons montré que l'immersion sèche engendre rapidement un nouvel état d'équilibre hydro-sodé. L'immersion ré-étalonne la « valeur seuil » du volume plasmatique à un niveau hypovolémique.

Après la diurèse et la natriurèse aigües au premier jour d'immersion, l'excrétion de sodium revenait au niveau initial. Dès la seconde journée, l'homéostasie hydrique était rétablie. La consommation d'eau dans ce protocole était imposée et ne variait pas, mais en conditions d'immersion, les systèmes de régulation la considéraient comme excessive. L'excrétion d'eau était augmentée, d'où la diminution de la balance hydrique partielle et l'augmentation de la clairance d'eau libre pendant l'immersion. L'absence de changement significatif des taux de rénine et d'aldostérone plasmatiques au troisième jour d'immersion démontrait que vers cet instant, les redistributions majeures hydro-sodées étaient déjà achevées et que la régulation s'était stabilisée.

Pendant la période de récupération, nous avons observé une augmentation significative du NT-proBNP. Cette augmentation pourrait refléter le niveau de déconditionnement cardiaque.

D'autres études sont à effectuer pour déterminer la valeur possible du NT-proBNP comme un marqueur biologique du déconditionnement cardio-vasculaire.

Title: NT-ProBNP levels, water and sodium homeostasis in healthy men: effects of seven days of dry immersion

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Abstract

Immersion is a useful tool for studying fluid-volume homeostasis. Natriuretic peptides play a vital role in renal, humoral, and cardiovascular regulation under changing environmental conditions. We hypothesized that dry immersion would rapidly induce a new steady state for water and sodium metabolism, and that serum NT-proBNP levels, a proxy measure for brain natriuretic peptide (BNP), would decrease during long-term dry immersion and increase during recovery. Eight healthy young men were studied before, during, and after seven days of dry immersion. Body weight, water balance, and plasma volume changes were evaluated. Plasma and serum samples were analyzed for active renin, NT-proBNP, aldosterone, electrolytes, osmolality, total protein, and creatinine. Urine samples were analyzed to determine levels of electrolytes, osmolality, creatinine, and free cortisol. A stand test was performed before and after dry immersion to evaluate cardiovascular deconditioning. Long-term dry immersion induced acute changes in water and sodium homeostasis on day 1, followed by a new steady state. Plasma volume decreased significantly during dry immersion. The serum levels of NT-proBNP increased significantly in recovery (10 ± 3 ng/L before dry immersion vs. 26 ± 5 ng/L on the fourth recovery day). Heart rate in the standing position was significantly greater after immersion. Results suggest that chronic dry immersion rapidly induced a new level of water-electrolyte homeostasis. The increase in NT-proBNP levels during the recovery period may be related to greater cardiac work and might reflect the degree of cardiovascular deconditioning.

Keywords: plasma volume, simulated weightlessness, natriuretic peptides, water immersion, body fluid regulation, deconditioning

Introduction

Water immersion is widely used in fundamental studies of renal physiology and fluid-volume homeostasis (Epstein 1996; Greenleaf 1984). The early effects of water immersion on body fluids are well known. Hydrostatic compression of superficial tissues and vessels lowers peripheral vascular capacity and causes continuous transcapillary reabsorption, resulting in the prompt redistribution of circulating blood with a relative central hypervolemia. Fluid centralization and increased stroke volume triggers an adaptive response with high natriuresis and diuresis. This response is associated with an increase in atrial natriuretic peptide (ANP) and suppression of arginine vasopressin (AVP) and the renin-angiotensin-aldosterone system. The maximal fluid loss with water immersion occurs during the first few hours (Epstein 1996, Somody et al. 1999, Stadeager et al. 1992). However there have been fewer studies on the long-term effects of water immersion on body fluids.

Modifying the water immersion protocol by separating the subject from the water with an elastic fabric allowed the experimental period to be lengthened and led to creation of the dry immersion (DI) method (Shulzhenko and Vil-Vilyams 1976). Dry immersion closely reproduces the centralization of body fluids and mechanical and support unloading observed in microgravity (Grigor'ev et al. 2004; Shulzhenko and Vil-Vilyams 1976). It is characterized by enforced physical inactivity (Grigor'ev et al. 2004).

Atrial natriuretic peptide and brain natriuretic peptide (BNP) are the members of the natriuretic peptides family directly involved in water-electrolyte regulation. Their physiologic actions include an increase in natriuresis and a decrease in systemic vascular resistance and central venous pressure. Both peptides are released from cardiac myocytes in response to acute cardiothoracic hypervolemia and stretching. Indeed, acute wall stretch induces activation of the BNP gene expression within 1 hour (Tokola et al. 2001). Natriuretic peptides

also reflect changes in cardiac wall stress in cases of chronic heart failure (Tokola et al. 2001). Inactive NT-proBNP (N-Terminal proB-type Natriuretic Peptide) is cleaved from the precursor peptide proBNP and is co-secreted in equimolar proportion to BNP. Compared to BNP, measurement of NT-proBNP is more reliable because of its longer half-life of 60-120 minutes versus 22 min for BNP, its stability (Cauliez et al. 2005), and lower intra-individual variations (Wu et al. 2003). Longer half-life is related to the differences in the elimination; NT-proBNP is excreted by the kidneys whereas BNP is mainly eliminated by a neutral endopeptidase and by a clearance receptor (NPR-C) (Cauliez et al. 2005).

Models of microgravity induce changes in blood volume and hemodynamic conditions. It is well known that initial phase response is an increase in ANP secretion (Epstein 1996). However, long-term effect and recovery effect are less known. During long-term immersion, decreases in plasma volume (Gogolev et al. 1980) and in cardiac load (decreased stroke volume, Shulzhenko et al. 1980) are observed. In recovery, plasma volume and cardiac work increase. All these changes might influence natriuretic factors.

The aim of this work was to study day-by-day water-electrolyte and volume homeostasis in healthy men under long-term dry immersion, with emphasis on NT-proBNP levels, a proxy measure of BNP. We hypothesized that DI would rapidly reset water and sodium metabolism to a new steady state with a stable decrease in blood volume and that NT-proBNP levels would decrease during long-term DI and increase in the recovery period.

Methods

Dry immersion method

Dry immersion involves immersing the subject into thermoneutral water covered with a special elastic waterproof fabric film (Shulzhenko and Vil-Vilyams 1976). The subject is kept dry, since the film isolates the body from the water. The film is thin and large enough to ensure that the water's hydrostatic pressure is equally distributed over the body surface, providing conditions similar to the complete lack of structural support experienced in microgravity.

Subjects

The investigation involved eight healthy non-smoking men (age 22.9 ± 0.5 years, height 175 ± 3 cm, weight 76 ± 4 kg, mean ± SEM). Based on their interview, they were non-athletic, but not sedentary: they had regular physical activity resulting from their daily life, occupation, and leisure sport. Subjects were enrolled after a thorough medical examination. All volunteers gave their informed consent in writing, in compliance with the Helsinki Declaration. The experimental protocol was approved by the Committee of Biomedicine Ethics of the Russian Academy of Sciences.

Procedures

The protocol used in this study is schematized in Figure 1. All subjects remained at the Dry Immersion Facility of the Institute of Biomedical Problems (Moscow, Russia) for 11 days, including two ambulatory control days before immersion (B-2 and B-1), seven days of dry immersion (DI 1 – DI 7), and two recovery days (R+1 and R+2). In addition, subjects returned to the facilities for 24 hours for urine collection on R+3 and blood sampling on R+4 and R+7. The subjects were immersed one after another. During immersion, they remained in

a supine position for all activities, except for two 15-minute periods for weighing (in a standing position) and hygienic procedures (at 9 a.m. and 9 p.m.). The water temperature was set between 32 and 33°C. The subjects were continuously observed by video monitoring. During immersion, daily caloric intake was 2270 ± 50 kcal, whereas before and after immersion it was 2880 ± 60 kcal. Water and salt consumption, normalized to body weight, were held constant throughout the experiment (before, during, and after immersion), as was protein intake. Total water consumption was 50 ml/kg/day (about 3.7 L/day), with direct water intake – drinking water, tea (without theine), juice - of about 2.6 L/day. Dietary sodium was set to 2.8 mmol/kg/day, daily calcium intake was adjusted to 1000-1150 mg, and protein intake to 1.15 g/kg/day.

The level of physical load before baseline, during baseline, and during recovery was not measured. However, daily physical activity was similar during these periods. The subjects did not exercise or perform heavy physical work before and after immersion. The tests with physical loads included 10-min active standing (on B-2, B-1, R+1, and R+2), and assessment of the kinematic characteristics of walking (on B-2 and R+3), and did not involve strenuous physical efforts. We postulate that physical load was approximately the same before and after immersion.

Sample collection and analyses

Blood sampling

Antecubital venous blood was taken in the morning before breakfast on B-2, DI 3, DI 7, R+4, and R+7 as the subjects rested in a supine position. Blood sampling in control and recovery periods was also performed after the subject had rested in the supine position for 30 min. Plasma (EDTA) and serum samples were immediately prepared and stored at -80°C until

analysis. Samples were analyzed for active renin, NT-proBNP, aldosterone, electrolytes (Na^+, K^+), osmolality, proteins, and creatinine.

Calculation of changes in plasma volume

Hemoglobin and hematocrit levels were determined immediately after blood collection using an MEK 6318 hematological auto analyzer (Nihon Kohden, Japan) to calculate percentage changes in plasma volume (delta plasma volume, DPV) using the Dill and Costill method:

$$DPV\ (\%) = 100 \times [HbB\ (1- 0.01Hcti) / [Hbi\ (1- 0.01HctB)] - 100 \quad (1)$$

In equation (1), HbB and HctB are hemoglobin and hematocrit levels at baseline, and Hbi and Hcti are the corresponding values on DI 3, DI 7, and R+4.

Urine sampling

Urine pools were collected over 24 hours each day of the study, then measured and stored at -20°C. The partial water balance was defined as the difference between consumed water and urine volume. Urine samples were analyzed to determine levels of electrolytes (Na^+, K^+), osmolality, creatinine content, and free cortisol. Creatinine, free water, and osmolal clearances were estimated on B-2 (using the 24-hour urine sample from B-2 and the morning blood sample on B-2), on DI 2 (using the 24-hour urine sample from DI 2 and the morning blood sample on DI 3), on DI 6 (using the 24-hour urine sample from DI 6 and the morning blood sample on DI 7), and on R+3 (using the 24-hour urine sample from R+3 and the morning blood sample on R+4).

Biochemical analyses

Active renin analysis was performed using a chemiluminescence immunoassay with two specific monoclonal antibodies and a commercial kit (DiaSorin) on the fully automated

"Liaison" analyzer (DiaSorin). Intra-assay and inter-assay variabilities were 2.6% and 7.3%. Plasma aldosterone levels were determined by a competitive radioimmunoassay (RIA) using a commercially available RIA kit (Immunotech, a subsidiary of Beckman Coulter) following the manufacturer's instructions. Intra-assay and inter-assay variabilities were 11.4% and 16.7%. Levels of NT-proBNP were evaluated by an electrochemiluminescence immunoassay using two monoclonal antibodies and a commercial kit (Roche); inter-assay variability was 4%. Urinary free cortisol was detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with an Applied Biosystems/MDS Sciex Api 3000 triple quad mass spectrometer equipped with a turbo ion spray source; intra-assay and inter-assay variabilities were 3.1% and 10.7%. Plasma and urine osmolality were measured by freezing-point depression relative to pure water. Intra-assay and inter-assay variabilities were 0.6% and 1.1%. All the other parameters from the blood and urine samples were evaluated using commercially available kits (Roche) on the Roche/Hitachi "Modular" automated clinical chemistry analyzer. Sodium and potassium values were measured with ion selective electrodes with intra-assay variability less than 0.5% and inter-assay variability less than 1.3%. Creatinine concentrations were measured by a modified Jaffe reaction to obtain high sensitivity and better precision; intra-assay and inter-assay variabilities were 0.7% and 2.3% for blood samples and 1.1% and 1.2% for urine samples. The total protein concentration was measured by a colorimetric assay with a Biuret reagent. Intra-assay and inter-assay variability were 0.7% and 0.95%.

Stand test

A ten-minute active standing test was performed on day B-2 and R+1 (first rising after the end of DI) by another team (Eshmanova 2009) and on day B-1 and R+2 - by our team. The stand test was performed in the morning in a quiet room at an ambient air temperature of 24-26°C.

The subjects were first placed in a supine position and allowed to rest for at least 30 min, and then data were recorded from this resting supine position for 10 minutes. Afterward, the subjects were asked to stand up. A 10-minute stand test was performed with continuous monitoring in order to stop the test upon any signs of presyncope. Blood pressure was measured throughout using the volume clamp method with a non-invasive finger cuff device on the third finger of the left hand (Finapres, Ohmeda, Englewood, Col). The finger cuff was maintained at the heart level in both supine and standing positions. To obtain the heart rate, a standard ECG was recorded continuously. For each studied parameter, mean values were obtained for 10 min in the supine and standing positions.

Data analysis

The data obtained during immersion and in the recovery period were compared with individual baseline data (controls). All values are presented as mean ± SEM. For the statistical analysis, a Friedman test was first used, followed by a post-hoc Wilcoxon signed-rank test if significant differences were obtained. These non-parametric 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 to be significant. Analyses were performed using SPSS 15.0 for Windows.

Results

At baseline, the values for all studied parameters were within normal limits. The seven days of dry immersion were well tolerated. All subjects, however, experienced backache at the beginning that disappeared by the third or fourth day of immersion. Body weight had already decreased significantly at the end of the first day of DI and continued declining until the seventh day (76.1±4.1 kg on B-1 vs. 75.2±4.2 kg on DI 1 vs. 73.8±4.0 kg on DI 7, p=0.017 and 0.01). Body weight increased significantly from DI 7 to R+1 (74.6±4.2, p=0.034). We also observed a significant decrease in plasma volume on the third and seventh days of immersion (-15±4% and -16±3%, respectively, p=0.012) (Fig. 2). Levels of serum Na^+, K^+, proteins, creatinine and osmolality remained within normal values for all measurements (Table 1).

One intolerant subject was found on R+1 while all the subjects completed a 10-min active standing test before DI and on R+2. Heart rate in the standing position was significantly higher after DI compared to baseline. On B-2 and R+1 it was 86±4 bpm and 128±3 bpm ($p<0.05$), respectively (Eshmanova 2009); on B-1 and R+2 it was 96±6 bpm and 108±6 bpm (p=0.036), respectively. Resting heart rate measured in the supine position did not change significantly (64±3 bpm at B-2 and 59±3 bpm at B-1 vs. 72±3 bpm at R+1 and 63±4 bpm at R+2, $p>0.05$). Mean blood pressure on the second day of recovery did not differ significantly from the control values either in the supine (80±3 mmHg on B-1 vs. 81±4 mmHg on R+2, $p>0.05$), or in the standing position (90±6 mmHg on B-1 vs. 90±5 mmHg on R+2, $p>0.05$).

Water consumption did not differ significantly throughout the entire experiment period. Daily diuresis was significantly increased on the first day of immersion (2 times vs. B-1, p=0.012) (Table 2), and a negative partial water balance was observed on this day (p=0.01) (Fig. 3).

Daily diuresis and partial water balance remained stable between DI 2 and DI 7 (Table 2, Fig. 3). On R+1 we observed a significant 30% decrease in diuresis (p=0.025) and increase in water balance (p=0.018) (Table 2, Fig. 3).

Free water clearance was significantly increased on DI 2 and DI 6 (0.18±0.4 ml/min on DI 2 and 0.04±0.14 ml/min on DI 6 vs. -0.70±0.19 ml/min on B-2, p=0.01); on R+3 it did not differ significantly from the baseline. Osmolal clearance was not modified.

On the first day of DI a significant increase in urinary sodium loss was observed (p=0.018), followed by a significant decrease on R+1 (136±13 mmol/24h on B-1 vs. 278±33 mmol/24h on DI 1 vs. 67±9 mmol/24h on R+1; p=0.028) (Fig. 3). Urinary potassium loss was not modified (Table 2). The urinary Na^+/K^+ ratio increased significantly only on DI 1 (p=0.018) and did not differ from the baseline during the remainder of the immersion period. Recovery was accompanied by a decrease in the Na^+/K^+ ratio (significant on R+2, p=0.018) (Table 2).

Urinary osmolality was significantly reduced for all seven days of immersion (p=0.01 to 0.04), with recovery on R+1 (Fig. 3). Urinary creatinine did not change significantly (Table 2). Creatinine clearance was not modified.

Plasma active renin and aldosterone were statistically unchanged from the basal level on DI 3, DI 7, R+4 and R+7 (Fig. 4). Urinary free cortisol was significantly increased only on DI 1 vs. B-1 (Table II). During DI NT-proBNP levels tended to decrease but did not change significantly. We observed a significant increase in serum NT-proBNP levels in the recovery period (10±3 ng/L on B-2 vs. 26±5 ng/L on R+4, p=0.036).

Discussion

During this DI study, we observed acute natriuresis and diuresis on day 1 of dry immersion, followed by a new steady state for water and electrolyte homeostasis. Plasma volume was significantly decreased at DI 3 and remained stable at DI 7. Heart rate in the standing position was significantly higher after dry immersion. During dry immersion NT-proBNP levels did not change significantly, although they did increase significantly during the recovery period.

Tolerance of DI and general effects

Dry immersion was well tolerated. The urinary free cortisol was elevated significantly only on the first day - probably reflecting the emotional stress at the beginning of immersion - but not thereafter. Long-term dry immersion induces cardiovascular deconditioning syndrome (Iwase et al. 2000) as evidenced in this study by the increased tachycardia in the standing position during the recovery period. Long-term dry immersion also induces muscular atrophy related to enforced physical inactivity and absence of support (Grigor'ev et al. 2004). The slight increase in plasma creatinine during DI is probably related to muscular changes rather than changes in renal functions, however creatinine clearance remains stable.

New steady state for sodium and water homeostasis between DI 2 and DI 7

Water and sodium

The osmolality of plasma and interstitial fluid is determined mainly by sodium. As it is the major extracellular cation, 85% of effective osmotic pressure depends on sodium and counter anions. So regulation of extracellular fluid osmolality can be schematically seen as maintaining the water-to-sodium ratio.

Acute losses of water and sodium with increased urinary Na^+/K^+ ratio and decreased urinary osmolality on DI 1 are in accordance with previous results (Epstein 1996; Iunusov et al. 1985; Somody et al. 1999). The focus of this current study was to examine chronic changes rather than acute ones.

Between DI 2 and DI 7, both sodium excretion and the urinary Na^+/K^+ ratio returned to pre-immersion level. While water consumption remained unchanged, a new homeostasis of water balance was established, with increased water excretion compared to the baseline period. This was manifested as a stable diminution in partial water balance between DI 2 and DI 7. Free water clearance estimated on DI 2 and DI 6, was increased, while osmolal clearance was not modified, suggesting that water intake during DI was considered as excessive relative to the new set points of active homeostatic control mechanisms. In other words, the same water consumption was treated as an increased water load. The decrease in urine osmolality and the increase in free water clearance imply a reduction in the urine concentration function of the kidneys in chronic immersion.

The absence of significant changes in plasma active renin and aldosterone relative to pre-immersion controls indicates that by that time the major redistributions were over, and regulation of osmotically active substances, water and electrolytes had already stabilized.

New level of plasma volume

Actual and simulated microgravity are known to re-establish plasma volume to a new lower set point (Truijen et al. 2010). In our protocol, the reduced plasma volume established by DI 3 (-15%) remained stable by DI 7 (-16%). The estimated plasma loss was in accordance with other DI studies. Gogolev et al. (1980) found a 12% decrease in plasma volume after the first day and a 16% decrease on the fourth day of DI.

Significance of the NT-proBNP increase during the recovery period

During the DI recovery, we observed a significant increase in NT-proBNP levels. Brain natriuretic peptide is produced by cardiac myocytes in response to an increase in cardiac wall stress.

Augmented cardiac work can increase BNP level in healthy subjects; in fact, an increase in the BNP pathway has been observed after strenuous exercise in endurance athletes (Scharhag et al. 2008).

In our study, the increase in NT-proBNP levels during the recovery period may be related to an increase in cardiac work during this period. Cardiac work depends primarily on wall properties and on heart rate. After exposure to dry immersion, cardiac wall properties might be impaired. To our knowledge, direct data on human cardiac muscle properties during and following DI are still lacking. However, recent bed rest studies indicate that the heart adapts to reduced myocardial work and loading conditions by an early decrease in left ventricular end diastole volume (Levine et al. 1997, Truijen et al. 2010) and then by a delayed reduction in muscle mass (Perhonen et al. 2001). During the recovery period we could postulate that when the subjects return to normal activity, the cardiac wall stress in turn increases with this remodeled cardiac wall.

Moreover, the tachycardia, which is characteristic of cardiovascular deconditioning, also directly increases the cardiac load. In the recovery period, the usual daily load seems to be excessive for a deconditioned heart after seven days of dry immersion. Thus, this increase in cardiac load during the recovery period might be related to the level of cardiovascular deconditioning.

The other major symptom of cardiovascular deconditioning is a decrease in maximal oxygen uptake ($\dot{V}O_2$ max). A specific measurement of exercise performance ($\dot{V}O_2$ max) was not

performed in our study. However, in another 7-day dry immersion trial, the post-immersion $\dot{V}O_2$ max decreased by 10.4 % as measured by cycle ergometry (Anashkin and Beliaev 1982). Thaman et al. (2006) demonstrated that VO_2 max was inversely correlated with NT-proBNP levels in patients with hypertrophic cardiomyopathy, suggesting that this biomarker measurement may be a reliable surrogate for assessing exercise performance. As the recovery period is considered as a return to normal activity levels, NT-pro BNP levels might be used to evaluate both the degree of cardiovascular deconditioning and exercise performance.

During dry immersion, NT-proBNP exhibited a decreasing trend that did not reach statistical significance. Contrary to our hypothesis, the decrease in cardiac load during dry immersion was not sufficient to induce a significant decrease in NT-proBNP.

Limitations of the study

The two daily standing sessions for weighing and hygiene could influence hemodynamic, hormonal, and electrolyte responses induced by dry immersion. However, DI experimental design classically includes authorized daily short-term standing with minimal physical activity. The effects of this brief standing might be limited compared to the effects of prolonged DI. Besides, plasma volume changes were only estimated indirectly. However, the Dill and Costill method had been validated in a bed rest model (Belin de Chantemele et al. 2006). We can assume that it is also applicable in DI. We interpret the increase in NT-proBNP in the recovery period as the result of the increase in cardiac work. However, cardiac work was only partially estimated by the increase in heart rate in the standing position. Our findings should be interpreted in light of these considerations.

In summary, our results suggest that chronic immersion rapidly induces a new level of water-electrolyte homeostasis. Increases in NT-proBNP levels during the recovery period may be

related to an increase in cardiac work during the normal daily load induced by increased cardiac wall stress and increased heart rate. NT-proBNP levels might reflect the degree of cardiovascular deconditioning after enhanced physical inactivity. Further studies aimed at determining the possible value of NT-proBNP as an index of cardiovascular deconditioning are clearly warranted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Blood biochemistry before, during and following dry immersion

	B-2	**DI 3**	**DI 7**	**R+4**	**R+7**
Na^+ (mM)	141.9±0.7	138.3±1.6	139.1±1.4	140.5±0.7	139.5±1.2
K^+ (mM)	4.2±0.07	4.41±0.12	4.4±0.06*	4.01±0.03	4.1±0.12
Proteins (g/L)	78.4±1.3	78.6±2.3	75.9±1.6	75.9±1.5*	76.9±1.9
Creatinine (µM)	87.8±2.3	98.8±4.1*	96.3±2*	95.3±4.2	88.5±4.9
Osmolality (mOsm/kg)	296±1.7	289±4.2	284±4.7*	288±2.8	292±4.4

B, baseline; DI, dry immersion; R, recovery; values are means ± SEM, * $p<0.05$ vs. baseline.

Table 2. Urine biochemistry before, during, and following dry immersion

	B-2	**B-1**	**DI 1**	**DI 2**	**DI 3**	**DI 4**	**DI 5**	**DI 6**	**DI 7**	**R+1**	**R+2**	**R+3**
K^+ (mmol/24h)	58±6	49±6	59±8	40±4	48±4	56±8	63±5	64±5	59±9	51±6	59±4	67±11
Na^+/K^+ ratio	3.2±0.5	2.9±0.3	4.8±0.2*****	3.3±0.4	2.9±0.2	2.6±0.1	2.2±0.1	2.3±0.1	2.2±0.3	1.8±0.7	1.5±0.2*****	2.1±0.2
Creatinine (mmol/24h)	12.2±1.1	11.5±1.1	12.7±1.1	11.6±0.9	11.8±1	12.2±1.1	13.8±1	13.9±0.8	10.3±0.8	11.9±1.6	12.6±1.2	11.6±1.9
UFC (µg/24h)	23±3	23±2	53±10*****	31±4	30±4	31±5	28±3	29±4	23±2	30±4	25±3	20±3
Diuresis (ml/24h)	1600±160	1840±230	3820±380*****	2710±280*****	2650±300*****	2680±280*****	2670±230*****	2810±200*****	2810±300*****	1300±90*****	1640±210	1550±200
Water intake (ml/24h)	3770±210	3710±210	3700±240	3620±250	3570±200	3710±230	3650±180	3610±180	3580±300	3800±210	3800±210	3610±210

B, baseline; DI, dry immersion; R, recovery; UFC, urinary free cortisol; values are means ± SEM, * $p<0.05$ vs. B-1

Legends for figures

Figure 1. The protocol. B-2 and B-1, baseline days; DI 1-DI 7, days of dry immersion; R+1 – R+7, recovery days; circles, days of blood and urine sampling; arrows, days of active orthostatic test

Figure 2. Plasma volume variations. Values are means ± SEM; * significant difference vs. baseline, $p<0.05$

Figure 3. Variation over time in partial water balance, urinary sodium, and urinary osmolality. B, baseline; DI, dry immersion; R, recovery; values are means ± SEM; * significant difference vs. B-1, $p<0.05$

Figure 4. Variation over time in levels of NT-proBNP, active renin, and aldosterone in plasma and serum. B, baseline; DI, dry immersion; R, recovery; values are means ± SEM; * significant difference vs. B-2, $p<0.05$

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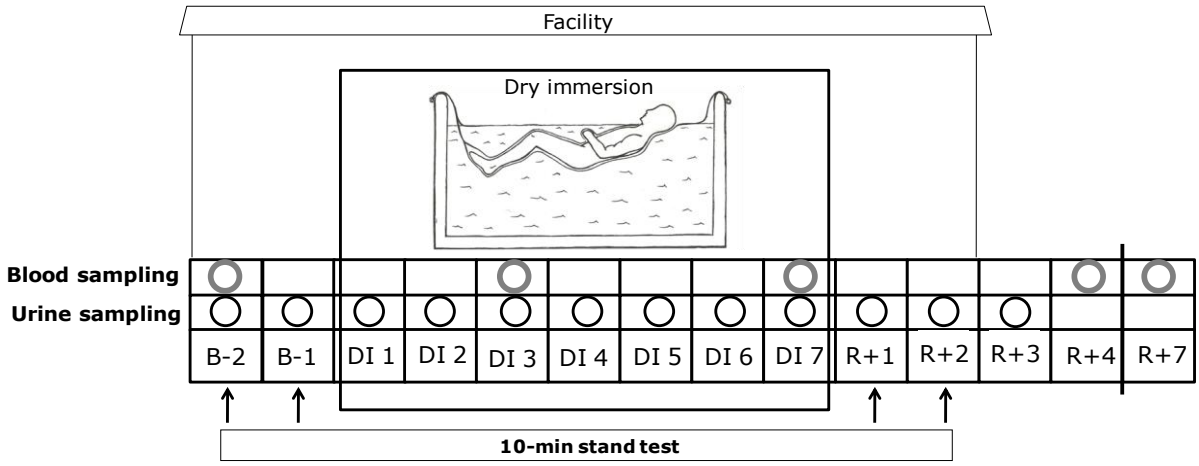
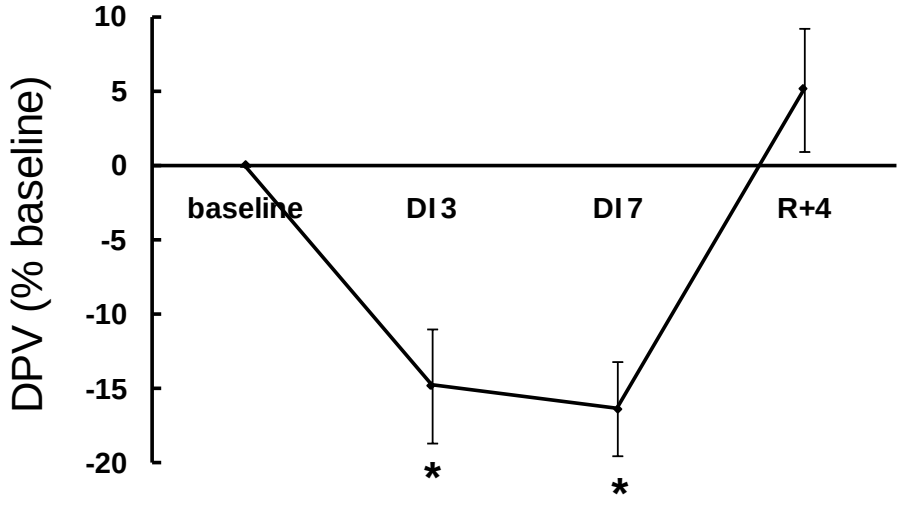


Figure 2. Plasma volume variations. DPV, delta plasma volume. Values are means ± SEM; * p<0.05 vs. baseline



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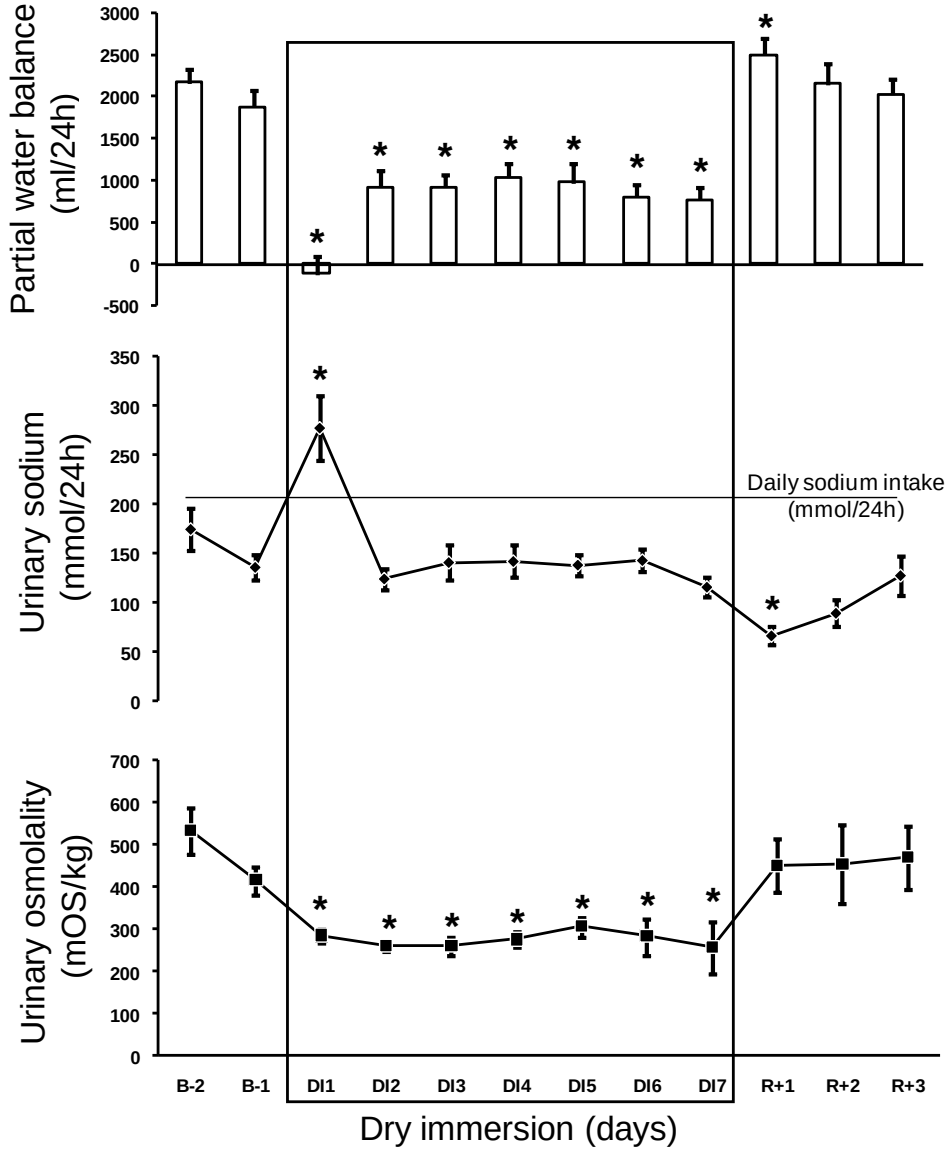

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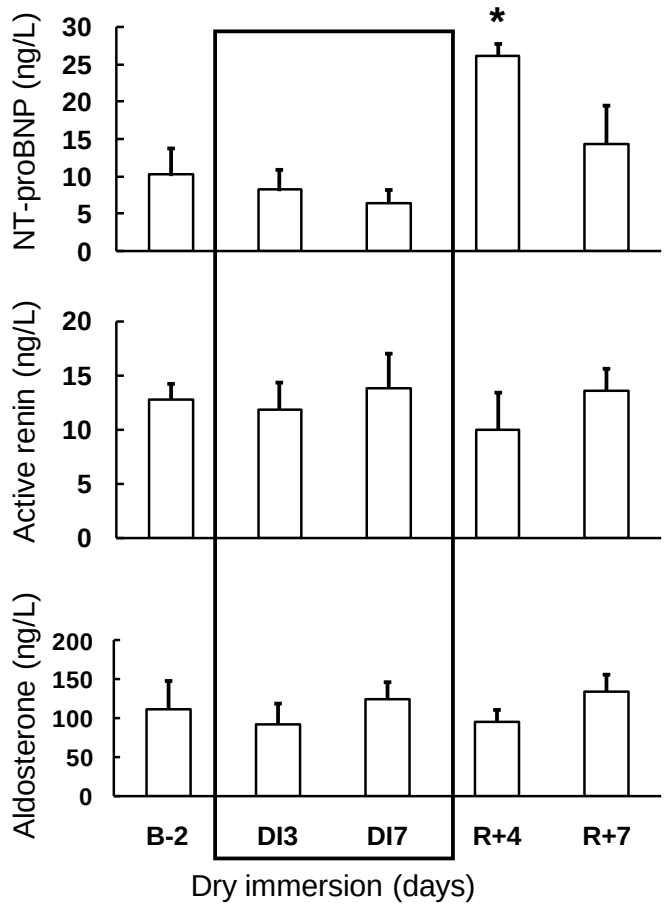

TRAVAIL EXPERIMENTAL CHEZ L'ANIMAL :

MECANISMES DE LA DYSFONCTION VASCULAIRE, NOUVELLES HYPOTHESES

A. Nécessité des modèles animaux de microgravité

B. Liens avec la dépense énergétique

C. Hypothèse des endotoxines

D. Modifications vasculaires ultrastructurelles

A. NECESSITE DES MODELES ANIMAUX DE MICROGRAVITE

Le modèle animal demeure un modèle d'étude nécessaire

"WHAT ARE THE FUTURE TOP PRIORITY QUESTIONS IN CARDIOVASCULAR RESEARCH AND WHAT NEW HARDWARE NEEDS TO BE DEVELOPED?"

Navasiolava N, Custaud MA. - Respiratory Physiology & Neurobiology 169 Suppl 1:S73-74, 2009

Le modèle animal demeure nécessaire pour les études de physiopathologie et les tests de contremesures potentielles. Les contremesures actuellement utilisées ou en cours d'évaluation pour le système cardio-vasculaire sont essentiellement des contremesures physiques (LBNP / exercice physique / centrifugation). Le modèle animal permet de tester de nouvelles contremesures, en particulier pharmacologiques. Un travail important reste à conduire sur ce modèle. Si les effets cardio-vasculaires, en particulier sur le remodelage vasculaire, ne font plus de doute, l'équivalent d'un déconditionnement cardio-vasculaire chez l'animal reste à prouver.

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Short communication

What are the future top priority questions in cardiovascular research and what new hardware needs to be developed?☆

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ABSTRACT

The final discussion that took place at the Cardiovascular Workshop in Strasbourg in November 2008 identified the following priorities for our future research and the need to develop new dedicated cardiovascular devices. Knowledge and treatment of cardiovascular deconditioning remain limited. The prospect of very long duration space flights could lead to the emergence of new cardiovascular risks. We need to maintain our efforts to standardize our experimental protocols and analysis equipment. We continue to rely on animal models for cardiovascular research. To continue our studies we recommend developing specific tools for the space environment so that they can be made available to the scientific and medical community.

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1. Introduction

The field of life sciences in microgravity is closely linked to Man's exploration of space. The work of our scientific community is intended to be directly applied to space medicine. These studies are also being conducted as part of a more fundamental approach, in order to understand how gravity affects physiology. We should not forget that gravity has shaped life over hundreds of millions of years. Both real and simulated microgravity conditions are being used for physiology research models.

The cardiovascular system is particularly sensitive to environmental conditions, especially gravity, and the countermeasures currently in use do not go far enough to prevent cardiovascular disorders being caused by the space environment.

For space agencies and research teams that study how the space environment alters the cardiovascular system, the last few years have been very significant:

- in terms of resources deployed (with international programs to simulate microgravity on the ground and the launch and commissioning of the Columbus module in the International Space Station);
- in terms of the international context, particularly with the development of an ambitious Chinese space program alongside long term programs from NASA, from the Russian Space Agency and from the European Space Agency;
- in terms of objectives, with a shift towards long duration flights, Man's return to the Moon and preparations for a mission to Mars.

This paper summarizes the discussion that took place at the Cardiovascular Workshop in Strasbourg in November 2008 to identify priority research themes and the new systems to be developed for studying the cardiovascular system in microgravity.

2. Research themes to be pursued

The cardiovascular deconditioning syndrome remains an operational problem that is not sufficiently prevented by current countermeasures. If long duration flights such as those needed to reach Mars are to become a reality, it will be essential to evaluate and take into account the specific vascular and cardiac risks incurred. The question of a possible vascular risk being caused by very long duration flights is an open one (the effects of intensive, prolonged lack of physical activity and the potentially harmful effects of radiation) as is that of a cardiac risk (rhythm disorders).

Several priority research themes have been identified:

- It is vital to continue developing countermeasures (optimizing current countermeasures, developing new integrated physical countermeasures, considering pharmacological or nutritional countermeasures).
- A consensus has not yet been reached on the consequences of microgravity on electrocardiograms and cardiac rhythm. It needs to be determined whether the space environment significantly increases the risk of cardiac rhythm disorders.

☆ This paper is part of a supplement entitled "Cardio-Respiratory Physiology in Space", guest-edited by P. Norsk and D. Linnarsson.

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- We should continue striving to understand the changes in vascular functions and regional circulation caused by the space environment. The study of renal perfusion is particularly important given the role played by the kidneys in regulating blood pressure, blood volume and fluid compartments more generally.
- The study of the sympathetic nervous system remains difficult. It is particularly hampered by the limited standardization of analysis methods. Despite the extensive work already undertaken, we still know very little about the effects of real or simulated microgravity on the autonomic nervous system. It is understood that the sympathetic nervous system is not activated globally but has specific regional characteristics that must be taken into account. This question can be added to the one about blood circulation, which also requires an approach at the organ level.
- Given the artificial environment recreated by humans inside space vessels, it is essential to investigate further circadian rhythms and their relationship with cardiovascular regulation.
- It is vital to continue our work with an integrated physiological approach by studying the relationships between, on the one hand, cardiovascular regulation, and on the other, neuro-vestibular regulation, regulation of energy metabolism, regulation of the body's fluid compartments and thermoregulation.

Alongside the essential work carried out on humans during ground simulations, we would like to stress the following aspects: standardization of tools, use of animal models and development of new devices for the space environment.

3. Efforts to standardize studies and data analysis

A harmonization process is already underway and has led to the definition of basic standardized measurements, which are taken systematically during the different experiments. The standardization work should be continued and in particular with respect to studies of the vegetative nervous system using methods that measure the variability of heart rate and blood pressure. Many studies have been conducted and there are several analysis tools which themselves depend on numerous variable factors. These study methods should be standardized for our community.

4. Development of animal models

Animal models remain necessary for pathophysiology studies and tests of potential countermeasures. The countermeasures currently in use or being tested for the cardiovascular system are essentially physical countermeasures (Lower Body Negative Pressure, physical exercise, and centrifugation). Animal models enable new countermeasures to be tested, particularly pharmacological ones. Much work remains to be done on this model; although there are no longer any doubts about the cardiovascular effects, particularly on vascular remodeling, the equivalent of cardiovascular deconditioning in the animal remains to be proven. The work on these animal models will mostly be done on the ground, but studies carried out in actual microgravity conditions will also be essential. There have been very few space flights with rodents (mice, rats, gerbils) in recent years and this lack should be compensated for over the next few years.

5. Development of new devices designed for the space environment to study the cardiovascular system and prevent cardiovascular disorders

To complement previous investigations, the following should be developed for a space environment:

- There is no doubt that new physical countermeasures need to be developed for interplanetary missions. The choices made will depend on the results of tests conducted on the ground (on systems currently being evaluated such as the Flywheel, the Lower Body Negative Pressure + Treadmill, or the short-arm centrifuge) and the development of new devices (such as the ICARE system).
- The precise study of cardiac functions will require a high-performance electrocardiogram, and would benefit from a three-dimensional ultrasound system.
- The study of regional circulation will require use of the Doppler ultrasound technique with high-performance devices for studying parenchymatous perfusions. The specific study of cutaneous microcirculation will require use of the laser-Doppler technique. This method, combined with the iontophoresis of pharmacological agents, will enable specific vascular functions to be studied, such as the endothelial function or the function of vascular smooth muscle.
- Measurements of fluid compartments using bioelectrical impedance analysis methods will also complement cardiovascular measurements. A precise knowledge of the body's composition is necessary for studying metabolism and cardiovascular regulation.

When designing new systems, we should continue to integrate or miniaturize systems using the latest technologies (development of portable and even implantable systems, transcutaneous systems for analyzing blood markers, analyzing blood parameters from a drop of blood, or even transcutaneously). Again with very long duration flights in mind, we should identify, among all the measurable cardiovascular parameters, those which are essential for the medical supervision of astronauts.

6. Conclusion

The cardiovascular system has been studied ever since the beginning of human space exploration, because cardiovascular supervision of astronauts is critical to space missions. The cardiovascular system must nevertheless remain a priority research theme if man is to succeed in conducting long and very long duration space missions.

B. LIENS AVEC LA DEPENSE ENERGETIQUE

La microgravité simulée induit l'inhibition du métabolisme couplé avec l'altération de cytokines proinflammatoires

"METABOLISM IN RATS DURING ANTIORTHOSTATIC HYPOKINESIA"

Novoselova AM, Custaud MA, Tsvirkun DV, Larina IM, Kulchitsky VA. - Bulletin of Experimental Biology and Medicine 146(1):38-40, 2008

Les variations d'intensité métabolique en microgravité demeurent mal connues. D'un côté, l'hypodynamie est caractérisée par la réduction des dépenses énergétiques ; de l'autre côté, la position anormale est accompagnée d'un stress qui augmente l'intensité du métabolisme.

Le but de ce travail était d'estimer l'effet de l'hypokinésie et de la microgravité simulée par l'inclinaison tête en bas (HDT) discontinu chronique de 14 jours sur l'intensité du métabolisme (production de la chaleur). L'objectif secondaire était d'étudier les relations entre dépenses énergétiques, le niveau de cytokines proinflammatoires et les paramètres caractérisant la thermorégulation (circulation régionale dans les tissus de la queue).

Nous avons observé une réduction de production de chaleur, la chute de l'interleukine-6 sérique et une vasodilatation prolongée dans les tissus de la queue chez les rats soumis au HDT discontinu chronique. Ces variations peuvent être les marqueurs d'atteintes des interactions entre les systèmes fonctionnels de thermorégulation physique et chimique et du système immunitaire lors de la microgravité simulée.

Metabolism in Rats during Antiorthostatic Hypokinesia

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O_2 consumption and CO_2 release in 3 groups of awake rats were studied on a MM-100 metabolic monitor system (CWE Inc.). The animals of 2 groups were preadapted to 4-h maintenance in special boxes (2 weeks). The rats could perform rotational movements and limited movements in the rostrocaudal direction (hypokinesia). The animals of one group were daily exposed to 4-h antiorthostatic load (≤45°) for 2 weeks. After 2 weeks, the intensity of metabolism in rats with antiorthostatic hypokinesia was lower than in hypokinetic specimens (by 15-20%, $p<0.05$) and freely moving animals (by 20-25%, $p<0.05$). Interleukin-6 concentration in rats with antiorthostatic hypokinesia (0.25±0.09 pg/ml) was lower than in hypokinetic (4.01±0.57 pg/ml) and freely moving animals (3.69±0.56 pg/ml). The decrease in the concentration of a proinflammatory cytokine interleukin-6 during experimental antiorthostatic hypokinesia reflects inhibition of metabolic processes, which are activated during antiorthostatism (but not hypokinesia).

Key Words: *antiorthostatism; hypokinesia; indirect calorimetry; interleukin-6; skin temperature*

Antiorthostatic hypokinesia (ANOH) is typical of clinical practice and serves as an experimental model in studies of the regulation of functional systems under conditions of weightlessness [1]. There is no general agreement about the processes occurring in mammals during experimental microgravity. Little is known about the mechanisms of adaptation to a specific posture [3,4,11,12]. Under these conditions, the factors of volume and pressure contribute to the increase in hemodynamic load on the heart and major reflexogenic zones in blood vessels. What is the period of adaptation? It remains unclear whether adaptation concerns only functional systems for the maintenance of homeostasis [6,9] or induces a variety of structural changes in organs and tissues. Specific variations in metabolic processes are unknown. On the one hand, hypodynamia is characterized by the reduced energy consumption. On the other hand, abnormal body position is accompanied by a stress response and activates heat production. Does blood flow redistribution during ANOH involve only the processes occurring in the sympathetic nervous system [1]? The question arises: do hormonal [4] and other regulatory factors of the vascular [9] or humoral nature play a role in the complex process of hemodynamic changes? Here we studied the role of proinflammatory cytokine interleukin-6 (IL-6) in metabolic transformations in rats with ANOH.

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats weighing 0.25±0.03 kg. The animals were maintained in a vivarium under standard conditions (21±1°C, 12/12-h artificial light-dark cycle) and had free access to food and water. The rats were preadapted

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to experimental conditions for 2 weeks. This procedure involved handling and 9-10 training sessions in special boxes. The time of training varied from 30 min to 3-4 h [10]. The rats could perform rotational movements and were restrained in the rostrocaudal direction. The animals were divided into 3 groups (8 rats per group). Group 1 rats were daily exposed to 4-h ANOH in boxes (P45°) for 2 weeks. Group 2 rats were daily subjected to 4-h hypokinesia in boxes (similarly to group 1 rats). Freely moving rats of group 3 served as the control. The intensity of metabolism in animals of 3 groups was studied on a MM-100 metabolic monitor system (CWE Inc.). Monitoring was performed before handling, 2 weeks after adaptation to experimental conditions, and 1 or 2 weeks after the start of ANOH. THE MM-100 system allowed us to measure air flow (liter/min) in the individual metabolic cell (total of 8 cells) and to estimate the concentrations of O_2 and CO_2 in the inspired and expired air (%). The respiratory exchange ratio was calculated from O_2 consumption (VO_2, ml/h) and CO_2 release (VCO_2, ml/h) as follows: $RER=VCO_2/VO_2$. The intensity of heat production was estimated taking into account the level of O_2 consumption per unit time, respiratory exchange ratio, and weight of animals (kcal/kg/h). The data were processed by MMCom software and recorded in the ASCII format.

On day 14, the intensity of metabolism in 24 rats was studied for 5 min. The animals were adapted to metabolic cages for 40-60 min. Group 1 and 2 rats were placed in the antiorthostatic position using special platforms in metabolic cages. The intensity of metabolism was studied after 40 and 80 min. Gas exchange parameters in all rats were recorded after resumption of the horizontal position. The intensity of metabolism in freely moving rats of groups 1 and 2 was studied after removal from boxes.

Copper-constatan thermocouples for recording of surface body temperature (Physitemp thermometer) were fixed on the ventral surface of tail skin in 3 rats by the 2nd week after adaptation to experimental conditions [10]. Skin temperature was monitored at 29°C for 4 h. The animals were exposed to ANOH for 3 h.

After the last recording of metabolic parameters, the rats were decapitated using a SAD 51330 guillotine. The blood was sampled and centrifuged at 1500 rpm for 15 min. Serum IL-6 concentration was measured by means of ELISA with Biosource Internat. kit for rats.

The results were analyzed by Student's *t* test and one-way analysis of variance (ANOVA). The differences were significant at $p<0.05$.

RESULTS

Parameters of heat production in rats of 3 groups on day 14 of the study are presented on (Fig. 1, *a*). The intensity of metabolism in group 1 rats decreased to 0.0026±0.0001 kcal/kg/h ($p<0.05$) and was lower compared to control animals (0.0035±0.0002 kcal/kg/h) and group 2 specimens (0.0033±0.0001 kcal/kg/h). On day 14, no differences in metabolic changes were revealed in group 1 and 2 rats during 40-min antiorthostasis in a metabolic cage (Fig. 1, *b*). After 80-min ANOH (Fig. 1, *c*) the intensity of metabolism in group 1 and 2 animals was 0.0036± 0.0002 and 0.0046± 0.0002 kcal/kg/h, respectively ($p<0.05$). Intergroup differences in the intensity of metabolism were found after the boxes were placed horizontally (0.0031±0.0001 and 0.0037±0.0002 kcal/kg/h, respectively, $p<0.05$; Fig. 1, *d*). No intergroup differences in metabolic processes were revealed in freely moving animals after removal from boxes.

On day 14, IL-6 concentration in group 1 rats decreased to 0.25±0.09 pg/ml ($p<0.01$) and was lower than in group 2 animals (4.01±0.57 pg/ml) and control specimens (3.69±0.56 pg/ml).

Tail skin temperature in rats varied from 30 to 33°C at 2-3-min intervals (atmospheric temperature 29°C). Skin temperature decreased to 29°C several seconds after the start of ANOH, remained unchanged for 10-15 min, and increased to 35-36°C over the next 2-3 min. Temperature remained unchanged for 1 h and progressively decreased to 31-32°C over 30 min. Tail skin temperature increased to 34-35°C after 5-10 min and remained unchanged for 30-40 min.

The concentration of proinflammatory cytokine IL-6 is affected by various factors, including stress, hemorrhage, trauma, infection, postural response, and endotoxin translocation from the gastrointestinal tract into the internal medium [5,7,8]. Among a variety of IL-6-producing cells in organs and tissues, particular attention is paid to vascular endothelium. ANOH is accompanied by changes in hemodynamic load on this structure [2]. It could be expected that repeated ANOH and hypokinesia for 2 weeks would increase the concentration of IL-6. However, IL-6 concentration in hypokinetic rats did not differ from that in control specimens (freely moving animals). However, serum IL-6 concentration decreased by more than 10 times during ANOH. The decrease in IL-6 concentration in the internal medium during ANOH was accompanied by the inhibition of metabolism. The increase in skin temperature during ANOH is related to prolonged dilatation of blood vessels and elevation of blood flow

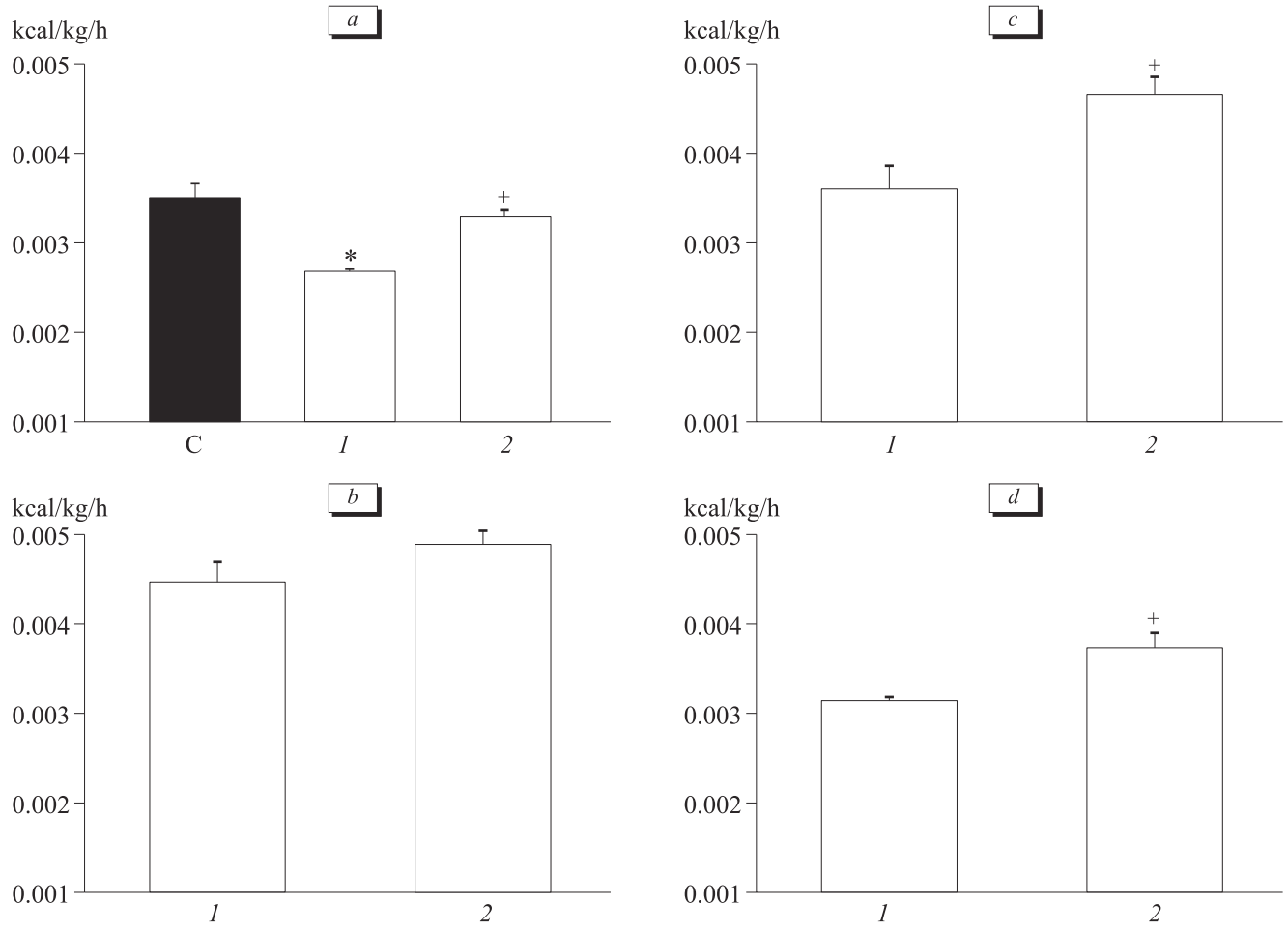

Fig 1. Heat production in rats on day 14. (*a*) Freely moving animals (*C*), ANOH (*1*), and hypokinesia (*2*). Antiorthostatic load test for 40 (*b*) and 80 min (*c*), and after the boxes were positioned horizontally after load test (*d*). $p<0.05$: *compared to the control (*C*); +compared to ANOH.

in tail tissues [10]. These changes are probably associated with the increased production of nitric oxide [7] and accompanied by intensification of heat emission [10]. These features (prolonged vasodilation, inhibition of metabolism, and decrease in serum IL-6 concentration during experimental ANOH) may serve as markers of processes, which reflect the impaired interaction between functional systems on the model of weightlessness. The observed changes are manifested in pathological disorders under natural conditions of space flight.

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C. HYPOTHESE DES ENDOTOXINES

L'une des raisons probables pour expliquer les dysfonctions endothéliales en microgravité est la translocation non-contrôlée d'endotoxines de l'intestin

"RESPONSE TO LPS INJECTION AFTER ANTI-ORTHOSTATIC HYPOKINESIA IN RATS, AN HYPOTHESIS FOR ENDOTHELIAL DYSFUNCTION"

Navasiolava NM, Pashkevich SG, Custaud MA, Emelianova AA, Kulchitsky VA. - Journal of Gravitational Physiology 15 (1):69-70, 2008

"ОСОБЕННОСТИ ПРОЦЕССА ТРАНСЛОКАЦИИ ЭНДОТОКСИНОВ В ОРГАНИЗМЕ ЧЕЛОВЕКА И ЖИВОТНЫХ / DES PARTICULARITES DE TRANSLOCATION DES ENDOTOXINES DANS L'ORGANISME HUMAIN ET ANIMAL"

Martusevich NO, Pashkevich SG, Navasiolava AM, Patseev SV, Custaud MA, Kulchitsky VA. - News of biomedical sciences 3:7-11, 2009

Les endotoxines (ou lipopolysaccharide, LPS) sont des toxines de nature glucido-lipo-protéique situées dans la membrane externe des bactéries Gram négatif. Le but de cette étude était de vérifier l'hypothèse que la translocation de LPS de l'intestin augmente avec la microgravité simulée, ce qui pourrait être l'un des facteurs de dysfonctionnement endothélial. Nous avons observé chez le rat une augmentation de LPS dans le sérum après head-down tilt. La réponse thermique et l'élévation de l'interleukine-6 à l'injection de LPS (exogène) après microgravité simulée par oscillation verticale et par head-down tilt étaient diminuées (le phénomène de tolérance). De plus, une décontamination préalable du tractus gastro-intestinal prévenait ces réponses. La conjonction de ces résultats peut indiquer une translocation augmentée de LPS par la microgravité simulée.

RESPONSE TO LPS INJECTION AFTER ANTI-ORTHOSTATIC HYPOKINESIA IN RATS, AN HYPOTHESIS FOR ENDOTHELIAL DYSFUNCTION

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ABSTRACT. The indirect estimation of lipopolysaccharide (LPS) translocation by measuring of IL-6 serum level after LPS injection was carried out. We found a decreased response to LPS injection as the result of previous LPS translocation in rats after head-down tilt -45° (HDT) during 7 days and in rats after the repeated injection of LPS in 7 days after the first injection. Under electron microscopy, fringe-looking protrusions in vessel lumen, disturbance of basal membrane integrity and infringement of interendothelial junctions, adhesion of leukocytes were found in arterioles of these groups of animals. So, we suppose that translocation of LPS from gut could occur in simulated weightlessness model in rats (HDT) and could be associated with endothelial dysfunction.

INTRODUCTION. The multifunctional role of endothelium, which forms an internal surface of blood and lymphatic vessels, is shown in the control of vasomotor functions and hemodynamic, permeability, proliferation, maintenance of immune balance [1]. In this approach it is clear, why endothelial cells are involved in formation of many protective reactions, and why their dysfunction after influence of extreme factors is reflected in the control of histohematic barriers [1, 5]. Decreasing of protective properties of barriers promotes increase of LPS translocation (endotoxemia) that is accompanied by development of systemic inflammatory process with increasing of cytokines, prostaglandins. In a case of repeated LPS translocation a tolerance usually develops [2, 3]. What are the changes of endothelial cells in the different vascular regions under the repeated HDT, when, according to above mentioned literary data, the conditions for development of tolerance could be created?

METHODS. . In experiments on rats (n=46) the shifts of IL-6 in serum and ultrastructural features of endothelium of brain, gut and hindlimb vessels were studied after hypokinesia for 7 days, 4 hours a day (I), HDT for 7 days, 4 hours a day (II), intraperitoneal (i.p.) injection of 10 or 100 µg/kg/ml *Escherichia coli* LPS once (IIIa and IIIb, accordingly), twice (IVa and IVb, accordingly), i.p. injection of 100 µg/kg/ml LPS in rats with HDT for 7 days, 4 hours a day (V), i.p. injection of 1 ml/kg Ringer solution (VI). Control animals were in the state of free behavior (VII). After 7 days of experiment LPS (groups IIIa, IIIb, IVa, IVb, V) or Ringer solution (group VI) injection was performed followed 3 hours later by decapitation for all the groups. The blood was collected for evaluation of IL-6 (ELISE method). The samples of brainstem, gut and hindlimb vessels were taken and fixed first in 2.5% glutaraldehyde and then in 1% osmium tetroxide. After fixation the material was washed with buffer, dehydrated in alcohols of graded strength, and embedded in Araldite. Sections were cut with a LKB-III Ultratome and scanned with a JEM-IOOB electron microscope (Japan). The data were statistically treated using the Student's t-test, followed by one-way and two-way ANOVA test. All data are presented as means ± SE.

RESULTS, DISCUSSION The level of IL-6 in group V consisted 1.89±0.06 pg/ml and was significantly lower ($P<0.001$), than in group IIIb (2.18±0.04 pg/ml). In group IVb where LPS was injected on the first and the seventh day of investigation, a level of IL-6 did not differ from that in group V with HDT and made 1.99±0.10 pg/ml, which testifies the development of tolerance to LPS in both groups. The mechanism of tolerance development in group IV is quite known and analogous to some processes after vaccination. In group V with HDT a LPS areactivity could be caused by previous penetration of LPS into the inner environment, f. e., from gastrointestinal tract due to endotoxin translocation during HDT. Under electron microscopy protrusions in vessels lumen similar to a small fringe, disturbance of basal membrane integrity and infringement of

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interendothelial junctions (Figs. 1-4), adhesion of leukocytes were found in arterioles of groups II, IIIa, and IIIb.

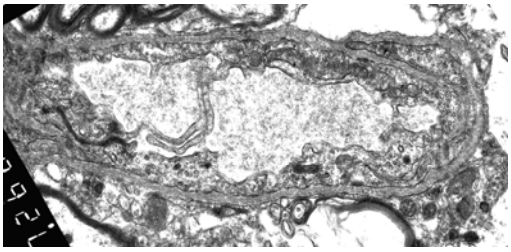

Fig. 1. Reduction of thickness and invaginations of an endothelial layer of the rat medulla oblongata blood vessel in 7 days after head-down tilt -45°. x 10 000.

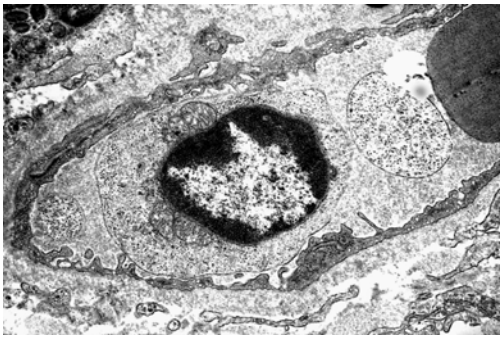

Fig. 2. Reduction of thickness of an endothelial layer of a colon blood vessel in 7 days after i.p. injection of 10 μg/kg LPS *E.coli*. x 4800.

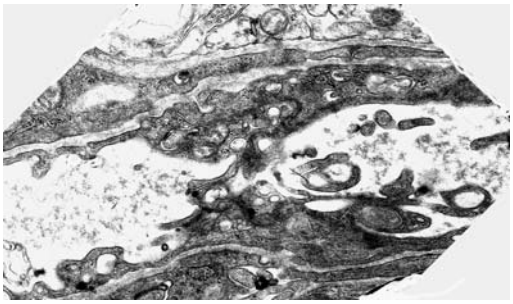

Fig. 3. Several invaginations in a blood vessel lumen of the rat medulla oblongata in 24 h after i.p. injection of 10 μg/kg LPS *E.coli*. x 10 000.

HDT alters blood flow in the regional vascular beds. This is accompanied by the response of the vascular wall, including changes in the activity of NO-synthases [6], endothelial secretion and reception, interactions of endothelium and smooth muscles [5]. Under these conditions the resistance of the gastrointestinal tract barriers is reduced, and endotoxin translocation is increased [4]. LPS induces the systemic inflammatory reaction, triggered by proinflammatory cytokines, in particular by IL-6 [3]. This shift is found in the experiments with single and double injections of LPS and with HDT without LPS injection, which testifies involvement of the endotoxins of the gastrointestinal tract in the development of reactions at HDT.

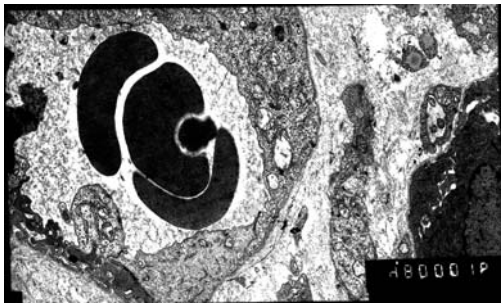

Fig. 4. Ultrastructural changes of a colon blood vessel in rat in 24 h after i.p. injection of 10 μg/kg LPS *E.coli*. There is an invagination below at the left in a vessel. Mitochondrial crists are destroyed. x 4800.

The observed endothelium dysfunction can be accompanied by not only local disturbance of microcirculation, but also by system shifts in an organism, first of all due to the LPS translocation.

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ОСОБЕННОСТИ ПРОЦЕССА ТРАНСЛОКАЦИИ ЭНДОТОКСИНОВ В ОРГАНИЗМЕ ЧЕЛОВЕКА И ЖИВОТНЫХ

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Среди сложных взаимоотношений желудочно-кишечного тракта – одной из важнейших функциональных систем организма, и всего организма в целом, выделяют аспект физиологической и патофизиологической роли микрофлоры кишечника в поддержании гомеостазиса в норме и при патологии [2, 4, 9, 11–13, 20, 23–26, 30]. Речь идет о естественной способности микрофлоры и эндотоксинов проникать в условиях нормы и патологии из кишечника во внутреннюю среду организма (процесс транслокации) [8, 9, 11, 23, 24]. В принципе, вопрос взаимоотношений микрофлоры и макроорганизма обсуждается в общем плане широко, но без акцента на конкретные механизмы этого взаимодействия. Необходимость детализации особенностей симбиоза микробов и организма и условий его распада, сопровождающихся затяжным противостоянием вплоть до летального исхода одной или обеих противоборствующих сторон, назрела давно, но, к сожалению, слабо проанализирована учеными. Выделяют как позитивные, так и негативные стороны данного процесса, механизмы которого фактически не изучены. Подтверждением этого вывода являются порой противоположные взгляды исследователей на суть процесса симбиоза. Так, по мнению ряда авторов, рассматривающих взаимоотношения микрофлоры и макроорганизма как эволюционно сформировавшийся симбиоз, транслокация эндотоксинов в физиологических условиях является необходимым условием для естественного функционирования всего организма, в частности, для поддержания оптимального уровня глубокой температуры тела у теплокровных животных [3, 6, 10, 17, 18, 26]. Другие исследователи на основании результатов экспериментов полагают, что ведущая функция микрофлоры кишечника заключается в постоянной активации нервных окончаний висцеральных органов. Это обеспечивает регуляцию и поддержание тонуса автономной нервной системы [22]. Существует также мнение о том, что процесс транслокации эндотоксинов из желудочно-кишечного тракта в кровоток является условием нормального функционирования иммунной системы организма, выступая в роли своеобразного триггера иммунокомпетентных клеток [1]. Если это действительно так, то процесс транслокации является неотъемлемым элементом системы защитных реакций. От интенсивности перемещения эндотоксинов во внутреннюю среду организма зависит способность организма противостоять действию повреждающих факторов экзогенного и эндогенного генеза.

Мнение экспериментаторов о позитивной роли процесса транслокации в большинстве работ клиницистов опровергается фактами негативных сдвигов в различных тканях и органах при эндотоксемии [11, 15, 16, 24–26, 28, 30]. Полагают, что именно транслокация токсинов и микробов и ассоциированная с ней эндотоксемия, являются причиной развития генерализованных форм инфекции (вплоть до сепсиса) и внекишечных эшерихиозов и септицемии [24–26, 28, 30]. Невольно напрашиваются ассоциации с философскими умозаключениями о переходе количества в качество, но со-

стояние больных, страдающих от такого «перехода» и от подобных рассуждений, не улучшается.

Известно, что транслокация бактерий и эндотоксинов вызывается множеством факторов (ишемия, гипоксия, кровопотеря, авитаминозы, колиты, иммунопатия, стрессы). Среди них можно выделить те, которые связаны с перемещением тела в пространстве, например, вертикальное укачивание [3] и антиортостатическую гипокинезию [5, 6]. Основываясь на ранее полученных данных о транслокации эндотоксина в лимфатические узлы и стенку кишечника после вертикального укачивания [2], мы заключили о стабильности развития разной степени эндотоксемии в указанных условиях в результате перемещения липополисахарида из желудочно-кишечного тракта во внутреннюю среду организма. Известно, что эндотоксин через сложные механизмы, включающие CD14, CD44, толл-рецепторы, активирует систему комплемента и монооксида азота, вызывает наработку и выброс провоспалительных цитокинов (TNF, IL_{β}, IL6), простагландинов группы Е и ряда гормонов [12, 17–19, 27, 29]. Каскад нейрогуморальных процессов, поддерживающих и следующих за активацией регуляторных субстанций, приводит к формированию системного воспалительного ответа, который в зависимости от выраженности эндотоксемии может привести к шоку и летальному исходу.

Есть еще один актуальный аспект проблемы транслокации эндотоксинов. Если гипотеза о развитии эндотоксемии эндогенного происхождения в условиях вертикального укачивания, при антиортостатической гипокинезии и иных вариантах изменения действия гравитационного фактора верна, то можно ожидать трансформацию защитных реакций организма при повторном введении эндотоксина крысам после моделирования этих состояний. При этом реально ожидать как усиление ответной реакции организма (гиперреактивность), так и ослабление (толерантность). Целью работы была проверка высказанных предположений.

Материал и методы исследования. Осуществляли вертикальное укачивание (0,3g; 45 минут) бодрствующих крыс-самцов (массой 220–300 грамм). После укачивания измеряли массу тела животных, ректальную температуру, артериальное давление, порог висцеро-соматических ноцицептивных реакций. С целью моделирования эндотоксемии в разные периоды наблюдения после вертикального укачивания (1, 3, 7, 14, 30 и 60 дней) вводили внутрибрюшинно 10 мкг/кг липополисахарида *Escherichia coli* [2]. После инъекции эндотоксина в термонейтральных условиях (29°С) регистрировали глубокую и кожную температуры. Осуществляли выделение и изучение свойств (морфологических, ферментативных, патогенных) культуры *Escherichia coli* кишечника крыс, определяли чувствительность кишечной микрофлоры к антибиотикам. Селективную деконтаминацию кишечника проводили путем внутрибрюшинного введения гентамицина (*Gentamycini sulfatis*). При моделировании антиортостатической гипокинезии в 12 сериях опытов на крысах забор крови для определения в сыворотке крови содержания эндотоксина осуществлялся в разные периоды времени в зависимости от задач опыта. [5, 6]. После последней регистрации показателей метаболизма крыс декапитировали с помощью гильотины (SAD 51330, США), осуществляли забор крови, которую центрифугировали 15 мин при 1500 об/мин. Сывороточную концентрацию ИЛ-6 измеряли методом иммуноферментного анализа (ELISA) с помощью набора для крыс (Biosource Internat., Camarillo, CA, США) [6]. Статистическую обработку данных проводили с использованием *t*-критерия Стьюдента и однофакторного дисперсионного анализа (*ANOVA*). Различия считались достоверными при $P<0.05$.

Результаты исследования и их обсуждение. На 7-й день после вертикального укачивания наблюдалось ослабление лихорадки по сравнению с контрольной группой животных. В частности, у крыс после укачивания к 90-й минуте наблюдения максимальный прирост глубокой температуры составил 0,5±0,1 °С, а у крыс, не подвергавшихся укачиванию, максимум прироста был зарегистрирован на 150–180-й минутах наблюдения и составил 1,1±0,2 °С ($P < 0,01$). Достоверная разница в ве́личине прироста колонической температуры почти в два раза в этих сериях опытов свидетельствовала о развитии толерантности к липополисахариду кишечной палочки после вертикального укачивания. Толерантность возникает при условии предшествующего поступления «порогового» уровня эндотоксина в кровь [2, 3, 10, 16–18]. В какой-то мере этот процесс ассоциируется с событиями, развивающимися в организме после прививки, но механизмы толерантности и иммунитета различные. Вертикальное укачивание крыс сопровождается развитием пролонгированной гипертензии (подъем артериального давления на 20–25% в течение суток) и гипоалгезии (ослабление висцеро-соматической реакции при раздражении кишки стандартным ноцицептивным стимулом).

Итак, эндотоксемия эндогенного происхождения может регулярно формироваться в процессе перемещения эндотоксинов, включая липополисахарид кишечной палочки и иных грамотрицательных микробов, из желудочно-кишечного тракта во внутреннюю среду организма при изменении действия гравитационного фактора. Этот процесс отражается на формировании защитных реакций организма.

Если эти рассуждения верны, то любые факторы, препятствующие развитию эндотоксемии эндогенного происхождения, в частности после вертикального укачивания, способны предотвратить развитие толерантности к эндотоксину. Одним из таких факторов может быть деконтаминация кишечника. Поскольку в экспериментальных условиях использовался эндотоксин кишечной палочки, то представлялось вполне адекватным применять для деконтаминации препараты, селективно блокирующие *Escherichia coli*. В процессе тестирования набора антибиотиков, аппликированных на культуру кишечной палочки, наиболее эффективными оказались левомицетин (*Laevomycetinum*) и гентамицин (*Gentamicini*). В качестве препарата для проведения деконтаминации был выбран гентамицин (*Gentamycini sulfatis)* в дозе 1 мг/кг. Гентамицин вводили внутрибрюшинно в течение 5 дней в одно и то же время суток. Через 5 дней в стерильных условиях были проведены посевы из слизистой оболочки тонкого и толстого кишечника крыс на дифференциально-диагностические среды, используемые для идентификации *Escherichia coli*. После проведения деконтаминации кишечника рост кишечной палочки на средах ни разу не был зафиксирован.

Далее в соответствии с протоколом исследования было проведено укачивание крыс, которые предварительно подвергались деконтаминации кишечника. Затем на 3-й и 7-й дни после такого гравитационного воздействия крысам внутрибрюшинно вводили 10 мкг/кг липополисахарида *Escherichia coli*. После деконтаминации и укачивания достоверных отличий в развитии лихорадки у крыс по сравнению с животными, которым эндотоксин кишечной палочки в дозе 10 мкг/кг вводился впервые, обнаружено не было. В отличие от этого в другой группе крыс, которые подвергались укачиванию, но у которых деконтаминация не осуществлялась, зафиксирована своеобразная ареактивность на липополисахарид, о чем свидетельствовало то, что развитие лихорадки было ослаблено или не наблюдалось. Подобную невосприимчивость можно объяснить лишь развитием устойчивости к повторному попаданию «пороговой дозы» эндотоксина во внутреннюю среду организма, то есть толерантностью (исходное проникновение эндотоксина в кровь обусловлено неконтролируемым процессом транслокации, когда из кишечника во внутреннюю среду организма перемещается первая «пороговая доза» липополисахарида). Таким образом, экспериментально подтверждена значимость неконтролируемого процесса транслокации эндотоксинов из желудочно-кишечного тракта во внутреннюю среду организма для трансформации функционального состояния систем, участвующих в формировании системного воспалительного ответа организма.

В тексте статьи сознательно используется термин «неконтролируемый процесс транслокации». Авторы полагают, что в условиях дизрегуляции во внутренней среде организма оказывается «патофизиологически» значимое количество эндотоксина. Одним из факторов, который способен вызвать выраженную эндотоксемию, может быть изменение направленности действия гравитации. Наоборот, в физиологических условиях «контролируемый» процесс транслокации липополисахарида из кишечника в кровь является атрибутом и признаком естественного симбиоза микрофлоры и организма. Этот симбиоз необходим для естественного тонуса защитных систем организма, включая иммунную.

Экспериментально проанализирован уровень эндотоксина в крови крыс разных серий опытов (всего 12), которые находились несколько часов (до 4-х) в течение недели в условиях горизонтальной или антиортостатической гипокинезии. Сравнивали уровни эндотоксина в крови крыс разных серий после предварительной деконтаминации, или деконтаминации и введения липополисахарида *Escherichia coli* в разные периоды наблюдения. Оказалось, что введение 10 мг/кг эндотоксина крысам на 7-й день антиортостатической гипокинезии не приводит к развитию лихорадки (толерантность). С другой стороны, инъекция такой же дозы эндотоксина крысам, подвергавшимся антиортостатической гипокинезии и деконтаминации, ведет к формированию типичной двухфазной лихорадки. Уровень эндотоксина в сыворотке крови крыс с семидневной антиортостатической гипокинезией был выше, чем в группе крыс, находящихся в свободном поведении. После предварительной деконтаминации и последующего предъявления семидневной ан-

тиортостатической нагрузки содержание эндотоксина в сыворотке крови крыс достигает контрольного уровня. Таким образом, накапливаются экспериментальные факты, подтверждающие точку зрения о важной роли транслокации липополисахарида из кишечника во внутреннюю среду организма при ситуациях, моделирующих микрогравитацию. Эти экспериментальные данные важны в первую очередь в прикладном аспекте, поскольку являются базисом для разработки конкретных мероприятий, направленных на коррекцию процесса транслокации в условиях микрогравитации и невесомости.

Итак, в ходе экспериментальных исследований установлен факт развития толерантности к эндотоксину в условиях действия факторов, вызывающих транслокацию эндотоксинов из кишки во внутреннюю среду организма. Верифицирована гипотеза о том, что эндотоксин, содержащийся в желудочно-кишечном тракте, может быть одной из основных причин инициации системного воспалительного процесса при определенных условиях (в частности, при вертикальном укачивании или анатиортостатической гипокинезии). Характер развития системного воспалительного ответа при повторном экспериментальном введении эндотоксина зависит от времени, прошедшего после изменения действия гравитационного фактора, продолжительности действия этого фактора, состояния микрофлоры кишечника и интенсивности процесса транслокации эндотоксина из кишечника во внутреннюю среду организма.

Эти заключения актуальны для рассмотрения еще одного важного физиологического вопроса. В период внутриутробного развития плод находится в околоплодных водах, что естественным образом изменяет действие гравитационного фактора и его влияние на эмбриональное развитие. При этом с увеличением массы плода действие микрогравитации снижается, так как возрастает соотношение массы плода и околоплодных вод. Это соотношение сводится к минимуму непосредственно перед родами. Недавно появились сведения о том, что нормальная микрофлора кишечника у плода закладывается во второй половине беременности от матери вследствие процесса бактериальной транслокации [7]. В связи с этим, вполне логично предположить, что изменение действия гравитационного фактора в процессе внутриутробного развития плода способно сказаться на интенсивности перемещения микрофлоры и эндотоксинов из желудочно-кишечного тракта плода в его внутреннюю среду. Есть еще один важный аспект этой проблемы. Не разработан вопрос о закономерностях проникновения эндотоксина из кровеносного русла матери во внутреннюю среду организма плода. Целесообразность ответа, несомненно, назрела, учитывая литературные сведения о проницаемости плацентарного барьера не только для эндотоксина, но для ряда микробов [7]. Анализ этих сведений побудил одного из соавторов данной статьи заняться разработкой вопроса о динамике уровня эндотоксина и провоспалительных цитокинов в сыворотке крови беременных женщин. В результате проведенных наблюдений установлена связь между уровнем ключевого цитокина системного воспалительной ответа (IL6) и выраженностью токсических проявлений у беременных. Эти факты инициируют появление новых вопросов о физиологических и патофизиологических аспектах процесса транслокации эндотоксинов и микробов в разные периоды онтогенеза человека. Появление этих вопросов лишь подчеркивает актуальность проблемы и целесообразность ее изучения в дальнейшем.

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THE FEATURES OF ENDOTOXIN TRANSLOCATION PROCESS IN HUMAN AND ANIMAL ORGANISMS

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Summary

There carried out of the analysis of endotoxin and microbes translocation from a gastrointestinal tract to the internal milieu of an organism in norm and at a pathology. The physiological role of endotoxin translocation in natural conditions is shown. At development of pathological conditions the translocation process is playing a pathophysiologic role and at the certain situations including acting as the trigger of pathological processes development.

D. MODIFICATIONS VASCULAIRES ULTRASTRUCTURELLES

L'hypokinésie antiorthostatique modifie l'endothélium de micro vaisseaux cérébraux

"УЛЬТРАСТРУКТУРНЫЕ ИЗМЕНЕНИЯ В СОСУДАХ МОЗГА ПРИ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ /

LES CHANGEMENTS ULTRASTRUCTURELS DE VAISSEAUX CÉRÉBRAUX INDUITS PAR HYPOKINÉSIE ANTIORTHOSTATIQUE"

Navasiolava NM, Pashkevich SG, Emelianova AA, Custaud MA, Kulchitsky VA. - Neuroscience: theoretical and clinical aspects 4(2):21-24, 2008

"ОСОБЕННОСТИ УЛЬТРАСТРУКТУРЫ МИКРОЦИРКУЛЯТОРНОГО РУСЛА МОЗГА КРЫСЫ ПОСЛЕ ДВУХНЕДЕЛЬНОЙ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ /

LES PARTICULARITES DE L'ULTRASTRUCTURE DE MICRO VAISSEAUX CEREBRAUX DU RAT APRES 2 SEMAINES DE L'HYPOKINESIE ANTIORTHOSTATIQUE"

Emelianova AA, Pashkevitch SG, Navasiolava NM. - The problems of regulation of visceral functions. NASB, Anthology of scientific papers, in two volumes. Minsk. Vol 1:24-27, 2008

L'hypokinésie antiorthostatique et l'endotoxémie expérimentale induisent des changements semblables dans l'endothélium de micro vaisseaux cérébraux. L'endothélium semble aminci et de nombreuses excroissances d'endothélium sont apparues dans le lumen vasculaire ; des zones de desquamation et dénudation de membrane basale apparaissent aussi.

Ces changements microstructurels pourraient être déclenchés par le même mécanisme: celui des endotoxines décrit au chapitre précédent. L'hypokinésie antiorthostatique pourrait induire une augmentation de la translocation de l'endotoxine vers le milieu intérieur.

Il s'agit d'une étude préliminaire n'étudiant que quelques coupes et qui nécessitera de plus amples investigations.

Нейронауки: теоретичні та клінічні аспекти

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УЛЬТРАСТРУКТУРНЫЕ ИЗМЕНЕНИЯ В СОСУДАХ МОЗГА ПРИ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ

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Реферат. *Установлено, что при антиортостатической гипокинезии и экспериментальной эндотоксемии наблюдаются сходные ультраструктурные изменения в микрососудистом русле, наиболее выраженные в эндотелиальном слое. Эндотелий истончается, происходит его десквамация, появляются многочисленные выросты в просвет сосудов и дефекты эндотелиального слоя с обнажением базальной мембраны. Высказано предположение, что триггером указанных структурных преобразований эндотелия в обоих случаях является эндотоксин, поступающий при антиортостатической гипокинезии во внутреннюю среду организма из желудочно-кишечного тракта в процессе транслокации.*

Ключевые слова: *антиортостатическая гипокинезия, эндотелий, эндотоксин*

Эндотелий, формирующий внутреннюю поверхность кровеносных и лимфатических сосудов, играет важную роль в контроле вазомоторного тонуса и гемодинамики, взаимодействии с форменными и иными элементами крови, проницаемости, пролиферации, поддержании иммунного и гемостатического балансов, секреции регуляторных субстанций [8]. Функции эндотелия фактически связаны со всеми процессами, происходящими на границе внутренней среды организма и тканей [7]. В связи с этим становится понятным, почему клетки эндотелия при развитии патологических состояний в целостном организме активно вовлекаются в формирование защитных реакций, включающих системные воспалительные ответы, аутоиммунные, пролиферативные и, в целом, гомеостатические процессы [8, 9, 18]. При этом воздействие экстремальных факторов (гипоксия, интоксикация, воспаление, орто- или антиортостатическая нагрузка) сопровождается нарушением функций эндотелия вплоть до возникновения его дисфункций, что отражается на эффективности контроля разнообразных процессов в организме, в том числе, и тех, которые связаны с деятельностью гистогематических барьеров [10, 15].

Гистогематические барьеры регулируют обменные процессы между внутренней средой организма и тканями, обеспечивая постоянство физико-химических и биологических свойств тканевой жидкости. Например, на пути субстанций, поступающих в организм извне или образующихся в процессе пищеварения, стоит ряд барьеров, включающих и слой эндотелия. Среди таких физиологических преград можно выделить эпителиальный и эндотелиальный барьеры, слой глико-, муко- и липопротеинов, гликокаликс, анаэробную микрофлору кишечника, лимфоидную ткань желудочно-кишечного тракта и др. [6].

Ослабление защитных свойств барьеров в отношении токсичных веществ как поступающих извне, так и образующихся в самом организме, может явиться причиной возникновения патологических процессов. Этому способствует изменение проницаемости тканевых барьеров, которое наблюдается при действии ионизирующего, электромагнитного и ультрафиолетового облучений, ультразвуковом воздействии, гипер- и гипотермии, ишемии, гипоксии, гипокинезии, интоксикациях, гипероксии, гипербарии, действии повышенного и пониженного атмосферного давления, деиннервации тканей, воздействии электрического тока, ожогах, травмах различной этиологии, экспериментальном шоке, стрессах [1, 2, 4, 11]. Установлено нарушение барьерных функций тканей при действии на организм гравитационных факторов. Так, одной из причин развития синдрома укачивания является проникновение бактерий и эндотоксинов из кишечника во внутреннюю среду организма (транслокация) [4]. По мнению ряда авторов [2, 6, 11], естественный процесс транслокации эндотоксинов является необходимым условием для поддержания иммунного статуса и оптимального уровня глубокой температуры тела у теплокровных животных. Высказано предположение, что микрофлора и эндотоксины желудочно-кишечного тракта являются естественными активаторами нервных окончаний висцеральных органов и, таким образом, участвуют в формировании как тонуса автономной нервной системы [13, 16, 17], так и иммунного статуса [3, 14]. Проникновение эндотоксинов в кровь (эндотоксемия) сопровождается активацией защитных реакций организма, в частности, развитием системного воспалительного ответа 12, 16], направленного на первых порах на сохранение жизнедеятельности организма. В случае повторного попадания эндотоксинов в системный кровоток происходит трансформация ответных реакций организма, когда может развиться как их угнетение (толерантность), так и чрезмерная активация [4, 12, 14]. Как будут реагировать клетки эндотелия разных регионов кровеносного русла в условиях многократного моделирования антиортостатической гипокинезии, когда согласно приведенным выше литературным сведениям могут сформироваться условия как для проявления толерантности, так и повышенной реактивности эндотелиальных клеток? Наличие противоречий в ответах на этот вопрос побудило авторов сформулировать цель работы следующим образом: провести анализ структурно-функциональных особенностей эндотелия кровеносных сосудов при моделировании антиортостатической гипокинезии (АНОГ) у

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крыс (АНОГ является одной из популярных экспериментальных моделей анализа медико-биологических закономерностей в условиях невесомости).

Материалы и методы

В условиях хронического опыта на 46 крысах экспериментальные процедуры начинали в 9.00-9.30 утра. Животных адаптировали к условиям эксперимента, в частности, пребыванию в металлических решетчатых боксах, ограничивающих их подвижность (7 ежедневных тренировок по 2-4 часа). В день проведения опытов боксы с крысами располагали в термостатах при температуре 29°С и относительной влажности 50%. Колоническую температуру тела (Тк) регистрировали медь-константановыми термопарами (электротермометр фирмы "Physitemp", США), которые располагали в толстой кишке (8-9 см от ануса). Мониторинг глубокой температуры проводили каждые 5 мин. в течение пяти часов после введения эндотоксина. Для моделирования системной воспалительной реакции [13, 17, 19] крысам внутрибрюшинно вводили эндотоксин – липополисахарид кишечной палочки *Escherichia coli* (ЛПС, 0111:B4, List Biological Laboratories, Campbell, CA; Lot No LPS-25E) в дозе 10 мкг/кг/мл.

С целью изучения ультраструктурных особенностей микрососудов мозга крыс после раушнаркоза осуществляли интракардиальную перфузию апирогенным физиологическим раствором, а затем 2,5% раствором глутарового альдегида (10–12°С) на фосфатном буфере (pH 7,4). В дальнейшем фиксацию кусочков сосудов и ткани мозга проводили согласно общепринятой методике: окрашивание раствором четырехокиси осмия, дегидратацией в спиртах возрастающей концентрации и заключением объектов в смесь эпонов [5]. Затем изготавливали срезы ткани с помощью ультрамикротома фирмы LKB (Швеция) и окрашивали их цитратом свинца. Анализ изображений проводили с помощью электронного микроскопа JEM – 100 CX (Япония).

Для определения уровня цитокинов в крови крыс декапитировали с помощью гильотины (SAD 51330, США), осуществляли взятие крови, которую центрифугировали 15 мин. при 1500 об./мин. Сывороточную концентрацию интерлейкина-6 (ИЛ-6) измеряли методом иммуноферментного анализа (ELISA) с помощью набора для крыс (Biosource Internat., Camarillo, CA, США) [12].

Статистическую обработку данных проводили с использованием t-критерия Стьюдента и однофакторного дисперсионного анализа (ANOVA). Различия считались достоверными при $P<0,05$.

Результаты и обсуждение

Исследования проведены на 46 крысах-самцах линии Вистар массой 0,25±0,03 кг. Одна часть животных была распределена на четыре группы по 8 крыс в каждой. Крыс первой группы (группа 1) ежедневно в течение 4-х часов подвергали АНОГ (Р 45°) в боксах на протяжении двух недель. Крысы второй группы (группа 2) ежедневно одновременно с животными первой группы находились 4 часа в боксах (гипокинезия). Животных третьей группы (группа 3) наблюдали в условиях свободного поведения. В четвертой группе отмечено, что температура кожи хвоста крыс при 29°С в окружающем воздухе колебалась каждые 2-3 мин. в диапазоне от 30 до 33°С. Через несколько секунд после начала АНОГ кожная температура снизилась до 29°С, оставаясь такой же на протяжении 10-15 мин. и затем в течение 2-3 мин. достигла 35-36°С. На этом уровне температура поддерживалась около часа, затем зафиксировано её плавное снижение до 31-32°С в течение получаса. Через 5-10 мин. вновь наблюдали подъем температуры кожи хвоста крыс до 34-35°С (на этом уровне температура сохранялась 30-40 мин.). На 14-й день эксперимента уровень ИЛ-6 снизился ($P<0,01$) в группе крыс с АНОГ до 0,25±0,09 пг/мл по сравнению с животными с гипокинезией (4,01±0,57 пг/мл) и теми, которые находились в условиях свободного поведения (3,69±0,56 пг/мл).

Изменение уровня провоспалительного цитокина ИЛ-6 в организме может быть вызвано многими причинами, в основном, ассоциируемыми со стрессом, в первую очередь, вследствие транслокации эндотоксина из желудочно-кишечного тракта во внутреннюю среду организма [12]. Систематическое моделирование АНОГ и гипокинезии в течение двух недель гипотетически должно было привести к возрастанию уровня ИЛ-6, однако при гипокинезии уровень ИЛ-6 не отличался от такового у крыс третьей группы, которые находились в условиях свободного поведения, а при АНОГ (группа 1), содержание ИЛ-6 в сыворотке крови снизилось более чем в 10 раз. При этом основой механизма увеличения температуры кожи при АНОГ является пролонгированная дилатация кровеносных сосудов и возрастание кровотока в тканях хвоста [19], что сопровождается увеличением теплоотдачи [11, 16] и может быть обусловлено повышенным образованием монооксида азота [10, 18]. Выявленные закономерности – пролонгированная вазодилатация и падение уровня ИЛ-6 в сыворотке крови при экспериментальной модели АНОГ – могут быть маркерами процессов, отражающими нарушение взаимодействия функциональных систем в условиях моделирования невесомости, что в условиях космического полета сопровождается развитием патологических состояний. Высказывается мнение, что дисфункция эндотелия является одним из этих состояний [8]. Для проверки этого предположения проведены морфологические исследования.

В процессе выполненного электронно-микроскопического анализа (n=7) в микрососудах мозга через неделю после АНОГ зафиксированы выраженные изменения, которые можно оценить как ультраструктурные сдвиги, отражающие развитие дисфункции эндотелия. На рисунке 1 представлены структурные особенности биазиллярного сосуда крысы через одну неделю АНОГ. Обращает внимание истончение эндотелиального слоя в некоторых участках сосуда, вплоть до обнажения базальной мембраны, появление сложной формы эндотелиальных выростов в просвете сосудистого русла, вблизи которых располагались форменные элементы крови. Эти процессы, наряду с изменением адгезивных свойств мемб-

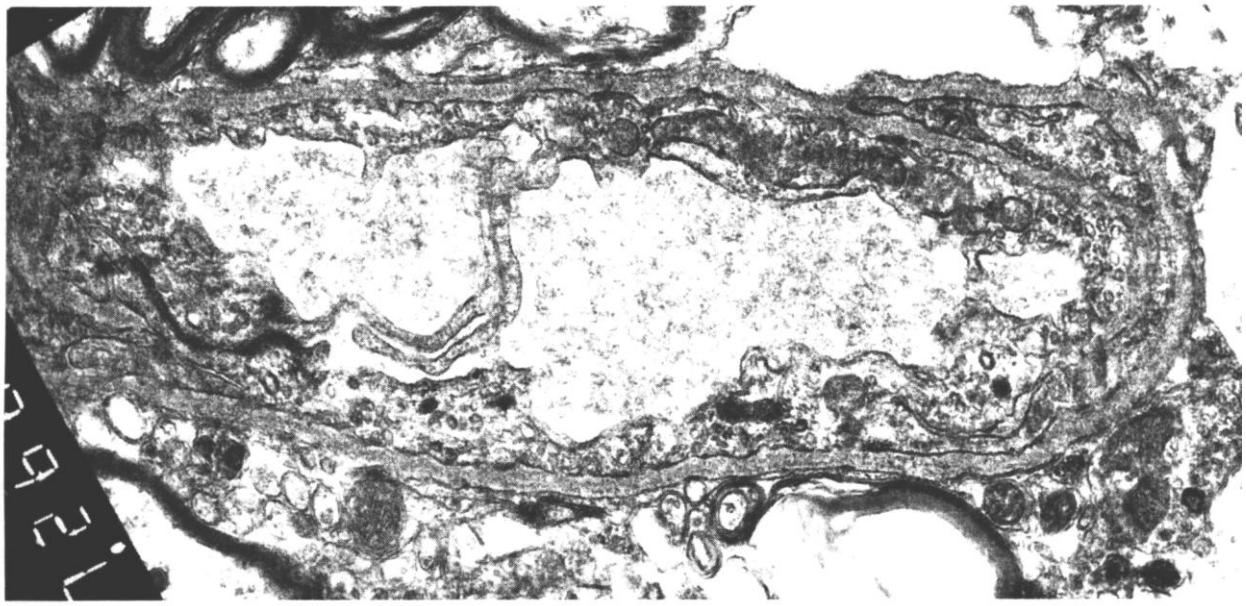

Рис. 1. Слущивание двух поперечно располагающихся в церебральном сосуде эндотелиальных клеток. На люминальной поверхности видны выросты разной величины и формы. Условия эксперимента – антиортостатическая гипокинезия. Увеличение: начальное – 7200, на рисунке – 10000

раны эндотелиоцитов, вполне могут приводить к нарушению гемодинамики и проницаемости сосудистой стенки.

Учитывая эти данные и с целью проверки выдвинутой гипотезы о роли процессов транслокации эндотоксинов из желудочно-кишечного тракта во внутреннюю среду организма при АНОГ, в дополнительной группе животных (n=7) осуществили однократное внутрибрюшинное введение ЛПС (10 мкг/кг/мл). В этой группе крыс анализировали особенности микрососудистого русла в толстой кишке, поскольку именно в этом отделе желудочно-кишечного тракта наблюдается наибольшее содержание микрофлоры [6, 11]. На рисунке 2 представлена электронно-микроскопическая картина сосуда толстой кишки через 2 часа после внутрибрюшинного введения 10 мкг/кг/мл ЛПС. Введение эндотоксина сопровождается изменениями в эндотелии, картина которых весьма напоминает сдвиги в стенке сосуда после АНОГ. Истончается слой эндотелия, выражены процессы адгезии форменных элементов крови вблизи трансформированной мембраны эндотелиоцитов. Столь близкая картина ультраструктурных перестроек в сосудах мозга и кишки при патогенетически разных процессах позволяет высказать гипотезу о близости их механизмов. Как уже упоминалось, основой предложенной авторами гипотезы является проникновение во внутреннюю среду организма эндотоксина из желудочно-кишечного тракта в результате «прорыва» барьеров (поломки защитных механизмов). Следовательно, можно заключить, что обнаруженные ультраструктурные особенности микрососудов после АНОГ и в условиях эндотоксемии являются одним из дополнительных аргументов в пользу высказанной гипотезы о роли эндотоксина в развитии дисфункции эндотелия при АНОГ.

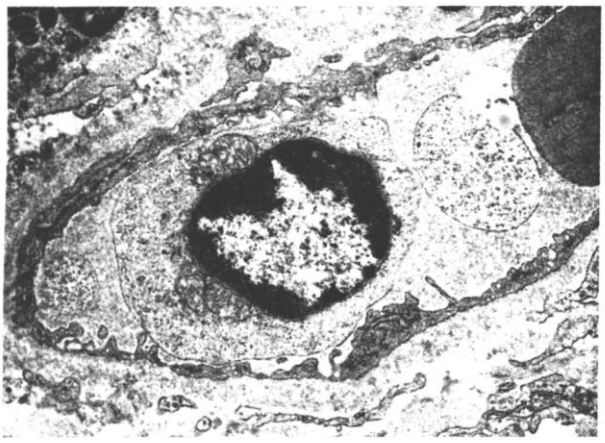

Рис. 2. Субмикроскопическая организация капилляра ободочной кишки крысы в условиях внутрибрюшинного введения липополисахарида E.coli в дозе 10 мкг/кг/мл. В пространстве сосуда располагаются лимфоцит и эритроцит. Стенка сосуда истончена, на его люминальной поверхности выявляются выросты, отдельные из которых отрываются и находятся в сосудистом пространстве. Увеличение: начальное – 4800, на рисунке – 10000

Установлено, что антиортостатическая гипокинезия у крыс сопровождается изменением в плазме уровня ИЛ-6 и нарушением регионарного перераспределения кровотока в сосудах хвоста. Высказано предположение, что триггером системного ответа организма при АНОГ является эндотоксин, поступающий во внутреннюю среду организма из желудочно-кишечного тракта в процессе транслокации. Эндотоксемия сопровождается структурными изменениями в сосудистом русле, наиболее выраженными и демонстративными в стенке кровеносных сосудов. Слой эндотелия истончается, происходит его десквамация, появляются многочисленные выросты в просвет сосудистого русла и дефекты эндотелиального слоя с обнажением базальной мембраны. Дальнейшие исследования позволят разобраться

в механизмах структурных и функциональных сдвигов в эндотелии при моделировании микрогравитации и предложить пути профилактики негативных последствий этих изменений, в том числе, и путем коррекции процессов транслокации эндотоксина, что актуально, в первую очередь, для космической медицины.

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Ultrastructural Changes in Brain Vessels During Antiorthostatic Hypokinesia

It was found that there are similar ultrastructural changes in brain microvessels, most pronounced in the endothelium, during antiorthostatic hypokinesia and experimental endotoxemia. The endothelium thinned, its desquamation occurred, numerous outgrowths into the vascular lumen and endothelial defects with exposed basal membrane appeared. It is suggested that these structural alterations in both cases are triggered by endotoxin that comes during antiorthostatic hypokinesia to the internal milieu from the gastrointestinal tract in the process of translocation. (Neuroscience: Theor. Clin. Asp. — 2008. — Vol. 4, № 2. — P. 21-24.).

Keywords: antiorthostatic hypokinesia, endothelium, endotoxin

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Ультраструктурні зміни в судинах мозку при антиортостатичній гіпокінезії

Встановлено, що при антиортостатичній гіпокінезії і експериментальній ендотоксемії спостерігаються схожі ультраструктурні зміни в мікросудинному руслі, найбільш виражені в ендотеліальному шарі. Ендотелій стає більш тонким, відбувається його десквамація, з'являються багаточисленні вирости в просвіт судин і дефекти ендотеліального шару з оголенням базальної мембрани. Висловлено припущення, що тригером вказаних структурних перетворень ендотелію в обох випадках є ендотоксин, що поступає при антиортостатичній гіпокінезії у внутрішнє середовище організму зі шлунково-кишкового тракту в процесі транслокації. (Нейронауки: теор. клін. асп.— 2008. — Т. 4, № 2. — С. 21-24.).

Ключові слова: антиортостатична гіпокінезія, ендотелій, ендотоксин

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ОСОБЕННОСТИ УЛЬТРАСТРУКТУРЫ МИКРОЦИРКУЛЯТОРНОГО РУСЛА МОЗГА КРЫСЫ ПОСЛЕ ДВУХНЕДЕЛЬНОЙ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ

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Введение. Сосудистая проницаемость является основой транскапиллярного обмена на уровне микроциркуляторного русла. Изменение проницаемости во многом зависит от субмикроскопической организации эндотелиальных клеток, в которых синтезируется часть факторов гомеостаза. На уровне эндотелия совершается много процессов [1]. В связи с этим, нарушения в структуре и, следовательно, функции эндотелия занимают одно из ключевых мест в развитии многих сердечно-сосудистых заболеваний, например, в патогенезе атеросклероза и его осложнений [2]. Все больше фактов за то, что в возникновении и развитии атеросклероза не последнюю роль играют воспалительные реакции. Синтез эндотелием целого ряда факторов роста, увеличение их продукции под действием медиаторов воспаления, относят также к факторам риска атеросклероза. Расшифровка механизмов, лежащих в основе сосудодвигательной дисфункции эндотелия и поиск лекарственных препаратов, предотвращающих и/или эффективно влияющих на уже сформировавшуюся патологию, далеки от завершения.

Задачей настоящей работы явилось изучение особенностей ультраструктуры элементов микроциркуляторного русла базиллярного сосудистого региона мозга крыс после 2-недельной тренировки в условиях антиортостатической гипокинезии..

Материал и методы исследования. Опыты проведены на половозрелых крысах массой 250–350 г. Материал для электронно-микроскопического исследования – кусочки базиллярной области мозга крыс – фиксировали в 4 % растворе глутаральдегида на 0,1 М фосфатном буфере с последующей фиксацией в 2 % растворе тетраокиси осмия. Обезвоживали в спиртах возрастающей крепости и ацетоне и заключали в смесь аралдитов [3]. Ультратонкие срезы, контрастированные уранилацетатом и цитратом свинца, анализировали в электронном микроскопе JEM 100 CX.

Результаты исследований и их обсуждение. В экспериментах установлено, что двухнедельная антиортостатическая гипокинезия сопровождается субмикроскопическими изменениями в микрососудах артериального и венозного русла вертебро-базиллярного бассейна каудальных отделов ствола головного мозга, которые, как правило, находятся в состоянии релаксации. В артериальном звене изменения ультраструктуры выражаются увеличением складок эндотелия и микроворсинок на его поверхности, а также ростом

числа микропиноцитозных пузырьков. В случаях отека эндотелиальных клеток (ЭК) может происходить нарушение структуры цитоплазматических органелл в виде деструкции митохондриальных крист, изменения объема эндоплазматического ретикулума, усиления вакуолизации цитоплазмы и появления локальных расширений подэндотелиального пространства. Ядра ЭК извилистой конфигурации с инвагинациями, хроматин сгруппирован у внутренней мембраны. Изменение структурно-функциональных свойств эндотелиальных клеток в условиях вышеназванных опытов способствует адгезии форменных элементов крови к ее люминальной поверхности (рис. 1).

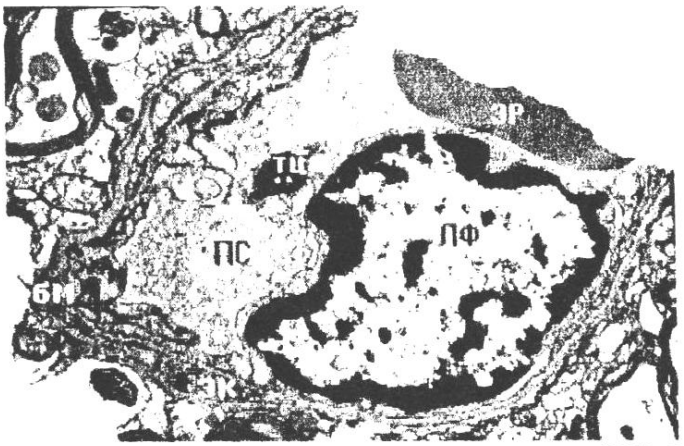

Рис. 1. **Изменение ультраструктуры эндотелиальных клеток (ЭК) базиллярной области мозга крысы в экспериментальных условиях антиортостаза. Описание в тексте. ЛФ – лимфоцит, ТЦ – тромбоцит, ЭР – эритроцит, БМ – базальная мембрана, ПС – просвет сосуда. Слева от тромбоцита - ЭК с нарушенной ультраструктурой. Ув 11200**

На рис. 1 виден тесный контакт форменных элементов крови – лимфоцита и лежащей рядом с ним тромбоцитарной пластинки, а также эритроцита – с эндотелием микрососуда. Деструкция расположенных слева от тромбоцита ЭК заключается в потере ими цитоплазматических органелл и превращении в окруженные мембраной и выдающиеся в просвет сосуда полости.

Микростазы и внутрисосудистые агрегаты эритроцитов способны образовывать микротромбы. Подобные нарушения сосудистого кровотока могут приводить к пенетрации форменных элементов крови в ткань мозга. Элементы крови обнаруживаются не только в периваскулярном пространстве, но и среди структур нейропиля (рис. 2, слева).

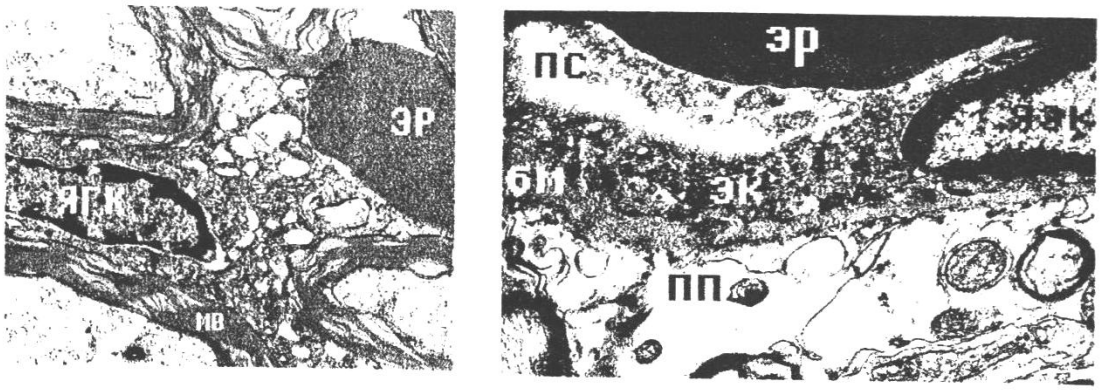

Рис. 2. **Нарушение ультраструктуры микроциркуляторного русла базиллярной области мозга крысы в условиях антиортостаза. Слева – появление эритроцита (ЭР) в межклеточном пространстве нейропиля. Справа – отек периваскулярного пространства (ПП), нарушение ультраструктуры эндотелиальной клетки (ЭК) и базальной мембраны (БМ). ЯГК – ядро глиальной клетки, МВ – миелинизированные нервные волокна. Увеличение соответственно – 10 200 и 13 000**

При нарушении целостности сосудистой стенки вследствие повреждения ультраструктуры эндотелиальных клеток и базальной мембраны (БМ), расположенные в отекшем периваскулярном пространстве элементы нейропиля частично разрушаются (рисунок 2, справа). На рисунке видны фрагменты разрушенных миелиновых волокон, отекшая митохондрия и многочисленные мембранные образования. В области нейропиля, находящегося вблизи сосуда, различить какие-либо дискретные структуры не представляется возможным.

Из анализа наших наблюдений следует, что часть микрососудов гипотетически выключена из кровотока по причине развития стаза. В некоторых сосудах кровоток приобретает характер гранулярного. Из-за наблюдаемого расхождения клеток в результате их сокращения межэндотелиальные щели расширяются, что неизменно приведет к увеличению проницаемости стенки сосуда. Подобный эффект повышения проницаемости отдельных участков сосудистой стенки принципиально не отличается от картины, которая наблюдается при выраженном истончении эндотелиальной выстилки микрососудов.

Базальная мембрана – мелкозернистая масса с низкой плотностью – в указанных экспериментальных условиях может незначительно утолщаться. В случаях же нарушения целостности клеточной мембраны и последующего разрыва сосуда она утолщается в несколько раз, а плотность становится настолько низкой, что ее зона выглядит «прозрачной». Таким образом, существенно меняется характеристика среды, с которой обязательно встречаются все вещества, проникающие из крови в ткань и в обратном направлении. Эти изменения могут касаться состояния воды как растворителя, состояния макромолекул как адсорбционных центров, состояния структурированности среды на молекулярном и надмолекулярном уровнях, что в совокупности

может определять степень проницаемости основного вещества как одного из структурных элементов гистогематического барьера [4].

Заключение. Итак, в условиях экспериментальной антиортостатической гипокинезии происходят нарушения не только ультраструктуры клеток микроциркуляторного русла базиллярной области мозга крыс, но и тонкой организации элементов, находящихся по обе стороны сосудистой стенки. Активация эндотелиоцитов веществами, синтезируемыми самим эндотелием и расслабляющими его гладкую мускулатуру [5], а также изменение физических и химических (усиление продукции цитокинов) свойств крови в условиях антиортостаза способствуют адгезии форменных элементов крови к люминальной поверхности сосуда. Недавно получены экспериментальные данные о нарушении регионарного кровотока и изменении интенсивности метаболизма у крыс в условиях антиортостатической гипокинезии [6]. Нарушение структуры эндотелиоцитов, их набухание и разрушение цитоплазматических органелл, расширение межэндотелиоцитарных щелей или значительное истончение эндотелиальной выстилки способствуют отеку периваскулярного пространства. Деструкция стенки сосудов, вплоть до нарушения их целостности, может явиться причиной локального расширения подэндотелиальных пространств, разрушения базальной мембраны и появле-

ельно, описанные эффекты антиорто- исследуемой области мозга. Следоват
нте отражают комплекс патологиче- статической гипокинезии в экспериме
ге, одним из показателей которых яв- ских процессов, развивающихся в моз
ональной организации элементов сис- ляется нарушение структурно-функци
зиллярной области мозга крыс. Даль- темы микроциркуляторного русла ба
экспериментальные, позволят предло- нейшие исследования, в том числе и
ии эндотелия при подобных патологи- жить способы восстановления функци
ческих процессах.

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DISCUSSION ET CONCLUSIONS

A. Discussion générale

1. Est-ce que l'immersion sèche est un bon modèle de microgravité ?
2. Déconditionnement cardio-vasculaire en immersion sèche, considérations méthodologiques
3. Quels seraient les mécanismes de l'atteinte vasculaire induite par la microgravité ?
4. Les atteintes vasculaires et le déconditionnement cardio-vasculaire général

B. Conclusions et perspectives

A. DISCUSSION GENERALE

1. Est-ce que l'immersion sèche est un bon modèle de microgravité ?

Aucun modèle terrestre ne peut reproduire tous les effets d'un vol spatial. Ainsi le modèle doit être choisi en fonction des systèmes que nous désirons étudier et des contremesures que nous souhaitons tester. L'atout le plus important de l'immersion sèche est l'absence de gradient de support : l'appui est reparti également sur toute la surface corporelle. Notre système de proprioception l'interprète comme l'absence complète d'appui, situation comparable à celle de la microgravité réelle. C'est donc un modèle unique permettant d'étudier les effets de la microgravité induits spécifiquement par l'absence d'appui. Dans le contexte de l'immersion sèche, il a été démontré que les signaux issus des zones d'appui des pieds déterminent par voie réflexe le « on / off » des muscles posturaux. C'est également le modèle de choix pour étudier la redistribution du tonus musculaire avec la chute du tonus des muscles extenseurs posturaux (Grigor'ev et al. 2004, Kozlovskaia 2008), la facilitation des réflexes spinaux (Sayenko 2007, Kozlovskaya 2002), les perturbations du maintien de la posture verticale (Sayenko et al. 2005) et de la locomotion (Mel'nik et al. 2006), et plus généralement le syndrome d'ataxie hypogravitationnelle (manque de coordination fine des mouvements volontaires) (Kozlovskaia 2008). L'évolution des désordres neuromoteurs en immersion sèche et en microgravité réelle sont très proches (Kozlovskaia 2008).

Ce modèle serait intéressant à l'avenir pour explorer les liens entre les zones d'appui et le système cardio-vasculaire. L'influx afférent des zones plantaires d'appui ou bien son absence pourrait influencer la régulation réflexe de la circulation et induire directement la redistribution sanguine parmi les différentes régions circulatoires.

En comparaison avec l'alitement prolongé, l'immersion sèche est un modèle accéléré. Il faut 60-90 jours d'alitement pour atteindre le même niveau de perte de fonctions contractiles musculaires qu'après 7 jours d'immersion (Grigor'ev et al. 2004). Cette perte en immersion

apparaît en raison de la détérioration de l'appareil neuromusculaire (« motor drive »), avant que l'amyotrophie ne survienne.

Concernant les changements de la balance hydro-sodée avec le modèle d'immersion sèche, nous avons montré une perte rapide du volume plasmatique plus importante qu'avec le modèle d'alitement et comparable à celle mesurée en vol spatial (selon les données obtenues dans la station « Mir », la diminution de volume plasmatique en vol est d'environ 15% - Grigoriev et al. 2002). Sans oublier que les mécanismes de cette diminution en immersion et en alitement antiorthostatique sont différents de ceux en vol réel (Norsk et al. 2001; Diederich et al. 2007).

Le modèle d'immersion sèche présente ses inconvénients. En comparaison avec un alitement prolongé, la pression d'eau sur les régions abdominales et thoraciques génère une résistance, aussi minime soit-elle, au fonctionnement des muscles respiratoires. La situation d'immersion est moins habituelle pour l'homme que la position couchée. Cette situation peut être vectrice d'un stress émotionnel, particulièrement à la phase initiale. Le confort thermique n'est pas toujours assuré (Kovalenko & Gurovski 1980). Finalement, l'alitement peut être maintenu beaucoup plus longtemps (l'immersion la plus prolongée était de 56 jours, et l'alitement - de 370 jours, Huntoon et al. 1998). L'alitement prolongé reste ainsi le meilleur modèle pour étudier l'amyotrophie et la perte osseuse, dont l'apparition nécessite de longs délais d'hypokinésie.

Les modèles d'immersion sèche et d'alitement prolongé se complètent bien pour apprécier l'intégralité des effets physiologiques de la microgravité.

2. Déconditionnement cardio-vasculaire en immersion sèche, considérations méthodologiques

L'immersion sèche induit incontestablement un déconditionnement cardio-vasculaire. La baisse rapide de la capacité à l'exercice (Anashkin & Beliaev 1982), l'intolérance orthostatique, la tachycardie majorée lors des tests orthostatiques (Iwase et al. 2000) et la diminution de tolérance à l'accélération (Vil-Viliams et al. 2001) ont été montrées avec ce modèle.

Pourtant, les effets de l'immersion sèche sur l'intolérance orthostatique ne sont pas clairs. Quel est le degré de diminution de tolérance induite par l'immersion par rapport à celui résultant de l'alitement ou du vol réel ? La réponse n'est pas évidente. La méthodologie du test orthostatique n'est pas toujours indiquée dans les publications. Elle varie selon les auteurs par le moment (premier levé, jour de récupération 1 ou 2), le caractère du test (stand ou tilt – avec appui sur les pieds ou sur la selle, angle d'inclinaison), la durée maximale du test. En outre, il manque des données sur le type d'intolérance. De plus, les protocoles d'immersion sèche utilisés diffèrent par la profondeur d'immersion (jusqu'à cou ou jusqu'aux aisselles), la période journalière hors baignoire, la consommation d'eau et de nourriture (*ad libitum* ou imposée).

Certaines **considérations méthodologiques** sont à souligner. Etudier des sujets jeunes facilitera la comparaison avec les données d'alitement. Les individus ayant bénéficié d'un entraînement physique soutenu sont prédisposés à l'hypotension et à l'intolérance orthostatique (Ogoh et al. 2003). Les sujets dont la masse musculaire est particulièrement développée souffrent davantage de douleurs dorso-lombaires au début de l'immersion.

La période ambulatoire qui précède l'immersion ne doit pas excéder 2 jours, sans quoi le déconditionnement risquerait de s'amorcer, en lien avec l'isolement, le confinement et la diminution de l'activité physique.

Les consommations d'eau et de nourriture, soigneusement notées, doivent être de préférence *ad libitum*. Un apport d'eau standardisé en quantité importante peut jouer le rôle de contremesure (Shulzhenko et al. 1977; Kokova 1984). Une alimentation inhabituelle et

une consommation d'eau imposée induisent de nouvelles conditions auxquelles chaque sujet devra s'adapter. De plus, une alimentation familière en quantité et en qualité crée un contexte émotionnel favorable, élément capital dans le cadre d'expériences internationales.

Pour la durée d'immersion, 5 ou 7 jours semblent être suffisants pour observer un déconditionnement cardio-vasculaire. Cette durée est suffisante pour engendrer des changements significatifs, tout en étant bien tolérée (jamais un volontaire n'a renoncé à l'expérimentation avant la fin d'une immersion de 7 jours dans l'expérience de l'IBMP).

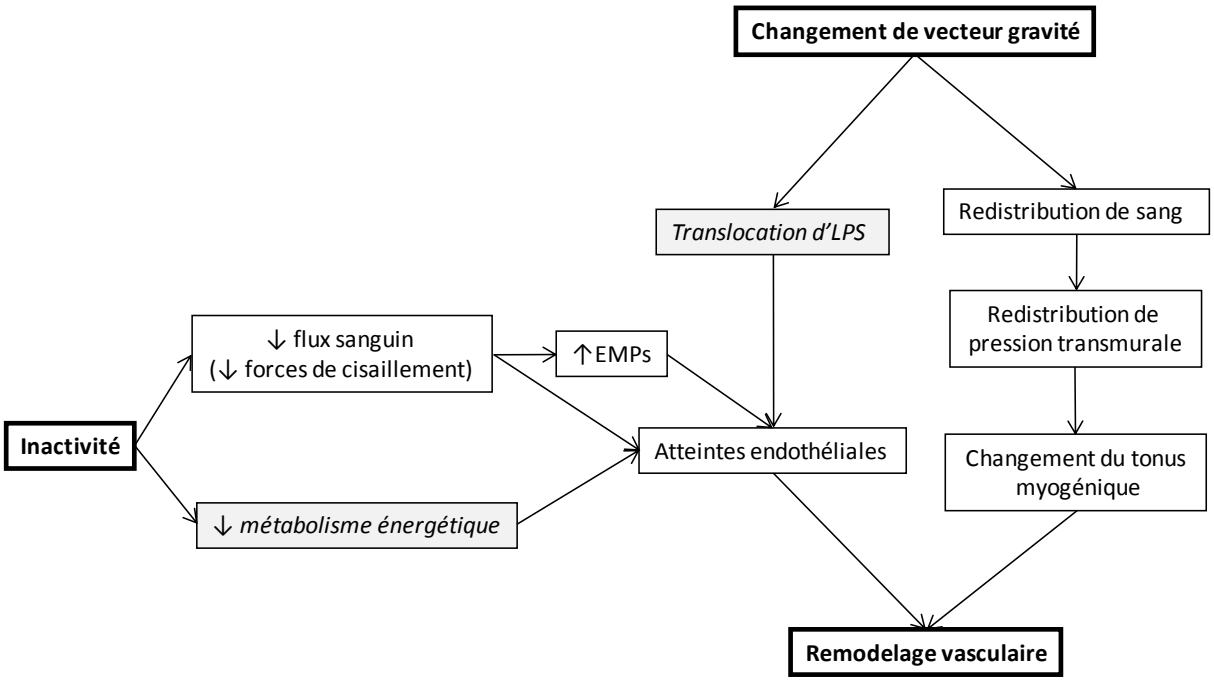
Il faut éviter de surcharger le protocole par des tests, surtout par ceux qui nécessitent l'extraction de volontaire de la baignoire. La profondeur d'immersion doit être jusqu'au cou, pour maximiser l'effet. Si l'on opte pour un levé quotidien de 15 minutes (ce qui est fait habituellement), il faudra nécessairement en comparer les résultats avec ceux des expériences d'alitement comprenant également un levé quotidien. Une brève stimulation orthostatique quotidienne peut jouer le rôle de contremesure (Greenleaf 1984, Vernikos et al. 1996). Si l'on veut maintenir le protocole « strict », il faut minimiser les périodes hors baignoire (douche, pesée) et au mieux, maintenir la position horizontale pendant ces périodes.

La mise en œuvre d'un test orthostatique standardisé lors du premier levé avec un enregistrement en continu de la pression artérielle et de l'électrocardiogramme permettra d'obtenir une image la plus complète possible de la tolérance orthostatique après l'immersion sèche.

3. Quels seraient les mécanismes de l'atteinte vasculaire induite par la microgravité ?

La Fig. 15 montre les mécanismes qui ont été étudiés dans ce travail. Les hypothèses étudiées exclusivement sur modèle animal sont en italique.

Figure 15. Le schéma des mécanismes étudiés. EMPs, microparticules circulantes endothéliales ; LPS, lipopolysaccharide



Modification des forces physiques

Comme nous l'avons indiqué, les modifications du vecteur gravité et l'inactivité physique modifient les forces agissant sur les vaisseaux. La microgravité annule la composante hydrostatique de la pression artérielle, tandis que l'inactivité physique diminue la composante hydrodynamique.

Il semble que les modifications soient différentes au niveau de la macro- et de la microcirculation lors des simulations par alitement prolongé (Demiot et al. 2007) et par immersion sèche (Iarullin et al. 1987). Après alitement, la réactivité vasodilatatrice des vaisseaux est plutôt augmentée pour la macrocirculation (augmentation de la vasodilatation induite par le flux dans les artères de conduction, Bleeker et al. 2005b) et diminuée pour la microcirculation (réduction de la réponse cutanée induite par l'iontophorèse de

vasodilatateurs, Demiot et al. 2007). Nous supposons que les atteintes microcirculatoires sont les manifestations les plus précoces, jouant ainsi un rôle important dans le déconditionnement cardio-vasculaire. Nous avons montré que l'immersion sèche induisait rapidement les changements microcirculatoires prédominants sur l'endothélium. La diminution des forces de cisaillement au niveau de la microcirculation liée à l'inactivité physique serait en partie responsable de cette dysfonction endothéliale.

EMPs

Nos résultats chez l'homme suggèrent un décalage de l'équilibre apoptotique endothélial en faveur de l'apoptose, en l'absence de signe d'inflammation endothéliale (estimée par le dosage de E-sélectine), d'activations leucocytaire et plaquettaire (estimées par les taux de microparticules correspondantes). La production de microparticules par les cellules endothéliales en immersion sèche peut alors, même partiellement, être provoquée par la diminution du tonus antiapoptotique.

Néanmoins, la production augmentée de EMPs pourrait être induite par les facteurs associés à l'activation du phénotype pro-inflammatoire et pro-thrombotique des cellules endothéliales : la diminution des forces de cisaillement, l'augmentation de cytokines proinflammatoires, le stress oxydatif excessif (Boulanger et al. 2006).

Les microparticules endothéliales semblent également jouer un rôle fonctionnel. Au niveau local, elles pourraient réduire la biodisponibilité locale du NO et donc altérer la fonction endothéliale. A l'étage systémique, elles pourraient diffuser la dysfonction endothéliale au sein de l'arbre vasculaire.

LPS

Des taux élevés de LPS dans le sang pourraient induire des atteintes endothéliales (Dauphinee et al. 2006). Les conditions de changement de vecteur gravité pourraient contribuer à une augmentation de la translocation de LPS. Avec le modèle animal nos résultats suggèrent directement (augmentation de niveau du LPS dans le sang) et indirectement (développement de tolérance au LPS et absence de cette tolérance en cas de décontamination préalable) que cette translocation est augmentée.

Métabolisme énergétique

Nos études animales ont relevé la diminution d'intensité du métabolisme avec le changement du vecteur gravité. Les changements de régulation du métabolisme énergétique pourraient induire des atteintes endothéliales. Il a été montré que l'inactivité physique chez les sujets sains induisait une hypertriglycéridémie et une diminution de la sensibilité à l'insuline (Bergouignan et al. 2010), en parallèle à une dysfonction endothéliale (Hamburg et al. 2007). Chez les rats, la suspension de 10 heures induisait une chute considérable (jusqu'à 95%) de l'activité de la lipoprotéine lipase musculaire (Bey & Hamilton 2003).

Dans un protocole d'immersion sèche, une augmentation significative de l'insulinémie à jeun a été constatée (Afonin & Sedova 2008), argumentant l'hypothèse de la résistance à l'insuline, mais ce thème est encore insuffisamment étudié dans ce modèle.

Rhéologie du sang

Un autre facteur potentiellement responsable des atteintes des fonctions vasculaires est la modification de la balance hydro-sodée. Les propriétés rhéologiques du sang, les forces de cisaillement et les conditions hémodynamiques en sont affectées. Un remodelage vasculaire peut en résulter.

Pourtant, les rhéologies sanguines macro- et microcirculatoires sont différentes. Une couche mince pariétale du plasma est formée avec le mouvement du sang et les érythrocytes sont "alignés" dans la coquille de plasma. En conséquence, les « gros » vaisseaux contiennent la plupart des érythrocytes (hématocrite 40-50%) et les capillaires représentent un réservoir de plasma (hématocrite 6-10%). Cette différence explique l'effet Fahraeus-Lindqvist – c'est-à-dire la diminution de la viscosité avec la diminution du diamètre du vaisseau (Chien 1982).

Ainsi, une augmentation de l'hématocrite, et donc de la viscosité sanguine, dans les macrovaisseaux pourrait ne pas avoir d'effet sur les microvaisseaux. Cependant, la majeure partie des cellules endothéliales réside dans la microcirculation. Cela pourrait expliquer, dans notre étude, l'altération des fonctions endothéliales malgré l'augmentation de l'hématocrite. Nous avons à prendre en compte la détérioration des conditions hémodynamiques locales comme un facteur majeur de dysfonction endothéliale.

4. Les atteintes vasculaires et le déconditionnement cardio-vasculaire général

Avec l'immersion sèche nous avons observé une dysfonction vasculaire au niveau microcirculatoire prépondérante sur l'endothélium et des changements cardio-vasculaires en général. Une tachycardie plus importante lors du stand test à R+2 témoignait du déconditionnement cardio-vasculaire. En outre nous avons observé une augmentation des taux de NT-proBNP pendant la période de récupération. BNP et NT-proBNP sont libérés en réponse à la distension ventriculaire. Cela reflète le niveau des contraintes pariétales s'exerçant sur le myocarde et le remodelage potentiel myocardique. L'augmentation du NT-proBNP peut être liée au travail cardiaque plus élevé après immersion. Cette augmentation peut apparaître comme un argument en faveur du déconditionnement cardio-vasculaire et apprécier le degré de celui-ci.

L'hypovolémie est l'un des mécanismes reconnus dans le déconditionnement cardio-vasculaire (Buckey et al. 1996, Convertino 1995). Nous avons observé une hypovolémie pendant l'immersion. L'inactivité physique accrue, comme formulée dans l'introduction, induit aussi des modifications fonctionnelles cardio-vasculaires.

Un lien possible entre une atteinte vasculaire et une baisse de la capacité à l'exercice serait une diminution de l'aptitude vasodilatatrice des microvaisseaux, ce qui pourrait participer à la diminution de consommation maximale d'oxygène ($\dot{V}O_2max$). En outre, nous pouvons suggérer une altération directe des échanges transcapillaires induits par les changements endothéliaux.

Les liens entre une atteinte vasculaire et une intolérance à l'orthostatisme peuvent être établis sur les bases d'un possible déficit d'augmentation des résistances périphériques en position debout et d'une altération de l'autorégulation de la circulation cérébrale.

L'insuffisance d'augmentation des résistances périphériques aux stimuli orthostatiques est un facteur majeur reconnu d'hypotension orthostatique induite par la microgravité (Zhang 2001). Après le vol, les astronautes intolérants à l'orthostatisme ne présentent pas l'augmentation adéquate des résistances périphériques totales en position debout (Buckey et

al. 1996), et aussi une moindre augmentation des taux plasmatiques de noradrénaline (Fritsch-Yelle et al. 1996). Une dégradation des mécanismes vasoconstricteurs peut apparaître au niveau des récepteurs, des centres intégrateurs, des voies du système nerveux autonome et des organes cibles (Blomqvist 1996), les organes cibles étant principalement les vaisseaux.

Nous avons observé une diminution du flux sanguin cutané basal en immersion sèche. Cela suggère une vasoconstriction basale plus importante et donc une diminution de la réserve de la vasoconstriction, qui peut conduire à une limitation de la réponse vasoconstrictive. Nous avons observé aussi une diminution du volume plasmatique estimé. Cette diminution peut être à l'origine d'une augmentation du tonus vasoconstricteur basal et donc d'une diminution des capacités de vasoconstriction supplémentaires. Selon l'hypothèse de Convertino 1999, la réserve vasoconstrictive diminuée peut être le mécanisme principal de l'insuffisance vasoconstrictrice en cas d'intolérance orthostatique. La capacité maximale de vasoconstriction ne s'altère pas en microgravité (Convertino 1999), mais la réduction du volume sanguin peut induire une vasoconstriction de base plus importante et ainsi diminuer la réserve vasoconstrictive. Néanmoins les résistances périphériques totales n'étaient pas modifiées dans notre étude. L'augmentation de la vasoconstriction cutanée pourrait compenser la vasoconstriction insuffisante d'autres lits vasculaires.

Les changements adaptifs des vaisseaux cérébraux en réponse à une redistribution liquidienne et une augmentation chronique des pressions artérielle et intracrânienne en conditions de microgravité pourraient également contribuer à l'intolérance orthostatique. Zhang et al. 1997 ont montré par mesure du flux cérébral que l'autorégulation cérébrale pendant un stress orthostatique (LBNP) est diminuée après 2 semaines d'alitement antiorthostatique. D'un autre coté, Iwasaki et al. 2007 ont trouvé une autorégulation cérébrale préservée, voire améliorée, après un vol spatial de courte durée.

B. CONCLUSIONS ET PERSPECTIVES

Les mécanismes potentiels de la dysfonction vasculaire induite par la microgravité sont complexes. L'immersion sèche reproduit fidèlement les effets physiologiques principaux de la microgravité. Notre travail montre que la microgravité simulée par immersion sèche induit des changements rapides au niveau microcirculatoire, ces changements prédominants sur l'endothélium. La dysfonction endothéliale repose probablement sur des mécanismes apoptotiques. Les modifications de la balance hydro-sodée apparaissent très rapidement en immersion avec un passage à un nouvel état d'équilibre dès le deuxième jour. Pendant la période de récupération, le NT-proBNP plasmatique augmente significativement. Le NT-proBNP pourrait servir de marqueur biologique du déconditionnement cardio-vasculaire. Nos études de la réponse cardio-vasculaire à l'orthostatisme permettent aussi de suspecter une plus grande sollicitation du réflexe veino-artériolaire après l'immersion.

Le modèle animal demeure un modèle d'étude nécessaire pour explorer de nouvelles hypothèses. Avec nos modèles d'hypokinésie horizontale et antiorthostatique discontinues chroniques chez le rat, nous avons proposé de nouveaux mécanismes de dysfonction vasculaire. Les études animales suggèrent une translocation accrue des endotoxines de l'intestin vers le milieu interne. Les modifications ultrastructurelles de l'endothélium des microvaisseaux cérébraux induites par endotoxémie expérimentale et par l'hypokinésie antiorthostatique sont très semblables. La translocation augmentée des endotoxines pourrait être l'une des raisons probables des dysfonctions endothéliales en microgravité. La réduction du métabolisme énergétique en microgravité pourrait aussi induire des atteintes endothéliales.

D'un point de vue fondamental, il est nécessaire d'approfondir la connaissance des mécanismes de cette atteinte endothéliale. En particulier, il conviendrait de regarder de plus près le rôle des progéniteurs endothéliaux - les cellules circulantes capables de se différencier en cellules endothéliales et donc capables de participer à la réparation des

atteintes endothéliales. Pour comprendre le lien entre atteintes endothéliales et intolérance orthostatique, il serait utile d'étudier les fonctions vasculaires cérébrales en détail.

D'un point de vue pratique, la dysfonction endothéliale doit être considérée comme une cible thérapeutique, dans le cadre des atteintes liées à l'inactivité physique et à la microgravité.

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Résumé

La gravité est une stimulation permanente pour l'organisme sur terre. La microgravité induit des modifications de nombreuses fonctionnalités, y compris celles du système cardio-vasculaire. Le but de ce travail était d'étudier les mécanismes des modifications vasculaires induites par les modèles de microgravité.
Nous avons conduit nos études chez l'homme à l'aide de l'immersion sèche qui est considérée comme un des modèles reproduisant au mieux la microgravité. L'immersion sèche est le seul modèle de microgravité prolongée à imiter l'absence d'appui. Notre travail montre que l'immersion sèche induit une atteinte au niveau microcirculatoire avec une dysfonction endothéliale (étude des microparticules circulantes) et une atteinte de la fonction vasodilatatrice cutanée (étude par iontophorèse couplée au laser Doppler). Cette atteinte vasculaire s'intègre dans un syndrome de déconditionnement cardio-vasculaire plus global que nous avons également étudié. Il comprend un nouvel équilibre hydro-sodé qui survient très rapidement, dans les 24 premières heures. Pendant la période de récupération, nous avons observé une augmentation significative du NT-proBNP qui pourrait être un marqueur biologique du déconditionnement cardio-vasculaire.
Nos études sur l'animal avec les modèles d'hypokinésie et d'inclinaison tête en bas discontinues chroniques suggèrent qu'une translocation augmentée d'endotoxine et une réduction du métabolisme énergétique en microgravité pourraient participer aux atteintes endothéliales.
La dysfonction endothéliale deviendrait ainsi une cible thérapeutique pour des atteintes liées à l'inactivité physique et à la microgravité.

Mots clés

Microgravité modelé, immersion sèche, déconditionnement, système cardio-vasculaire, dysfonction vasculaire, microcirculation, dysfonction endothéliale, endotoxine

Title

THE MECHANISMS OF VASCULAR DYSFUNCTION INDUCED BY SIMULATED MICROGRAVITY

Abstract

Gravitational stimulation is an inherent part of terrestrial environment. Modified gravity changes functioning of numerous body systems, including cardiovascular system. The aim of this work was to study the mechanisms of vascular modifications induced by modeled microgravity.
The human studies were performed using dry immersion. This model is considered as one of the most appropriate to reproduce the effects of prolonged microgravity and especially to mimic the lack of support. We found that dry immersion induces microcirculatory impairment with endothelial dysfunction (study of circulating microparticles) and impaired skin vasodilation (study by iontophoresis of vasoactive substances coupled with laser Doppler flowmetry). This vascular impairment is integrated to more general cardiovascular deconditioning syndrome. The global deconditioning was also considered in this work, including the rapid reset of water and sodium balance to a new steady state. During the recovery period, we observed a significant increase of plasmatic NT-proBNP which might reflect the degree of cardiovascular deconditioning.
Our studies with animal models of horizontal and antiorthostatic hypokinesia suggest that increased endotoxin translocation and reduced energy metabolism in microgravity could contribute to endothelial impairment.
Endothelial dysfunction may be regarded as one of the therapeutic targets for pathologies associated with physical inactivity and microgravity.

Keywords

Simulated microgravity, dry immersion, deconditioning, cardiovascular system, vascular dysfunction, microcirculation, endothelial dysfunction, endotoxin

Spécialité

Physiologie et pathophysiologie humaine